

Original Article

Anti-inflammatory evaluation of *Spatoglossum variabile* by GC-MS, *in silico* and *in vitro* assessment

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ABSTRACT

Objectives: Inflammation is a crucial component of various diseases such as arthritis, asthma and cancer. *Spatoglossum variabile* is a brown algae species that has not been extensively studied for its anti-inflammatory potential. This study aimed to evaluate the *in vitro* anti-inflammatory activity of the ethanolic extracts of *S. variabile* in protein denaturation and human red blood cell (HRBC) assay.

Materials and Methods: *S. variabile* was collected and extracted using ethanol, and bioactive compounds were identified using gas chromatography–mass spectroscopy (GCMS) techniques. Moreover, the *in silico* studies were carried out to check its binding affinity. The anti-inflammatory activity was evaluated by measuring the percentage inhibition of protein by *in vitro* studies. The bioactive compounds responsible for the anti-inflammatory activity were identified using GC-MS analysis. Based on the GCMS report, the ethanolic extract contains more bioactive compounds, so the extract is chosen for further study.

Results: The ethanolic extracts of *S. variabile* showed significant dose-dependent percentage inhibition of protein. The ethanolic extract was found to be more potent, with inhibition rates of 70%, 80% and 90% for the egg albumin and HRBC assay. The GC-MS analysis identified bioactive compounds in the ethanolic extracts that were found to have minimum binding affinity.

Conclusion: The results suggest that *S. variabile* extracts possess potent anti-inflammatory activity *in vitro*, which could be attributed to the presence of bioactive compounds such as hydroxy-2-methyl-4-pentanone (diacetone), Tetradecanoic acid, Neophytadiene, n-Hexadecanoic acid, Oleic acid, Octadecanoic acid, Behenic alcohol, 4,8,12,16-Tetramethylheptadecan-4-olide, 1-Heptacosanol, Oleoyl chloride, 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E) and Pregnane Silane Derivatives. These findings provide evidence for the potential use of *S. variabile* as a natural source of anti-inflammatory agents.

Keywords: Antibacterial, Anti-inflammatory, Antimicrobial, Gas chromatography mass spectroscopy, Molecular docking, Phytochemicals screening, *Spatoglossum variabile*

INTRODUCTION

A complicated biological reaction of vascular tissue in response to noxious stimuli, characterised by pathogens and irritants are known as inflammation.^[1] Chemical and physical factors such as bacteria, poisons, radiation, injuries and caustic chemicals are also responsible for inflammation.^[2] A defensive mechanism, the inflammatory response seeks to limit hazardous substances. Another objective is the removal of damaged cells to promote tissue or organ repair.^[3] The beginning, progression, modulation and eventual resolution of the acute stage of inflammation are all

mediated by a variety of chemical mediators that are released from neutrophils and macrophages. The removal of cell debris is primarily accomplished by monocytes. There will be a chronic stage if the acute stage is not resolved.^[4] Rheumatoid arthritis, atherosclerosis, hay fever and ischaemic heart diseases are all caused by chronic inflammation^[5-7] and inflammation is a common symptom of infectious diseases such as leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vascularitis, celiac diseases and autoimmune diseases. Recently, chronic inflammatory diseases have been identified as the leading cause of death worldwide, accounting for more than half of all fatalities. These conditions include ischaemic heart disease, chronic kidney disease, cancer, diabetes, neurodegenerative diseases and autoimmune diseases. However, non-steroidal anti-inflammatory medicines (NSAIDs) have a variety of side effects, including increased risk for heart disease and gastrointestinal discomfort.^[7] Therefore, an in-depth study was done on several plant species and their active components in an effort to find new chemicals with anti-inflammatory capabilities that are less expensive and side effect free. Furthermore, research was not just done to assess the anti-inflammatory efficacy. As a result, numerous studies were conducted to identify all possible therapies.

The nuclear factor kappa B (NF- κ B) pathway has long been considered a prototypical proinflammatory signalling pathway, largely based on the role of NF- κ B in the expression of proinflammatory genes, including cytokines, chemokines and adhesion molecules. NF- κ B's intricate role in inflammation is discussed in this article.^[8] However, these most recent results indicate that this pathway may prove to be a challenging target in the treatment of chronic disease. NF- κ B has long been regarded as the 'holy grail' as a target for new anti-inflammatory medications. The activation of NF- κ B by proinflammatory cytokines such as interleukin-1 and tumour necrosis factor- α , as well as the role of NF- κ B in the expression of other proinflammatory genes such as cytokines, chemokines and adhesion molecules, which have been extensively reviewed elsewhere, has led to the long-held belief that NF- κ B is the prototypical proinflammatory signaling pathway.^[9] However, the inflammatory response involves a complicated physiological process called NF- κ B and inflammation is one of these processes. Through its capacity to trigger the transcription of proinflammatory genes, nuclear translocation of cytoplasmic complexes, which activate the NF- κ B/Rel transcription family, plays a crucial role in inflammation.^[10] The proper cellular stimulation, which is typically caused by signals relating to infections or stress, causes this route to become active. That is how different NF- κ B proteins are specialised, how they play a part in inflammatory diseases, how I κ B proteins and I κ B kinase control NF- κ B activity and how NF- κ B inhibition therapy techniques are being developed. In response to a

specific stimulus, the production of NF- κ B proteins can offer site- and event-specificity.^[8,11,12]

Spatoglossum variabile belongs to the Dictyotaceae family and has a wide range of beneficial benefits. Ninety per cent of the world's living biomass is found in the oceans, with marine species comprising approximately half of the total global biodiversity. Marine vegetation is a potentially abundant source of highly bioactive secondary metabolites that could serve as helpful leads in the creation of novel pharmacological medicines.^[13] Algae can be divided into two primary categories: macroalgae (seaweeds), which include green, brown and red algae and microalgae (blue-green algae, dinoflagellates, Bacillariophyta).^[14] Marine algae are abundant in dietary fibre, minerals, lipids, proteins, vital amino acids, polysaccharides and Vitamins A, B, C and E. The main components of brown and red algal cell walls are phycocolloids, which are extensively employed in industry and include agar, alginic acid and carrageenan. In the last four decades, a large number of new compounds have been identified from marine creatures, and many of these compounds have been shown to exhibit fascinating biological activity.^[15] Their sizes range significantly, from microscopic unicellular organisms to huge kelps up to 70 m long that can grow as fast as 50 cm/day.^[16] Algae are present almost everywhere on the planet, including the ocean, rivers, lakes, soil and cliffs. Lately, microalgae metabolites have drawn a lot of attention, and several researchers have written on the subject. Many health-promoting properties, such as anti-oxidative, anti-inflammatory, antibacterial and anti-cancer effects, have been identified by studies on the bioactivities of marine algae.^[17] Anti-inflammatory properties are well-known for many marine-based natural products that contain antioxidants.^[18] *Sargassum vulgare*, *Spatoglossum schroederi* and *S. variabile* also have anti-inflammatory effects. Recent epidemiological and clinical research has demonstrated that consumption of plant-derived foods and beverages may lower the risk of oxidative damage-related diseases, such as aging and other lifestyle diseases.^[19] A lot of emphasis has been placed on the isolation and study of new bioactive components with biological activity from marine algae. According to their colour, marine algae were largely divided into three primary types known as brown, red and green algae, which are referred to as *Phaeophyceae*, *Rhodophyceae* and *Chlorophyceae*, respectively.^[20,21] It has been discovered that different pigments extracted from marine algae have a variety of biological activities and potential health perks. Due to this, there has recently been a surge in interest in adopting complementary and natural therapies to treat a variety of disorders; however, there is a lack of sufficient scientific data. Interest in medicines derived from nature has increased to create novel drugs with greater therapeutic potential.^[22,23]

MATERIALS AND METHODS

Collection and authentication

Algae were collected from Rameshwaram, India. The herbarium was made, and authentication was carried out at Madras Christian College, Chennai, India.

Extraction of the plants

The algae were cleaned and shade dried before extraction. The dried material was then ground into powder using an electric blender. The coarse powders obtained were extracted in high polarity order with n-Hexane, chloroform, ethyl acetate and ethanol at 80°C to obtain extracts using a Soxhlet apparatus. These extracts were then concentrated in a Rota evaporator under reduced pressure and constant temperature at 65–80°C and dried to powder, and their extractive yields were measured.^[24] Based on the extractive yield, the solvent was chosen for the entire extraction process. The determination of the extraction yield of *S. variabile* was calculated by the following equation.^[25]

$$\text{Extraction yield (\%)} = W1/W2 \times 100$$

Where W1 is the mass of crude extract

W2 is the mass of the sample.

Phytochemical screening

The phytochemical study of extracts from *S. variabile* revealed a wide variety of phytochemicals. The key components, such as flavonoids, tannins, alkaloids, glycosides, steroids and saponins were present in the extracts [Table 1].^[25]

Successive extraction solvent	Weight of algae	Yield
N-hexane	250 g	0.02 g
Chloroform	250 g	0.15 g
Ethyl acetate	250 g	0.5 g
Ethanol	250 g	1.5 g

Table 2: Phytoconstituents present in the extract.

S. No.	Phytoconstituents	N-hexane	Chloroform	Ethyl acetate	Ethanol
1.	Carbohydrates	–	–	+	+
2.	Proteins	+	+	–	+
3.	Alkaloids	+	+	+	+
4.	Flavonoids	–	–	–	+
5.	Glycosides	–	+	–	+
6.	Tannins	–	+	+	+
7.	Steroids	+	–	+	+
8.	Reducing sugars	+	–	–	+

Gas chromatography/mass spectroscopy (GC/MS) analysis

The GC-MS analysis was conducted on a GC-MS QP2010 Plus equipped with a flame ionisation detector. The GC is equipped with a fused silica (30 m × 0.25 mm ID × 0.25 mm) capillary column. The oven temperature was programmed at 60°C for 2 min, then increased to 300°C for 6 min at the rate of 10°C/min. Helium was used as a carrier gas at a flow of 1.0 mL/min. The injector temperature was 250°C, injection size 1.0 µL neat, with a split ratio 10:1. Mass detector turbo mass gold-Perkin Elmer was used as the detector. The phytoconstituents were identified after comparison with those available in the WILEY 8. LIB is attached to the instrument and reported [Figure 1].^[26]

Identification of compounds

The National Institute of Standards and Technology (NIST)-08 LIB and WILEY-8 LIB library sources were utilised for matching the detected components from the plant material containing more than 62,000 patterns, and they were employed for the interpretation of the mass spectrum of the GC-MS. The *S. variabile* fraction's acquired spectrum of the unidentified components was compared to the standard mass spectra of the known components kept in the NIST collection (NISTII). Based on this, the test sample names, molecular weights and structures were determined.^[27]

Swiss absorption, distribution, metabolism and excretion (ADME) property explorer

From the ChemDraw Ultra, all of the ligands' smiles were obtained, and the Swiss ADME programme was then used. The representation of a boiled egg, physicochemical properties, lipophilicity, water solubility, drug similarity and pharmacokinetics are all provided.^[28]

Docking studies

Preparation of ligands

The phytoconstituents found in the extract identified by GCMS analysis and standard drugs available in the market

were selected as ligands.^[29] Extracting the International Union of Pure and Applied Chemistry (IUPAC) names into ChemDraw Ultra transformed into structures. The structures were then copied, pasted and optimised in Chem3D Pro before being saved as PDB files.

Preparation of protein

Using the protein data bank, structures of the protein used in this study were retrieved (<https://www.rcsb.org/>) in 3D structure format. A transcription factor known as nuclear factor- κ B (NF- κ B) is essential for many biological processes, such as immune response, inflammation, cell growth and survival and development. The protein structure of NF- κ B PDB ID: 1SVC was retrieved from the Protein Data Bank. Other than the water, heteroatom and ligands which were imported in Biological Visualisation and Analysis (BIOVIA) Discovery studio was exported as PDB format.

Docking analysis

The AutoDock version 2.4.1 software was utilised to perform the docking of structures. Both ligands in mol2 format and the protein structure in PDB format were uploaded for this process. Each run constituting the docking experiment was individually configured to conclude after 31 evaluations. In the BIOVIA Discovery Studio, the prediction of target receptor binding attributes was carried out using the receptor cavity technique. This involved assessing the inhibitory properties of amino acid residues within the binding site to identify active sites on the target receptor. The molecular docking experiment between the receptor and ligands was executed using the AutoDock Vina 4.2.6 programme from The Scripps Research Institute. The docking protocol is initiated with the ligand and receptor to identify potential binding sites on the target protein. Standard procedures, such as the addition of polar hydrogen atoms to protein targets and the application of Kollman unified atomic charges, were followed. Hydrogen atoms were added to the ligands before applying Gastiger partial charges. After removing the existing crystal ligand, bond ordering was verified (Trott and Olson 2010). Subsequently, the results from individual docking runs for each ligand were obtained in terms of binding energies. The Biovia Discovery Studio was employed to compile configurations with the highest free energy values and lowest root mean square deviation, and to illustrate the molecular interactions.^[30]

In vitro anti-inflammatory studies

Using the Egg albumin denaturation assay and the human red blood cell (HRBC) membrane stabilisation assay, ethanolic extracts were evaluated for their ability to reduce inflammation.

EGG albumin denaturation assay

Fresh egg albumin, Phosphate Buffered Saline (PBS) buffer phosphate saline (pH 6.4), and various extract concentrations (10, 20, 40, 80 and 100 μ g/mL) were all included in the reaction mixture (5 mL). The positive control contained 2.0 mL of diclofenac sodium solution at various doses (10, 20, 40, 80 and 100 μ g/mL), 0.2 mL of freshly prepared egg albumin, and 2.8 mL of PBS (pH 6.4). The equivalent quantity of egg albumin and PBS was used in the negative control samples, along with 2.0 mL of distilled water. After being incubated at 37°C for 15 min, the mixture was heated to 70°C for 5 min to produce denaturation. Following cooling off, the rate of absorption was measured using the water as a blank at 660 nm (ultraviolet mini 1240, Shimadzu). The procedure was run in triplets throughout. Using the formula, the % inhibition for the denaturation of proteins was determined [Supplementary 2].^[29]

$$\text{Inhibition (\%)} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

HRBC membrane stabilisation

The experimental approach of Aidoo DB *et al.* was used to evaluate the effects of the *S. variabile* extracts on heat-induced HRBC haemolysis.^[31]

Preparation of solutions

Alsever's solution

Alsever's solution consists of 0.8 g of sodium citrate, 2 g of dextrose, 0.05 g of citric acid and 0.42 g of sodium chloride diluted in 100 mL of distilled water.

Hypotonic saline

Hypotonic saline embody of 0.36 g of sodium chloride was dissolved in 100 mL of distilled water to create hypotonic saline.

Isotonic saline

Isotonic saline embody of 0.85 g of sodium chloride was dissolved in 100 mL of distilled water to create isotonic saline.

PBS buffer

PBS buffer, embody of 0.19 g of potassium dihydrogen phosphate, 2.38 g of disodium hydrogen phosphate and 8 g of sodium chloride were taken as phosphate buffer saline in 100 mL of distilled water (pH 7.4, 0.15 M).

Preparation of red blood cells suspension

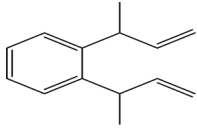

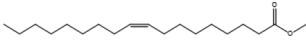
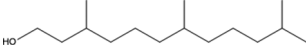
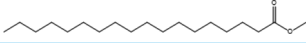
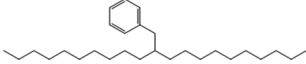
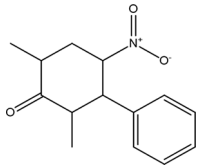
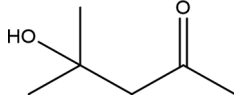
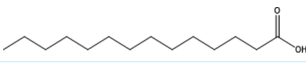
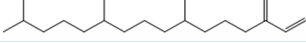
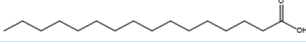
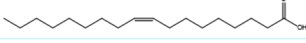
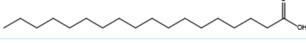

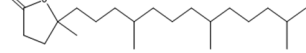
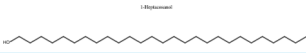
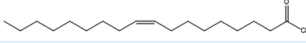
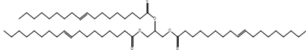
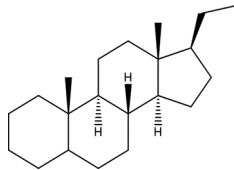
The fresh blood was drawn from healthy volunteers, who

Table 3: GCMS identified compounds.

S. No.	Compound name	Structure	Retention time	Area%
Chloroform				
1.	2-hydroxy-2-methyl-4-pentanone (diacetone)		4.359	5.45
2.	1-isopropyl-4-methyl-3-cyclohexen-1-ol		9.956	2.11
3.	Ethyl cyclopropanecarboxylate		12.494	9.62
4.	Phenol, 3,5-bis (1,1-dimethylethyl)-		14.694	2.13
5.	Dodecyl acrylate		16.855	4.24
6.	Tetradecanoic acid		17.709	4.33
7.	9-octadecene, (e)-		17.944	3.11
8.	Dodecane, 2,7,10-trimethyl-		18.005	1.33
9.	Neophytadiene		18.467	3.00
10.	N-Hexadecanoic acid		19.854	20.79
11.	1-nonadecene		20.076	6.91
12.	9-Octadecenoic acid (Z)-, methyl ester		21.221	1.17
13.	Oleic acid		21.629	1.89
Ethyl acetate				
14.	N-Tetracosanol-1		22.026	4.09
15.	2-hydroxy-2-methyl-4-pentanone (diacetone)		4.389	26.38
16.	Neophytadiene		18.485	2.46
17.	Hexadecanoic acid, methyl ester		19.454	7.01
18.	Pentadecanoic acid		19.890	4.19
19.	(1-methylene-5-phenyl-5-hexenyl) benzene		19.960	5.85
20.	Hexadecanoic acid, ethyl ester		20.124	3.99

(Contd...)

Table 3: (Continued).

S. No.	Compound name	Structure	Retention time	Area%
Ethyl acetate				
21.	1,2-bis (1-methyl-2-propenyl) benzene		20.301	1.37
22.	5,8,11-heptadecatriynoic acid, methyl ester		20.354	2.43
23.	9-octadecenoic acid (z)-, methyl ester		21.228	5.07
24.	1-Dodecanol, 3,7,11-trimethyl-		21.360	1.89
25.	Methyl stearate		21.451	1.54
26.	Benzene,(2decylidodecyl)-		21.877	2.93
27.	2,6-Dimethyl-4-nitro-3-phenyl-cyclohexanone		23.092	1.74
Ethanol				
28.	2-hydroxy-2-methyl-4-pentanone (diacetone)		4.356	1.08
29.	Tetradecanoic acid		17.701	6.14
30.	Neophytadiene		18.461	5.14
31.	N-Hexadecanoic acid		19.931	44.27
32.	Oleic acid		21.657	8.98
33.	Octadecanoic acid		21.832	3.42
34.	Behenic alcohol		22.021	1.55
35.	4,8,12,16-Tetramethylheptadecan-4-olide		23.634	1.11
36.	1-heptacosanol		23.808	1.11
37.	Oleoyl chloride		24.204	1.13
38.	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E, E, E)-		24.615	1.08
39.	Pregnane, silane deriv		24.717	1.72

GCMS: Gas chromatography–mass spectroscopy.

did not take any alcohol, steroids, and NSAIDs for at least 1 week. Blood samples were drawn into centrifuge tubes and centrifuged for 10 min at a speed of 3,000 rpm to separate the packed red blood cells. To prepare a 10% v/v suspension for later usage, separated packed cells were flipped using an equal sample of normal saline solution to supernatant and retransformed as a suspension with PBS (pH 7.4).

The test solution consisted of 1 mL of phosphate buffer, 2 mL of hypotonic saline, 0.5 mL of crude extract/fractions at different concentrations and 0.5 mL of 10% w/v HRBCs.

As a test control, 1 mL of phosphate buffer, 2 mL of water and 0.5 mL of a 10% suspension of HRBCs in isotonic saline were utilised.

Standard solutions included 1 mL of phosphate buffer, 2 mL of hypotonic saline, 0.5 mL of standard medication solutions with different concentrations and 0.5 mL of 10% w/v HRBCs (diclofenac sodium).

All of the assay mixtures were centrifuged at 3,000 rpm after 30 min of incubation at 37°C, and the supernatant was poured out to determine the haemoglobin content using a spectrophotometer at 560 nm. The following formula was used to calculate the percentage of protection against haemolysis [Supplementary 2].^[32]

$$\text{Percentage protection} = 100 - \left[\frac{\text{optical density sample}}{\text{optical density control}} \times 100 \right].$$

RESULTS

Percentage yield of extract

Choosing a solvent for an extraction can be a complex process that involves many factors, including the type of algae being extracted, the desired compounds to be extracted and the intended use of the extract. The percentage yield of the extract is one factor that can be considered when selecting a solvent. For extraction, the solvent is used according to the polarity order from N-hexane to ethanol [Table 1].

Preliminary phytochemical analysis

The algae extract in different solvents tested for the presence or absence of various phytochemicals in the qualitative form is noted in Table 2. The results show that algae contain phytochemical groups, such as - alkaloids, flavonoids, proteins, carbohydrates, steroids, glycosides, phenols, saponins and terpenoids. The results of preliminary phytochemical analysis should be considered as a preliminary indication of the chemical composition of the ethanolic extract. Quantitative analysis and further validation are

necessary to confirm the presence of specific compounds and their potential biological activity.

GC-MS analysis

The GC-MS chromatogram of the extracts of *S. variabile* in ethanol, ethyl acetate and chloroform revealed 155 compounds in ethanol and 112 peaks in the other two solvents. According to the bioactive compounds that were identified [Table 3] by comparing their mass spectral fragmentation patterns, peak retention times, peak areas (%) and heights (%) to those of the known compounds listed in the NIST collection. Results revealed that 13 compounds were found in chloroform and ethyl acetate; 14 compounds were found in ethanol extracts. Overall, the 39 main phytochemicals were identified in chloroform, ethyl acetate and ethanol. Overall, the successive extraction was effective in identifying the diverse range of compounds from the ethanol extract. The ethanolic extract contained more polar compounds when compared to chloroform and ethyl acetate. Moreover, several of these compounds have been shown to have potential anti-inflammatory properties. Overall presence of these compounds 2-HYDROXY-2-METHYL-4-PENTANONE (DIACETONE),

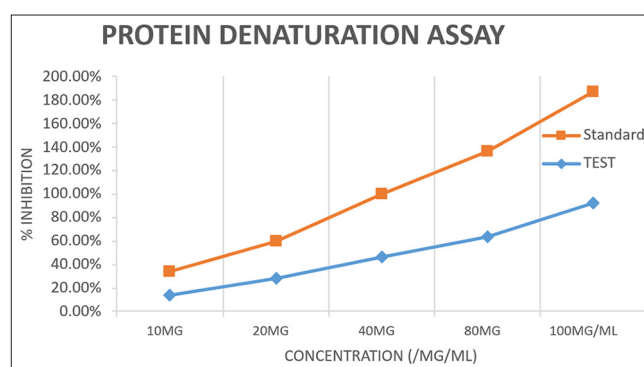


Figure 1: Graphical representation of protein denaturation.

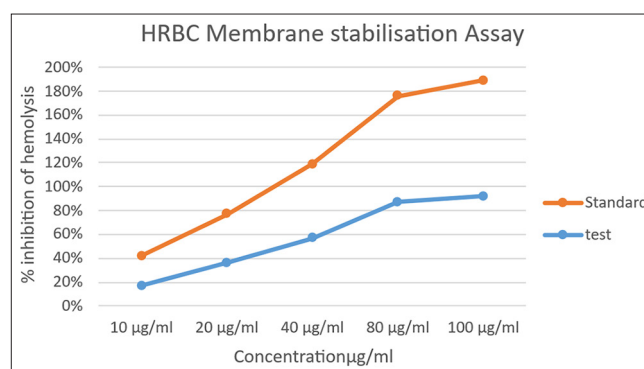


Figure 2: Graphical representation of human red blood cell assay.

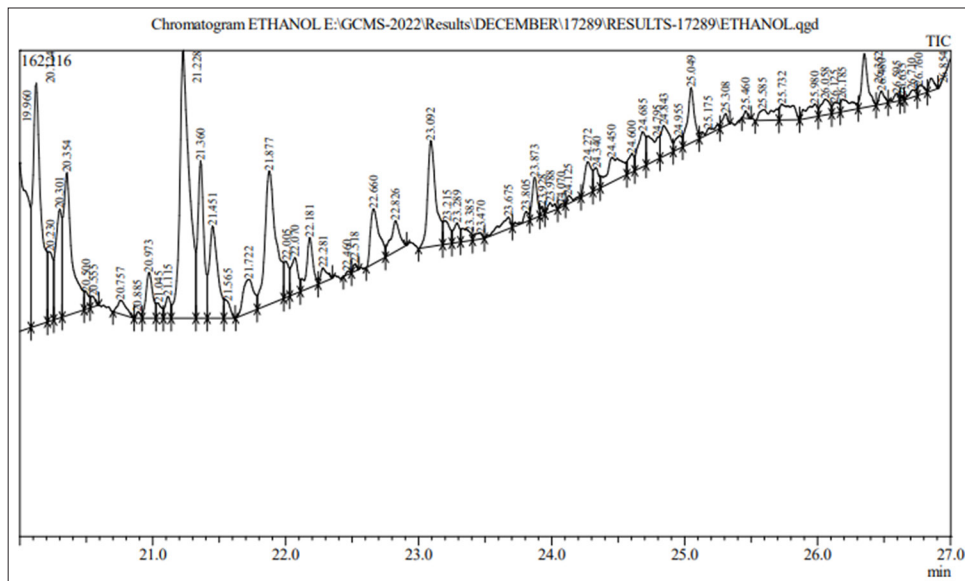


Figure 3: GCMS chromatogram of the ethanolic extract of *Spatoglossum variabile*

Tetradecanoic acid, Neophytadiene, n-Hexadecanoic acid, Oleic acid, Octadecanoic acid, Behenic alcohol, 4,8,12,16-Tetramethylheptadecan-4-olide, 1-Heptacosanol, Oleoyl chloride, 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-, Pregnane Silane Derivatives in the ethanolic extract suggest that it has the potential to promote wound healing through several different mechanism, including reducing inflammation, promoting tissue repair, increasing collagen production and preventing infection.

Swiss ADME analysis

From the ChemDraw Ultra, all of the ligands' smiles were obtained, and the Swiss ADME programme was then used. The representation of a boiled egg [Figure 2], physicochemical properties, lipophilicity, water solubility, drug similarity and pharmacokinetics are all provided. Drug-like compounds should have a good aqueous solubility, which is predicted by three methods: ESOL, (ALI) logs and (SILICOS-IT) logs.^[33]

In silico studies

Docking studies

In silico docking studies can be used to predict the binding affinity of small molecules to a target protein for anti-inflammatory purposes. The binding affinity of each compound for the target protein is expressed as a binding energy or score [Table 4]. The predicted binding mode of the compound to the target protein, including the amino acid residues involved in the interaction and the type of interaction (e.g., hydrogen bonds, van der Waals forces) [Supplementary 1]. Interpretation of the results and their significance in relation to the anti-inflammatory activity

Table 4: Binding energy of the compounds by *in silico* docking.

Compound name	Binding energy
	NF-kappa b
Diclofenac sodium	-7.1
2,6-Dimethyl-4-nitro-3-phenyl-cyclohexanone	-6.5
2-(2-aminoanilino)-5-methyl-2-thiazoline	-6.2
10-heneicosene, 11-phenyl-	-6.2
N-benzyl-2-([5-(2-furyl)-1,3,4-oxadiazol-2-yl] sulfanyl) acetamide	-7.3
2-((e)-[[(e)-2-[(e)-(2-hydroxyphenyl) methylidene] amino) propyl] imino] methyl) phenol	-7.1
1,1'-(4-methyl-1,3-phenylene) bis[3-(5-isopropyl-1,3,4-thiadiazol-2-yl) urea]	-9.0
2-(3-hydroxy-1-propynyl)-2-adamantan-2-ol	-5.6

Table 5: Egg albumin assay.

Concentration	Standard (%)	Test (%)
10 µg	20	13.6
20 µg	32	28
40 µg	54	46
80 µg	72	64
100 µg	95	92

of the compounds studied. The results obtained from *in silico* docking studies should be considered as preliminary indications of a compound's potential anti-inflammatory activity. Additional experimental data and validation are necessary to confirm these predictions.^[34]

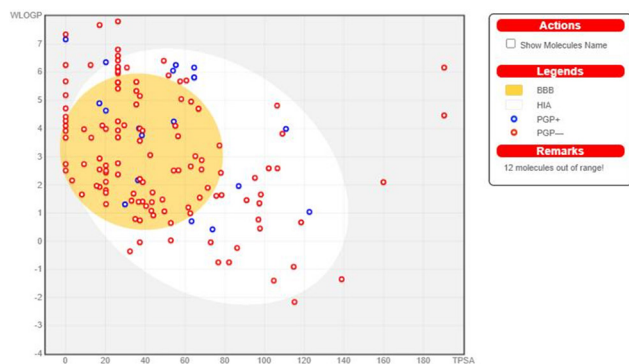


Figure 4: SwissADME BOILED-Egg model representing the predicted gastrointestinal absorption. (HIA) and blood-brain barrier (BBB) permeability of the identified phytoconstituents

***In vitro* studies**

Protein denaturation assay

EGG albumin

Egg albumin assay is a common *in vitro* test used to assess the protein content of egg white. The egg albumin assay is very important to assess the protein content of egg white. The results of the egg albumin assay, a graph showing the standard curve generated using known concentrations of albumin and their corresponding absorbance values [Figure 3]. The protein concentration of the egg white sample, expressed in mg/mL or percentage. The results from replicates of the assay, including mean values and standard deviations. The results from control samples, including positive and negative controls. Interpretation of the results and their significance in relation to the protein content of the egg white sample. Comparison with previously reported studies, limitations and future directions. The results obtained from *in vitro* egg albumin assays should be interpreted with caution, as there may be variations in the protein content of eggs due to factors such as diet, age and storage conditions. Additional experimental data and validation may be necessary to confirm the protein content of the egg white sample [Table 5].

HRBC assay

The HRBC assay is an *in vitro* test used to evaluate the anti-inflammatory activity of compounds. The HRBC assay is very important to evaluating the anti-inflammatory activity of compounds. The absorbance values obtained for the control group may consist of untreated HRBCs or HRBCs treated with a known pro-inflammatory agent [Figure 4]. The absorbance values obtained for the test groups, which consist of HRBCs treated with different concentrations of the test compound. The concentration of the test compound that inhibits 50% of

haemolysis, calculated using a dose–response curve. Results and their significance in relation to the anti-inflammatory activity of the test compound were interpreted [Table 6]. Comparison with previously reported studies, limitations and future directions.

DISCUSSION

Microalgae contain several different types of potent, naturally occurring bioactive compounds. The extract from *S. variabile* was used in the current study to examine anti-inflammatory activity. An HRBC assay and albumin denaturation were used to measure the extract's anti-inflammatory activity.^[35,36]

In addition to fucoxanthin, the most significant component found in marine brown algae, the spectral analysis of the extract from *S. variabile* revealed the existence of numerous other active elements.^[37] Using GC MS analysis, it is feasible to identify the major non-volatile chemicals. Unprocessed ethanolic extract displayed a high peak intensity molecule that was significant to numerous other essential compounds, including pentadecanoic acid, 14-methyl-, methyl ester and methyl stearate, among other derivatives.^[38,39] Minor substances such as 10-methyl-, methyl ester, methyl tetradecanoate, tetradecanoic acid, 12-methyl-, methyl ester, 9-hexadecenoic acid, methyl ester, (Z)-. By using GC MS analysis, the main non-volatile chemicals are recognised.^[40] The high peak intensity compound dominating the presence of important compounds, including Pentadecanoic acid, 14-methyl-, methyl ester and methyl stearate among other derivatives, was indicated by the crude ethanolic extract.^[20] Minor compounds were also found. They were 10-methyl-, methyl ester, methyl tetradecanoate, tetradecanoic acid, 12-methyl-, methyl ester, 9-hexadecenoic acid, methyl ester, (Z)-, hexadecanoic acid, 14-methyl-, methyl ester, 10-octadecenoic acid, methyl ester and heptadecanoic acid. These substances have been shown to have antioxidant, antifungal, antimicrobial, nematocidal, hypercholesterolemic and cancer-preventive properties.^[41] Furthermore, the compounds identified from GCMS analysis of the algae extract have revealed many active compounds which are responsible for anti-inflammatory activity. Certain literature has also reported that these active constituents have been present in certain plant species and have anti-inflammatory activity. By this, we are also confirming that the extract of *S. variabile* also has anti-inflammatory activity by performing the egg albumin and HRBC assay.^[42] Several investigations have led researchers to the conclusion that the ethanol extract of seaweeds contains phenolics, alkaloids and amino acids that may be the cause of the anti-inflammatory activity.^[43]

For *in silico* docking, the protein 1SVC was chosen based on the pathophysiological steps it is engaged in, and 7 compounds were chosen and docked with this protein. The compounds were chosen based on the peak produced from GCMS analysis. In addition, the standard drug Diclofenac

Table 6: HRBC assay values.

Concentration	Standard (%)	Test (%)
10 µg	25	17
20 µg	40	36
40 µg	62	57
80 µg	104	97
100 µg	129	113

HRBC: Human red blood cell

was docked with these proteins, and the binding energies of the standard drug were compared to the binding energies of the seven compounds obtained from the extract. All 7 compounds have excellent binding energies compared to standard drugs with proteins. This was further evaluated by an *in vitro* anti-inflammatory protein denaturation assay in which most proteins lose their biological function when they are denatured, a process known as albumin denaturation. Inflammation can be predicted to be caused by the denaturation of proteins. The capacity of the ethanolic extract of *S. variable* to inhibit protein denaturation was intended as a component of the research into the mechanism of the anti-inflammation activity. With a maximal inhibition of 92% seen at 100 µg/mL, it was successful in preventing albumin denaturation. Diclofenac, a common anti-inflammatory medication, had a maximal 95% inhibition at the same dose. Protein denaturation was intended to be avoided by the ethanolic extract of *S. variable*. At 100 µg/mL, the maximum level of inhibition is 92%. It has shown efficacy in halting albumin denaturation. The common anti-inflammatory drug diclofenac demonstrated a maximal inhibition of 95% at the same dose. Since the erythrocyte membrane and the lysosomal membrane have a similar structural makeup, the HRBC membrane stabilisation method was used to evaluate the *in vitro* anti-inflammatory efficacy.^[26] Lysosomal stabilisation is essential for limiting the release of lysosomal contents, which in turn helps to regulate the inflammatory response. Lysosomal enzymes generated during inflammation are responsible for a wide range of diseases. At lesser concentrations like 100 g/mL, the standard medication had more activity (25%) than the test sample (17%), but at this concentration, the standard drug had more activity (129%) than the test drug's 113%.

The ethanolic extract of *S. variable* prevented heat-induced haemolysis at a number of doses, including 10, 20, 40, 60, 80 and 100. According to the results at a 100 g/mL concentration, the test sample shows a considerable inhibition of 113% to the common drug Diclofenac, which yields 129%. The inhibition values were found to be 113% and 129% for the test samples and the standard, respectively.

CONCLUSION

The evaluation of the ethanolic extract of *S. variable* indicates the existence of an *in vitro* study that was utilised to demonstrate the potent anti-inflammatory activity of *S. variable* and aids in the discovery of active metabolites. The current study's findings indicate that *S. variable* is a potent antibiotic that can successfully cure a wide range of bacterial infections as well as diseases such as cancer, neurological disorders and aging. It is also a potent anti-inflammatory drug that can be used. More *in vivo* testing on the chemical is required to determine the extract's mode of action as a cutting-edge medicinal agent.

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Declaration of patient consent: Patient's consent not required as there are no patients in this study.

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