

SECONDARY METABOLITE PROFILE AND BIOACTIVE POTENTIAL OF A NATIVE *Synechococcus elongatus* STRAIN FROM SOUTHEAST SULAWESI AS A NUTRACEUTICAL CANDIDATE

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ARTICLE HIGHLIGHTS

- A native *Synechococcus elongatus* strain was isolated from coastal waters of Southeast Sulawesi, Indonesia.
- Molecular identification confirmed 99.70% sequence identity with *S. elongatus*. High total phenolic (249.81 mg GAE/g) and flavonoid (78.43 mg QE/g) contents were recorded
- LC-MS analysis identified 30 bioactive compounds, including palmitic and juniperic acids.
- The methanolic extract showed moderate antioxidant activity (IC₅₀ 114.34 mg/L) and anticancer potential (LC₅₀ 170.07 mg/L).
- Toxicity testing yielded an LC₅₀ of 254.06 mg/L, supporting further investigation of its potential for sustainable nutraceutical and pharmaceutical applications.

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ABSTRACT

Microalgae represent a potential biological resource for the development of functional raw materials due to their ability to produce a diverse range of bioactive compounds. This study aimed to identify, characterize the secondary metabolite content, and evaluate the bioactive potential of the microalga *Synechococcus elongatus* isolated from the coastal waters of Tambo Watu Beach, Konawe, Southeast Sulawesi. Sample identification was performed using morphological and molecular methods. Molecular analysis using BLASTn revealed that the WW1 isolate shared a 99.70% identity with the species *S. elongatus*. The methanolic extract from the microalgal biomass yielded 13.5%. Qualitative phytochemical screening indicated the presence of phenolic compounds, flavonoids, alkaloids, and terpenoids/steroids, but was negative for saponins. Quantitative analysis revealed a total phenolic content of 249.81 mg GAE/g extract and a total flavonoid content of 78.43 mg QE/g extract. Compound identification via LC-MS highlighted significant functional compounds including palmitic acid, juniperic acid, and xanthine, which have potential applications in the cosmetic and pharmaceutical fields. Bioactivity testing demonstrated that the *S. elongatus* extract possessed moderate antioxidant activity with an IC₅₀ value of 114.34 mg/L. Toxicity assessment using the Brine Shrimp Lethality Test (BSLT) showed weak toxicity with an LC₅₀ value of 254.06 mg/L, indicating its safety profile. Furthermore, the extract exhibited moderate preliminary cytotoxicity with an LC₅₀ value of 170.07 mg/L. However, the extract showed no antibacterial activity against *E. coli* and *S. aureus*, nor antifungal activity against *C. albicans*, at concentrations up to 100 mg/disk. In conclusion, the *S. elongatus* isolate from Konawe waters is a rich source of phenolic and flavonoid compounds, exhibiting moderate antioxidant and cytotoxic bioactivities with low toxicity. These results affirm its potential for further development as a promising candidate for functional raw materials in the nutraceutical, pharmaceutical, and cosmetic industries.

Keywords: antioxidant, bioactive compounds, cytotoxicity, phytochemistry, Southeast Sulawesi, *Synechococcus elongatus*

INTRODUCTION

Concurrent with the rising global awareness of health and environmental sustainability, a significant paradigm shift has occurred across various industrial sectors towards the utilization of renewable natural resources (Philp *et al.* 2013). This phenomenon has driven an exponential demand for safe and effective natural products, particularly within the functional food, pharmaceutical, and cosmetic industries (Atanasov *et al.* 2021; Carroll *et al.* 2023). Consequently, bioprospecting the systematic exploration of biodiversity to discover novel bioactive compounds has become increasingly crucial. Among the various biological resources, marine ecosystems harbor immense potential, much of which remains untapped, especially at the microbial level, such as microalgae and cyanobacteria (Demay *et al.* 2019).

Indonesia, as a country with the world's second-longest coastline and a center of marine megabiodiversity, possesses coastal ecosystems that are home to thousands of uncharacterized microalgal species (Larasati *et al.* 2023). These microscopic organisms can be regarded as highly efficient "biochemical factories," capable of synthesizing a diverse array of secondary metabolites with unique chemical structures as adaptive mechanisms to competitive and often extreme environmental conditions (Singh *et al.* 2016). These compounds, including polyphenols, flavonoids, alkaloids, and terpenoids, have been empirically shown to possess various beneficial biological activities, such as antioxidant, anti-inflammatory, and cytotoxic effects against cancer cells (Spolaore *et al.* 2006).

Cyanobacteria of the genus *Synechococcus*, as one of the oldest phototrophs on Earth, present a highly compelling potential for exploration. Their high adaptability allows *Synechococcus* to thrive in diverse aquatic habitats (Flombaum *et al.* 2013). However, the biochemical profile and bioactive potential of *Synechococcus* strains are significantly influenced by geographical factors and specific environmental pressures (Rastogi & Sinha 2009). Strains isolated from tropical waters rich in solar radiation, such as those in Southeast Sulawesi, are hypothesized to have evolved to produce protective compounds, particularly antioxidants and UV-screening phenolics, in higher concentrations as a defense mechanism against intense solar radiation and oxidative stress (Richa *et al.* 2011; Assunção *et al.* 2022).

Despite this vast potential, a significant knowledge gap exists concerning the microalgal resources of Indonesian waters, particularly in the scientifically underexplored region of Southeast Sulawesi. Many local strains have never been accurately identified using molecular methods, comprehensively analyzed for their chemical profiles, or tested for their bioactive potential. This lack of foundational scientific data serves as a primary barrier to their utilization.

Therefore, this study was systematically designed to bridge this gap. The research aimed to conduct a comprehensive isolation, identification, and characterization of a *Synechococcus elongatus* strain from the coastal waters of Konawe, Southeast Sulawesi. Specifically, this study was designed to: 1) identify the microalgal isolate through morphological and molecular methods; 2) analyze its phytochemical profile both qualitatively and quantitatively; 3) identify specific bioactive compounds within it using Liquid Chromatography-Mass Spectrometry (LC-MS); and 4) evaluate its functional bioactive potential as an antioxidant and antimicrobial agent, as well as assess its toxicity and preliminary cytotoxic potential.

Thus, the urgency of this research was in its role as one of the first comprehensive studies to unveil the potential of a *Synechococcus elongatus* strain from a local genetic resource. It is anticipated that the findings were expected not only to contribute new data to the body of scientific knowledge but also to provide a robust scientific foundation for the development of high-value, sustainable, natural functional ingredients. In turn, this knowledge could unlock opportunities for industrial innovation and enhance the economic value of Indonesia's marine biodiversity.

MATERIALS AND METHODS

Isolation and Cultivation of Microalgae

Seawater samples containing phytoplankton were collected from the coastal waters of Tambo Watu Beach, Konawe Regency, Southeast Sulawesi Province. The isolation of microalgal strains was performed using the serial dilution and streak plate methods on enriched Walne agar medium (Andersen & Kawachi 2005). The successfully isolated pure culture was then cultivated on a laboratory scale using liquid Walne medium (Walne 1966). The cultivation was carried out in

Erlenmeyer flasks under controlled conditions: a temperature of 24–26 °C, continuous illumination from fluorescent lamps (approximately 3000 lux), and constant aeration to supply CO₂. Biomass was harvested during the stationary phase (approximately day 14) via centrifugation. The collected biomass was subsequently dried using the freeze-drying method to preserve the integrity and stability of heat-sensitive bioactive compounds (Tzovenis *et al.* 1997).

Identification and Characterization of the Isolate

Morphological Characterization

The cell morphology of the pure microalgal isolate was observed using Scanning Electron Microscopy (SEM). The dried biomass sample was placed on a stub, coated with a thin layer of gold, and examined under the electron microscope (Boekema *et al.* 2001).

Molecular Characterization

Molecular identification was conducted through DNA sequence analysis of the 16S rRNA gene region. Genomic DNA was extracted using a commercial DNA extraction kit. The target DNA fragment was amplified using PCR with phytoplankton-specific primers (PS_F, U_R and PS_R, U_F). The purified PCR product was sequenced and analyzed using BLASTn against the NCBI database (Altschul *et al.* 1990). A phylogenetic tree was constructed using MEGA software to determine the kinship relationships between sequences (Kumar *et al.* 2018).

Extract Preparation

The dried biomass of *S. elongatus* was extracted using the maceration method with analytical grade methanol. Methanol was chosen as the sole extraction solvent due to its broad polarity range, allowing for the efficient extraction of a wide spectrum of metabolites from polar to semi-polar classes (Sasidharan *et al.* 2011). The biomass was soaked in methanol for 3 x 24 hours at room temperature. The resulting filtrate was concentrated using a rotary evaporator at 40 °C to obtain a crude extract. The extraction yield was calculated as the percentage of the extract weight relative to the initial dry biomass weight, derived from pooled triplicate cultivations.

Phytochemical Analysis

Qualitative Phytochemical Screening

The methanolic extract was tested for phenolics, flavonoids, alkaloids, terpenoids/steroids, and saponins following standard procedures (Harborne 1998).

Quantitative Analysis

Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu method (Singleton & Rossi 1965). Total Flavonoid Content (TFC) was determined using the aluminum chloride (AlCl₃) colorimetric method (Chang *et al.* 2002).

Analysis of Compound Composition by LC-MS

The bioactive compound profile was analyzed using an LC-ESI-MS system (positive and negative ionization modes) equipped with a C18 chromatography column. Compound separation was performed with a gradient mobile phase of water and acetonitrile. Identification was conducted based on m/z values, retention times, and fragmentation patterns compared against the METLIN and MassBank databases (Smith *et al.* 2005).

Bioactivity Assays

Antioxidant Activity Assay

Antioxidant activity was measured using the DPPH free radical scavenging method (Blois 1958). Activity was expressed as the IC₅₀ value.

Antimicrobial Activity Assay

Antimicrobial activity was tested using the Kirby-Bauer disk diffusion method (Bauer *et al.* 1966) against *E. coli*, *S. aureus*, and *C. albicans*.

Toxicity and Preliminary Cytotoxicity Assay

An acute toxicity assay was conducted against brine shrimp (*Artemia salina*) larvae (BSLT). Mortality data were used to determine the LC₅₀ value through probit analysis (Finney 1971). The same BSLT platform was utilized as a preliminary screen for cytotoxicity (anticancer potential) in separate assays, as established in literature (Meyer *et al.* 1982).

Statistical Analysis

To ensure the reliability and validity of the research findings, a systematic statistical approach was applied to all quantitative data obtained. All assays, including the analysis of total phenolic and flavonoid content, as well as bioactivity tests (antioxidant, toxicity, and anticancer), were performed in triplicate. The numerical data from these replications were processed and are presented as mean $\pm$ standard deviation (Mean $\pm$ SD).

The standard deviation was used as a measure of the variability or dispersion of the data from the mean value, indicating the precision of the measurements. These calculations were performed using spreadsheet software, such as Microsoft Excel. More specific statistical analyses were applied to determine key bioactivity parameters, as follows.

Linear Regression Analysis for IC_{50} and LC_{50} Determination

For antioxidant activity, the IC_{50} value was determined by plotting a series of extract concentrations (as the independent variable, x-axis) against the percentage of DPPH radical inhibition (as the dependent variable, y-axis). A simple linear regression model ($y = mx + c$) was applied to this data. The coefficient of determination (R^2) was calculated to ensure the linearity and reliability of the model. The IC_{50} value was then calculated mathematically from the regression equation by setting the y-value to 50.

For the toxicity and anticancer potential assays, a similar approach was used to determine the LC_{50} value. The percentage of brine shrimp mortality was plotted against the logarithm of the extract concentration. Probit analysis, a standard method for analyzing binary dose-response data (Finney 1971), was used to transform the mortality data to linearize the dose-response curve, which was then analyzed with linear regression to obtain a more accurate LC_{50} value.

Descriptive Analysis

Data from the antimicrobial assay (diameter of inhibition zone) were analyzed descriptively. The results were reported as absolute values (in mm) without further inferential statistical analysis, as the findings showed no activity (0 mm) at all tested concentrations, making statistical comparison irrelevant.

The results from the qualitative phytochemical screening were also analyzed descriptively and reported as positive (+) or negative (-) based on visual observation of color changes or precipitation.

Bioinformatic Analysis

The DNA sequence data from the molecular characterization were analyzed using bioinformatic software. BLASTn (Altschul *et al.* 1990) was used for homology analysis to identify the species by comparing the sequence against the GenBank database. Meanwhile, MEGA (*Molecular Evolutionary Genetics Analysis*) software (Kumar *et al.* 2018) was used for phylogenetic analysis, which involves statistical algorithms to construct an evolutionary tree and determine the kinship relationships between sequences.

RESULTS AND DISCUSSION

Identification and Characterization of *S. elongatus*

A fundamental step in bioprospecting research is the accurate identification of the target organism. This study commenced with the isolation and characterization of a microalga from the coastal waters of Tambo Watu Beach, Konawe, Southeast Sulawesi, a location known for its unique marine biodiversity. Initial morphological identification using Scanning Electron Microscopy (SEM) revealed rod-shaped (bacilli) microalgal cells, a characteristic feature of the genus *Synechococcus* (Fig. 1).

However, morphological identification alone is insufficient for definitive species determination, necessitating confirmation at the molecular level. For molecular validation, genomic DNA was successfully extracted from the isolate. The amplification process using the Polymerase Chain Reaction (PCR) provided crucial insights into the challenges of microalgal genetic analysis. The use of several universal primers did not consistently yield the expected DNA bands, which could be attributed to various factors such as primer mismatch, the concentration and purity of the DNA template, and suboptimal annealing temperature. The failure of amplification or the appearance of primer dimers underscored the importance of selecting appropriate primers. Success was ultimately achieved using a primer pair designed more specifically for phytoplankton (PS_F, U_R and PS_R, U_F), which successfully

amplified the target DNA fragment, appearing as a thick and clear band at a size of 750-1000 bp.

Morphological identification using SEM revealed rod-shaped cells characteristic of the genus *Synechococcus*. Molecular validation confirmed the isolate (WW1) as *S. elongatus* with 99.70% identity based on the 16S rRNA gene sequence (Table 1).

The phylogenetic tree (Fig. 2) indicated that this local isolate occupies a unique position compared to reference strains, suggesting genetic adaptation to the tropical waters of Konawe.

The use of a dual approach, combining morphological characterization via SEM with molecular confirmation, represents the gold standard in modern microalgal taxonomy (Leliaert *et al.* 2014). Although the rod-shaped cell morphology is consistent with the general description of the genus *Synechococcus*, molecular identification is crucial due to the phenomenon of cryptic species (morphologically identical but

genetically distinct species) in microorganisms, which is commonly reported in cyanobacteria (Sciuto & Moro 2015).

The successful DNA amplification using phytoplankton-specific primers after the failure of universal primers highlights the importance of precise primer selection for the target organism. The sequencing result, showing a 99.70% identity with *S. elongatus* (Table 1), provides a very high degree of confidence in the species identification. A similarity level above 97% for the 16S rRNA gene is generally accepted as the threshold for species identification at the molecular level (Stackebrandt & Goebel 1994). Furthermore, the slight divergence observed in the phylogenetic tree (Fig. 2) suggests that this local isolate is likely a unique strain that has genetically adapted to the specific environmental conditions of the Konawe waters, a critical finding in bioprospecting as unique strains have the potential to produce unique metabolite profiles (Puglisi *et al.* 2014).

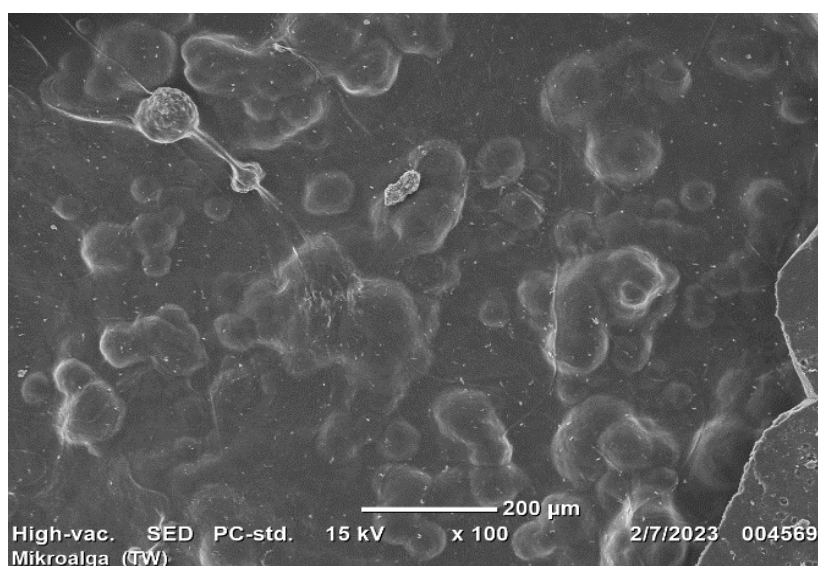


Figure 1 SEM micrograph of a *S. elongatus* cell from the waters of Tambo Watu, Konawe

Table 1 BLAST results based on NCBI for the species identification of the WW1 isolate

Sample ID	Species	Query Cover	Per. Ident
WW1	<i>Synechococcus elongatus</i>	90%	99.70%

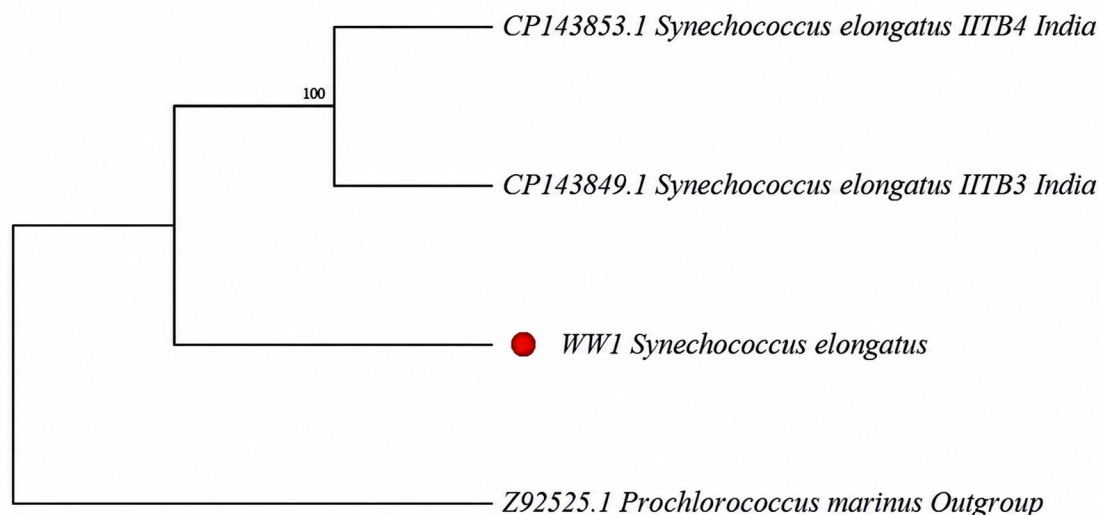


Figure 2 Phylogenetic tree showing the relationship of the WW1_Konawe isolate with reference *Synechococcus elongatus* strains

Phytochemical Analysis: Unveiling a Wealth of Secondary Metabolites

Following its confirmed identification, the biochemical potential of the *S. elongatus* biomass was explored. The extraction process using methanol, an effective solvent for dissolving polar to semi-polar compounds, yielded an extract of 13.5% of the dry biomass weight (Table 2). This figure is quite favorable and indicates that the cultivation of *S. elongatus* has the potential to produce an adequate amount of extract for further-scale applications. The methanolic extract yielded 13.5%. Phytochemical screening was positive for phenolics, flavonoids, alkaloids, and terpenoids/steroids (Table 3).

Quantitative analysis revealed an exceptionally high TPC of 249.81 ± 6.43 mg GAE/g extract (Table 4) and TFC of 78.43 ± 2.49 mg QE/g extract (Table 5). While this TPC value is significantly higher than some global reports (e.g., Goiris *et al.* 2012), it is important to note that the value is reported per gram of crude extract, which concentrates the metabolites compared to dry weight. Furthermore, the high phenolic accumulation is likely a specific adaptive response to the intense UV radiation in the tropical Southeast Sulawesi region, where phenolics serve as primary photoprotectants (Richa *et al.* 2011). Potential matrix interference from other reducing agents in the crude extract may also contribute to the high Folin-Ciocalteu response.

An extract yield of 13.5% is quite favorable and comparable to other studies that use polar solvents for cyanobacterial extraction. The presence of various secondary metabolite classes (Table 3) confirmed the potential of *S. elongatus* as a source of bioactive compounds. The most prominent finding was the total phenolic content, which reached 249.81 mg GAE/g. This value was significantly higher than several reports on other cyanobacteria. For instance, a study by Goiris *et al.* (2012) that tested 32 microalgal species found that the phenolic content in a *Synechococcus* sp. strain was only about 3.4 mg GAE/g of dry weight. Even in *Arthrospira* (Spirulina), known for its rich nutritional profile, the phenolic content was reported in a much lower range, around 5.5 mg GAE/g of extract (Colla *et al.* 2007).

The high phenolic and flavonoid content in the Konawe isolate was likely an adaptive response to the high intensity of UV radiation in tropical waters, where these compounds functioned as natural sunscreens and antioxidants to protect cells from oxidative damage, a hypothesis supported by Rastogi & Sinha (2009). A study by Richa *et al.* (2011) also confirmed that cyanobacteria exposed to environmental stress, including UV, tend to accumulate phenolic compounds in larger quantities as a defense mechanism. This high content directly implies a strong antioxidant potential, which will be validated in the bioactivity assays.

Table 2 Extraction results of the *Synechococcus elongatus* sample

Sample	Initial Weight (g)	Extract Weight (g)	Yield (%)
<i>Synechococcus elongatus</i>	2	0.3	13.5

Table 3 Phytochemical screening results of the *Synechococcus elongatus* extract

Sample	Phenolics	Flavonoids	Alkaloids	Terpenoids/ Steroids	Saponins
<i>Synechococcus elongatus</i>	+	+	+	+	-

Table 4 Results of total phenolic content determination

Sample	Replication	Absorbance	Total phenolic (mgGAE/g eks)	Average of total phenolic content (mgGAE/g eks)
<i>Synechococcus elongatus</i>	1	0.479	251.21	249.81 ± 6.43
	2	0.462	242.79	
	3	0.486	255.42	

Table 5 Results of total flavonoid content determination

Sample	Replication	Absorbance	Total flavonoid (mgQE/g eks)	Average of total flavonoid content (mgQE/g eks)
<i>Synechococcus elongatus</i>	1	0.204	75.57	78.43 ± 2.49
	2	0.213	79.64	
	3	0.214	80.09	

Comprehensive Metabolite Profiling by LC-MS: A Roadmap to Functional Applications

To advance beyond merely qualitative and quantitative analyses, a metabolomic analysis was performed using Liquid Chromatography-Mass Spectrometry (LC-MS). This sophisticated technique enables the specific identification of individual compounds within the extract. The resulting chromatogram (Fig. 3) revealed a highly complex compound profile in the *S. elongatus* extract, characterized by numerous peaks detected at various retention times. The analysis results were presented as a chromatogram, which visualizes the chemical profile of the extract. The horizontal axis (x) of this chromatogram represents the retention time (in minutes), which was the time required for each compound to pass through the separation column. The vertical axis (y) indicated the relative abundance, which represented the intensity or relative concentration of each detected compound.

The chromatogram above clearly demonstrates that the *S. elongatus* extract possesses a very rich and complex metabolite profile. This is evidenced by the appearance of dozens of sharp and well-defined peaks throughout the analysis time range

(0 to 33 minutes). Each of these peaks represents at least one unique chemical compound. Several key observations from this chromatogram include:

- 1. Compound Diversity:** The numerous peaks with different retention times (e.g., at 1.12, 6.94, 9.71, 18.85, and 24.14 minutes) indicate that this microalga synthesizes a wide variety of metabolites with varying chemical properties, ranging from highly polar (eluting early) to more non-polar (eluting later).
- 2. Dominant Compounds:** There are several peaks with very high intensity, particularly the peak at a retention time of 18.85 minutes, which is significantly higher than the others. This signifies the presence of one or more compounds that are the main or most abundant components in the extract. Other dominant peaks are also visible at retention times of 1.12 and 24.14 minutes. It is highly probable that these dominant compounds contribute significantly to the overall bioactivity of the extract.
- 3. Potential of Minor Compounds:** In addition to the major peaks, there are numerous minor peaks with lower intensity that are still clearly detected. Often, it is these minor compounds

that possess very potent and unique biological activities, despite their low concentrations.

LC-MS analysis identified 30 compounds (Table 6). Significant peaks were observed for palmitic

acid and juniperic acid, which are key fatty acids for cosmetic applications. The presence of xanthine further supports the extract's antioxidant and anti-aging potential.

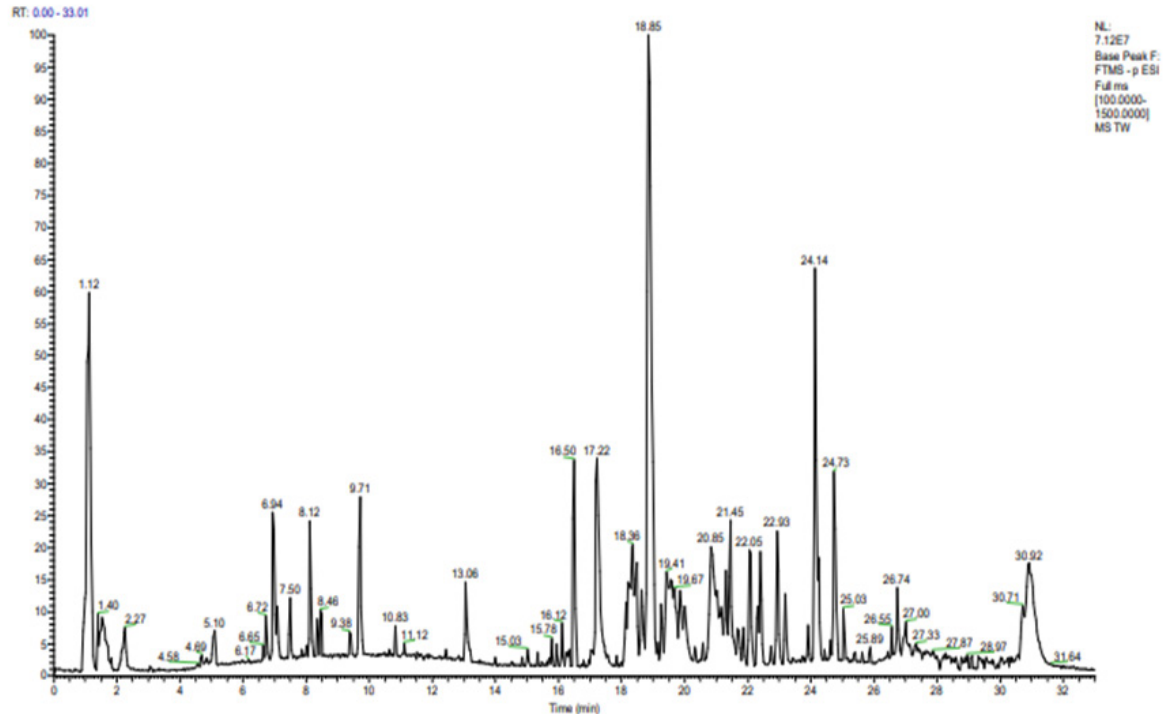


Figure 3 LC-MS chromatogram of the methanolic extract of *S. elongatus*, showing the compound separation profile based on retention time

Table 6 Compounds identified in the *Synechococcus elongatus* extract through LC-MS analysis

No.	Compound name	Potential activity
1	2-Hydroxyethyl 12-hydroxyoctadecanoate	Fatty acid for soap products
2	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	Fatty acid
3	Glycol stearate	Emollient, moisturizer, skin brightener
4	6-keto Prostaglandin F1 α	Anti-inflammatory, blood pressure regulator
5	3-oxopalmitic acid	Maintains skin moisture and smoothness
6	Suberic acid	Anti-acne, anti-inflammatory, antioxidant
7	16-Hydroxyhexadecanoic acid	Maintains skin moisture and smoothness
8	N-Isovaleryl-glycine	Enzyme metabolite
9	Juniperic acid	Moisturizer, anti-aging, skin brightener
10	NP-008993	Peptide, anti-inflammatory
11	3-Hydroxypentadecanoic acid	Fatty acid, antimutagenic
12	C12-Carnitine	UV protectant, skin brightener, anti-aging
13	6-Hydroxycaproic acid	Ingredient for anti-aging treatments
14	Deoxycholic acid	Skin brightener, exfoliant, anti-aging
15	L-gamma-Glutamyl-L-leucine	Supplement for immune system and stress
16	4-Amino-7-(α -D-ribofuranosyl)	Anticancer, antiviral, immunomodulator
17	N-Oleoyl-4-Aminobutyric Acid	Neurotransmitter for anxiety and stress
18	NP-008309	Antioxidant, anti-aging, immunity booster
19	Palmitic acid	Emollient, maintains skin moisture
20	4-Indolecarbaldehyde	Tryptophan metabolite

No.	Compound name	Potential activity
21	MFCD00083370	Vitamin (osteoporosis, infections)
22	2-Aminooctanedioic acid	Protein nutrition, heart protectant
23	2-Hydroxy-myristic acid	Maintains skin moisture and elasticity
24	DL-Malic acid	Exfoliant (AHA), skin brightener
25	12-HSA	Bacterial xenobiotic metabolite
26	Thymidine	Moisturizes skin, anti-free radical
27	NP-016455	Vitamin (Hb formation, immunity)
28	16-Heptadecyne-1,2,4-triol	Antioxidant, anticancer
29	Xanthine	Anti-aging, antioxidant, fades dark spots
30	DL- β -Leucin	Amino acid, regulator of cell metabolism

Compounds for Cosmetic and Skincare Applications

A large number of the identified compounds have functions relevant to cosmetics. Among them are fatty acids such as palmitic acid, juniperic acid, and 16-hydroxyhexadecanoic acid, all of which are known as effective emollients that can moisturize, smoothen, and support the skin's barrier function. Additionally, deoxycholic acid and DL-malic acid (an alpha-hydroxy acid, AHA) were found, which are popular in exfoliating products for brightening the skin and fading acne scars. The presence of xanthine, an antioxidant compound, also supports the potential of this extract as an anti-aging ingredient that can combat free radicals and reduce dark spots. This aligns with the fact that *S. elongatus* is an oleaginous microalga capable of producing significant amounts of lipids, which can be utilized as raw materials for moisturizing and skin-brightening cosmetics.

Compounds with Pharmacological Potential

Beyond cosmetic applications, several compounds demonstrated strong pharmacological potential. For instance, the detection of 6-keto Prostaglandin F₁ α , which plays a role in inflammatory processes and blood pressure regulation, and 4-Amino-7-(alpha-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine-5-carbonitrile, a nucleoside analog reported to have anticancer and antiviral activities. Furthermore, amino acids like DL- β -Leucine and the dipeptide L-gamma-Glutamyl-L-leucine were also identified, which are crucial for cell metabolism and immune system function. The diversity of these compounds indicates that the *S. elongatus* extract is a complex and promising "biochemical factory" for the development of new pharmaceuticals.

The chromatogram in Figure 3 clearly visualizes the metabolomic richness of the *S. elongatus* extract, characterized by dozens of distinct peaks. The identification of 30 specific compounds in Table 6 provides a clear roadmap for its potential applications. The presence of various fatty acids such as Palmitic acid and Juniperic acid is highly relevant for the cosmetic industry, as these compounds function as natural emollients (Purnamawati *et al.* 2017). This finding aligns with metabolomic studies on cyanobacteria, which consistently identify palmitic acid as one of the most abundant saturated fatty acids (Coates *et al.* 2014).

The detection of compounds like xanthine and DL-Malic acid (AHA) further strengthens the extract's potential as an active ingredient for anti-aging and skin-brightening products. Xanthine and its derivatives are known to have antioxidant and skin-protective activities (Shapiro & Saliou 2001), while AHAs like malic acid are popular chemical exfoliants in dermatological products for skin rejuvenation (Smith 1996). More compelling is the identification of compounds with strong pharmacological potential, such as the nucleoside analog 4-Amino-7-(alpha-D-ribofuranosyl).

Natural nucleoside analogs, especially from marine organisms, have become a major focus in drug discovery for their ability to act as potent antiviral and anticancer agents (Newman & Cragg 2007). This finding is highly significant as it suggests that the *S. elongatus* extract is not only beneficial for topical applications but also holds potential for the development of systemic drugs.

Evaluation of the Functional Bioactivity of the *S. elongatus* Extract

Armed with the rich chemical composition data, this study proceeded with empirical bioactivity testing to validate its functional potential.

Antioxidant Activity

The antioxidant activity assay using the DPPH method directly confirmed the initial hypothesis. The *S. elongatus* extract exhibited an IC_{50} value of 114.34 mg/L, which is classified as moderate activity (Table 7). This result is scientifically consistent with and can be explained by the high total phenolic and flavonoid content measured previously. This moderate antioxidant activity is significant enough to provide a protective effect against oxidative stress, positioning it as a promising candidate for raw material in functional foods or health supplements.

Antimicrobial Activity

An interesting result was found in the antimicrobial testing. The *S. elongatus* extract, even at the highest concentration (100 mg/disc), showed no inhibitory activity (0 mm inhibition zone) against the bacteria *E. coli* and *S. aureus*, nor the fungus *C. albicans* (Table 8). This absence of activity does not necessarily mean the microalga

lacks antimicrobial compounds, but it can be interpreted in several ways: 1) the responsible active compounds may not be optimally soluble in methanol; 2) the concentration of active compounds in the crude extract was insufficient to produce an effect; or 3) the existing compounds have a very specific mechanism of action that does not target the tested microbes.

Cytotoxic Activity and Anticancer Potential

Toxicity testing is a crucial aspect for determining the safety of a substance. Using the Brine Shrimp Lethality Test (BSLT), the extract showed an LC_{50} value of 254.06 mg/L, classifying it as a substance with weak toxicity (Table 9).

This is a very positive finding, as a high safety level is a primary prerequisite for applications in products consumed or used by humans. Interestingly, despite its low general toxicity, the extract exhibited moderate anticancer potential with an LC_{50} value of 170.07 mg/L against the target cells (Table 10).

Table 7 IC_{50} antioxidant activity values of the extract

No.	Sample	IC_{50} (mg/L)	Activity
1	<i>Synechococcus elongatus</i>	114.34	Moderate
2	Vitamin C (Control)	7.99	Very Strong

Table 8 Results of the antimicrobial activity test

Sample	Treatment group	Diameter of inhibition zone (mm)		
		<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
<i>Synechococcus elongatus</i>	10 mg/disc	0	0	0
	50 mg/disc	0	0	0
	100 mg/disc	0	0	0
	Control + (10 mg/disc)	17	22	18
	Control -	0	0	0

Table 9 Acute toxicity test (BSLT) results

No.	Sample	LC_{50} (mg/L)	Activity
1	<i>Synechococcus elongatus</i>	254.06	Weak
2	Potassium dichromate (Control)	13.96	Very Strong

Table 10 Anticancer potential test results

No.	Sample	LC_{50} (mg/L)	Activity
1	<i>Synechococcus elongatus</i>	170.07	Moderate
2	Fluorouracil (Control)	5	Very Strong

The difference between the general toxicity and anticancer activity indicates selectivity, where the compounds in the extract are more toxic to cancer cells than to normal cells (represented by the shrimp larvae). This anticancer activity is likely the result of a synergistic effect of the various identified compounds, such as flavonoids, terpenoids, and especially the pyrimidine nucleoside analog, which is known to interfere with DNA synthesis in rapidly replicating cells like cancer cells.

The extract exhibited moderate antioxidant activity (IC_{50} 114.34 mg/L) and weak general toxicity (LC_{50} 254.06 mg/L). Interestingly, the extract showed moderate preliminary cytotoxicity with an LC_{50} of 170.07 mg/L (Table 10). This cytotoxic effect may arise from the synergistic interaction between identified fatty acids and nucleoside analogs, which can disrupt cellular membranes and interfere with DNA replication, respectively.

An antioxidant activity with an IC_{50} value of 114.34 mg/L directly confirms the hypothesis derived from the phytochemical data. This moderate activity, while not as potent as Vitamin C, is highly consistent with the high phenolic and flavonoid content. This result is comparable to other reports for crude microalgal extracts, where antioxidant activity varies widely among species. For comparison, a study by Goiris *et al.* (2012) found that a *Synechococcus* sp. extract had moderate antioxidant activity (ORAC), which they also correlated with its phenolic content.

The absence of antimicrobial activity presented in Table 8 is a significant result. Although some studies have reported antimicrobial activity from *Synechococcus*, many have also reported negative results or very weak activity, depending on the strain and solvent used. Abdel-Raouf *et al.* (2012), for instance, found that methanolic extracts of several marine cyanobacteria showed no activity against *E. coli* and *S. aureus*. This suggests that antimicrobial compounds may not be produced by this specific strain under the culture conditions used, or they were not effectively extracted by methanol.

The most significant findings were in the cytotoxicity tests. A high general toxicity LC_{50} value (> 250 mg/L) indicates that the extract is relatively safe, according to the criteria of Meyer *et al.* (1982), which stated that an extract with an LC_{50} above 1,000 $\mu\text{g/mL}$ is considered non-toxic. However, the lower anticancer potential LC_{50} value

(170.07 mg/L or 170.07 $\mu\text{g/mL}$) indicates selective cytotoxicity.

According to the American National Cancer Institute (NCI) criteria, an extract with an IC_{50} below 30 $\mu\text{g/mL}$ is considered promising for further fractionation. Although the value obtained here is still above that threshold, this result shows clear potential. This moderate activity is likely the result of a synergistic effect of various identified compounds, such as flavonoids, terpenoids, and especially the pyrimidine nucleoside analog, which is known to interfere with the replication of cancer cells (Newman & Cragg 2007). The correlation between BSLT and anticancer activity has been widely reported, making this result highly promising for further research using specific cancer cell lines.

Overall, this study comprehensively demonstrated that the local isolate of *S. elongatus* from Konawe waters is a highly potential biological resource. With its wealth of phenolic compounds, flavonoids, and various other unique metabolites, this extract exhibits moderate antioxidant and anticancer bioactivities with a good safety profile, making it a very strong candidate for further development as a functional raw material in the nutraceutical, pharmaceutical, and cosmetic industries.

CONCLUSION

The WW1 isolate from Konawe was definitively identified as *S. elongatus* (99.70% identity). The extract is rich in phenolics and flavonoids, exhibiting moderate antioxidant and preliminary cytotoxic activities with low general toxicity. The diversity of compounds identified via LC-MS, particularly fatty acids and xanthine, underscores its potential as a nutraceutical and cosmetic candidate. Future research should focus on in vitro testing against specific cancer cell lines and in vivo safety studies to move beyond preliminary BSLT results.

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