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Phytochemical Screening of Secondary Metabolites Extracted from *Spirogyra subsalsa* as a Renewable and Sustainable Source of Biologically Active Compounds

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Abstract:

This study examined the potential of *Spirogyra subsalsa*, a freshwater green alga collected from the Tigris River (Al-Rashidiya, Baghdad) during January 2024, as a natural source of bioactive secondary metabolites. The water's physicochemical parameters—such as pH, salinity, conductivity, and key minerals—indicated a moderately alkaline, low-nutrient environment, which may favor secondary metabolite production. The ethanolic extract obtained via Soxhlet extraction was analyzed using FTIR spectroscopy and standard phytochemical screening tests. The results revealed the presence of alkaloids, glycosides, flavonoids, and terpenoids, while tannins and saponins were absent. These findings support the potential of *S. subsalsa* as a renewable, eco-friendly source of compounds with possible pharmaceutical ,environmental and Biological applications.

Keywords: Secondary metabolites, *Spirogyra subsalsa,* Eco-friendly source.

الفحص الكيميائي النباتي للمركبات الثانوية المستخلصة من طحلب *Spirogyra subsalsa* كمصدر متجدد ومستدام للمركبات الحيوية الفعالة

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مستخلص:

هدفت هذه الدراسة إلى استكشاف الإمكانات الحيوية لنوع *Spirogyra subsalsa*، وهو طحلب أخضر مياه عذبة جُمع من نهر دجلة (منطقة الراشدية، بغداد) في كانون الثاني/ يناير 2024. أظهرت التحاليل الفيزيوكيميائية لمياه النهر بيئة قلوية معتدلة منخفضة المغذيات، وهي ظروف قد تعزز من إنتاج المركبات الثانوية. تم الحصول على المستخلص الإيثانولي للطحلب باستخدام جهاز الاستخلاص Soxhlet، ثم جرى تحليله باستخدام مطيافية الأشعة تحت الحمراء (FTIR) إلى جانب اختبارات الكشف النباتي القياسية. كشفت النتائج عن وجود مركبات ثانوية مهمة مثل القلويدات، والجليكوسيدات، والفلافونويدات، والتربينويدات، في حين لم تُكتشف العفصات (التانينات) والصابونينات. تدعم هذه النتائج إمكانية استخدام *S. subsalsa* كمصدر طبيعي مستدام وصديق للبيئة للمركبات النشطة بيولوجيًا، ذات التطبيقات المحتملة في المجالات الدوائية والبيئية والحيوية.

الكلمات المفتاحية: مركبات الايض الثانوي ، *Spirogyra subsalsa* ، المصادر الصديقة للبيئة.

Introduction :

Recently, exploring natural sources for biologically active compounds has been growing interest , especially as the world shifts toward safer and more sustainable alternatives to synthetic chemicals. Among these natural resources, freshwater green algae have gained attention for their remarkable ability to produce a wide variety of beneficial compounds (AlRrubaie *et al*.,2019). These algae are rich in secondary metabolites including flavonoids, alkaloids, phenolics, terpenoids, and saponins—that are known for their antioxidant, antimicrobial, anti-inflammatory, and even anticancer properties (Harborne, 1998; Parekh & Chanda, 2007; El Gamal, 2010; Ibrahim et al., 2023). One such alga is *Spirogyra subsalsa*, a filamentous green alga found abundantly in freshwater ecosystems like rivers, ponds, and lakes , it holds promise as a sustainable source of biomass (Costa et al., 2022). *S. subsalsa* has received limited scientific attention compared to more studied species like *Spirulina* or *Chlorella*. Its potential chemical diversity and bioactivity remain largely unexplored (Demiriz Yücer, 2024).

Previous studies on related *Spirogyra* species have shown that they can produce a variety of useful secondary metabolites with pharmaceutical, agricultural, and environmental applications (Sofowora, 1993; Edeoga et al., 2005). Additionally, using environmentally friendly solvents such as ethanol and non-destructive analysis methods like Fourier-transform infrared spectroscopy (FTIR) aligns with the goals of green chemistry and sustainable research (APHA, 2017; Harborne, 1998).

This study, therefore, seeks to examine the phytochemical content of *Spirogyra subsalsa* using ethanolic Soxhlet extraction and FTIR analysis. By doing so, it aims to evaluate the alga's potential as a renewable, eco-friendly source of biologically active compounds—offering insight into a largely overlooked but potentially valuable freshwater species.

Materials and Methods

Sample Collection and Preparation

Spirogyra subsalsa was collected from the Tigris River at the Al-Rashidiya region, Baghdad, Iraq, during the January of 2024. Collection sites were selected based on algal abundance and accessibility. Samples were carefully scraped from submerged rocks and sediment using sterilized tools to avoid contamination. The algal biomass was transported to the laboratory in clean polyethylene containers filled with river water to maintain freshness. Upon arrival, samples were thoroughly rinsed with distilled water to eliminate debris, sediments, and associated microorganisms, then prepared for extraction and biochemical analyses.

Physicochemical Analysis of Water

Water samples from the collection site were gathered in sterilized polyethylene bottles and analyzed immediately using standard protocols (APHA, 2017). The measured parameters included:

The physicochemical parameters of the water samples were measured following standard methods (APHA, 2017). **pH** was determined using a calibrated digital pH meter (HANNA HI98107), and **temperature** was recorded on-site with a portable thermometer (Thermo Scientific TCT100). **Electrical conductivity (EC)** and **salinity** were measured using a conductivity/salinity meter (WTW InoLab Cond 7110). **Nitrate (NO_3^-)** and **phosphate (PO_4^{3-})** concentrations were analyzed spectrophotometrically using a Shimadzu UV-1800 spectrophotometer. **Calcium (Ca^{2+})** and **magnesium (Mg^{2+})** were quantified by EDTA complexometric titration, while **chloride (Cl^-)** was determined by argentometric titration using silver nitrate following the Mohr method.

Each parameter was measured in triplicate to ensure accuracy and reproducibility.

Ethanolic Soxhlet Extraction of Secondary Metabolites :

Dried algal biomass was finely powdered using a mechanical grinder. Approximately 50 grams of the powder underwent Soxhlet extraction using

95% ethanol for 6 hours. Continuous solvent reflux facilitated effective dissolution of bioactive compounds. The resulting extract was concentrated using a rotary evaporator under reduced pressure at 40°C to remove ethanol, yielding a crude extract. This extract was stored at 4°C in amber-colored bottles for further analyses (Handa et al., 2008).

FTIR Analysis of Functional Groups:

To identify functional groups associated with bioactive compounds, Fourier-transform infrared spectroscopy (FTIR) was conducted. A small portion of the dried extract was mixed with potassium bromide (KBr) and pressed into a thin pellet. Spectral analysis was performed over the range of 4000–400 cm^{-1} using an FTIR spectrometer at room temperature. The absorption peaks were interpreted to identify functional groups such as hydroxyl, carbonyl, amine, and aromatic rings—indicative of classes like phenolics, flavonoids, and alkaloids (Silverstein et al., 2014).

Phytochemical Screening :

Preliminary screening of the ethanolic extract was performed using standard qualitative methods to assess the presence of key secondary metabolites. The tested groups included alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, and terpenoids (Harborne, 1998).

Results and Discussion

Spirogyra subsalsa - Kuetzing. Filaments of slender cells 26-28 Mm in Liameter 148-35 .Mm long. with plane anel walls. Chloroplast solitary, making 1/2-3 turns in one cell, as showed in figure (1) and this agree with (Al-hussieny, 2018) and (Xiong *et al.,* 2022) (Prescott,1982) Zygospores ellipsoid with median spore wall Smooth.

The classification of genus as below:

Division*:* ***Chlorophyta***

Class*:* ***Chlorophyceae***.

order*:* ***Zygnematales***.

Family, ***Zygnemataceae***

Genus : ***Spirogyra***

Species: ***Subsalsa***

Spirogyra subsalsa were include classed based on visual characteristics and microscopic examinations. Fila-

mentous green algae does not branch. Pyrenoids are usually present in a cell's thick chloroplast. Cells often have one end that is broader than the other. In other cases, cells seem bulbous or almost spherical (Hirn, 1900). The key distinguishing feature is the rings at the broader extremities, which develop during cellular division. This appearance is caused by each ring representing a cell division, which can be seen inside the filament with careful focusing under favorable conditions (Xiong *et al*, 2021).

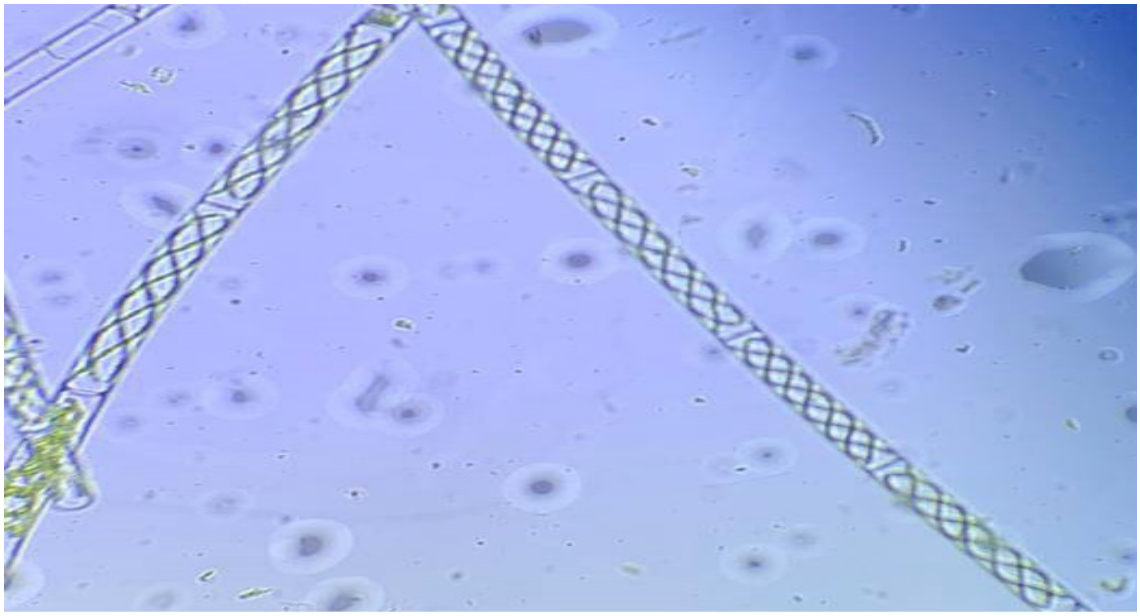

Figure (1): *Spirogyra subsalsa* under microscope 40 X.

Water Sample Characteristics:

The physicochemical parameters represent the water from which *Spirogyra subsalsa* was isolated table (1). These measurements provide important context for understanding the environmental conditions influence the alga's growth and biochemical composition (APHA, 2017; Hem, 1985).

Table (1):Test Physic-chemical Parameters Values of the Water sample

Test	Mean ± SE
Air tem.	30 ± 2
Water tem.	20 ± 2
Ec(µS/cm)	1754 ± 18
pH	8.2 ± 0.4
Ca(mg/l)	175 ± 5
Mg(mg/l)	49 ± 3.5
Cl (mg/l)	85 ± 4.3
SAL%	0.7 ± 0.1
PO4(mg/l)	BDL
NO3(mg/l)	BDL
NO2(mg/l)	BDL

SE: Standard error, BDL : below detective limit

These physicochemical characteristics reflect a typical freshwater environment with moderate mineral content and low nutrient pollution, conditions that are conducive to the growth of *Spirogyra subsalsa* (Sawyer *et al.,* 2003; WHO, 2017).

Environmental conditions such as pH, temperature, mineral content (Ca^{2+}, Mg^{2+}, Cl^{-}), salinity, and nutrient levels significantly affect the production of secondary metabolites in *Spirogyra subsalsa*. Slightly alkaline pH and moderate temperatures promote enzymatic activities involved in metabolite biosynthesis. Minerals like calcium and magnesium act as cofactors essential for metabolic processes. Low nutrient availability and mild salinity can induce stress responses in the alga, stimulating the synthesis of bioactive secondary compounds as defense mechanisms (Singh *et al*., 2018; Patel *et al*., 2021).

The FTIR spectrum of the *Spirogyra subsalsa* ethanolic Extract :

By revealing key functional organizations indicative of bioactive compounds. Below figure (2) are outcomes of the spectral functions and their clinical implications.

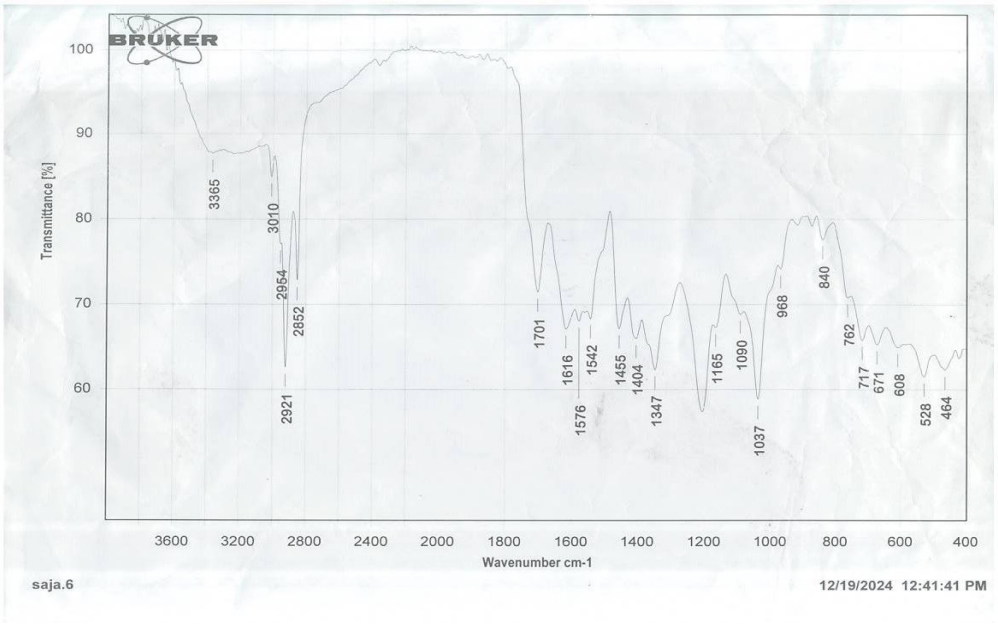

Figure (2): FTIR analysis for *Spirigyra subsalsa* extract

A broad absorption band around ~3300 cm^{-1}, corresponding to O–H or N–H stretching vibrations, indicates the presence of hydroxyl-containing compounds (e.g., phenols, alcohols) or amine groups (e.g., proteins) (Silverstein et al., 2014). Phenolic compounds, commonly found in algae, are known for their antimicrobial properties, primarily through disruption of microbial membranes (Cowan, 1999). Absorption peaks near 2921 cm^{-1} and 2850 cm^{-1} are associated with C–H stretching vibrations, typical of aliphatic hydrocarbons, such as those found in fatty acids or lipids (Larkin, 2017). Algal fatty acids, such as lauric acid, have been reported to lyse bacterial cell membranes, contributing to their antimicrobial action (Desbois & Smith, 2010).

A strong peak around ~1650 cm^{-1} is attributed to C=O stretching, particularly amide I bands, indicative of proteins or peptides, or possibly ketones (Silverstein et al., 2014). Antimicrobial peptides (AMPs), for instance, often function by targeting and disrupting bacterial cell walls (Hancock & Sahl, 2006). Additionally, peaks at 1452 cm^{-1} and 1375 cm^{-1} correspond to C–H bending vibrations, typically

from methyl or methylene groups, supporting the presence of lipids. These groups may interact synergistically with other bioactive compounds to enhance antimicrobial efficacy (González et al., 2021).

Active Compounds Detected in ***Spirogyra subsalsa*** Extract:

The phytochemical screening of the ethanolic extract of *Spirogyra subsalsa* revealed the presence of several important secondary metabolites, including alkaloids, glycosides, flavonoids, and terpenoids, while saponins and tannins were absent table (2).

Table (2):Active compounds in *Spirogyra subsalsa* crude extracts

Chemicals Compounds	Ethanolic Spirogyra Extract
Alkaloids	+
Glycosides	+
Saponins	-
Tannins	-
Flavonoids	+
Terbenoid	+

+ Presence of active compound , - Absent of active compound

The presence of alkaloids in *Spirogyra subsalsa* suggests it may possess important pharmacological properties, such as antimicrobial and analgesic effects. Alkaloids are well-known for their biological activity and play a key role in plant defense mechanisms (Harborne, 1998). Similarly, the detection of glycosides points to the presence of compounds that may have cardiotonic or other therapeutic activities. These molecules are commonly found in both plants and algae and contribute to a variety of biological effects (Sofowora, 1993 , Mohd & Fadzureena, 2016).

Flavonoids were also identified and are particularly notable for their strong antioxidant properties, which help protect cells from oxidative stress caused by free radicals. Additionally, flavonoids are known to have anti-inflammatory and antiviral actions (Parekh & Chanda, 2007). The detection of terpenoids suggests the presence of volatile and non-volatile compounds with a wide range of health-promoting effects, including antibacterial and anticancer activities (Al-Fatlawi & Hassan, 2022). On the other hand, the absence of saponins and tannins might be due to the specific nature of the ethanolic extraction process or the envi-

ronmental conditions under which the alga was collected. These compounds are sensitive to both ecological factors and extraction methods, which can influence their presence or concentration (Singh et al., 2018).

Overall, these findings confirm that *Spirogyra subsalsa* is capable of producing a diverse range of bioactive secondary metabolites, making it a promising sustainable source of pharmacologically and environmentally valuable compounds.

The physicochemical properties of the water where the alga was collected—such as a slightly alkaline pH (8.2 ± 0.4), moderate temperature (20 ± 2°C), and moderate electrical conductivity (1754 ± 18 µS/cm)—create favorable conditions for its growth and metabolism. These environmental parameters significantly affect the biosynthesis of secondary metabolites, including the alkaloids, glycosides, flavonoids, and terpenoids detected in the ethanolic extract. Essential mineral ions like calcium (175 ± 5 mg/L), magnesium (49 ± 3.5 mg/L), and chloride (85 ± 4.3 mg/L) serve as cofactors in enzymatic reactions, supporting metabolic processes that lead to the production of bioactive compounds (Guedes & Malcata, 2012). Interestingly, low levels of nutrients (phosphate, nitrate, and nitrite below detection limits) may act as mild stressors, stimulating the alga's defense mechanisms and encouraging the accumulation of protective secondary metabolites with antioxidant functions (Patel et al., 2021).

The moderate salinity (0.7 ± 0.1%) and mineral composition of the environment also shape the alga's metabolic profile, which could explain the absence of compounds like saponins and tannins in this study. These results highlight the important role of environmental conditions in influencing the chemical makeup of *Spirogyra subsalsa* and support its potential as a renewable source of biologically active substances.

Conclusions:

Spirogyra subsalsa **shows great potential as a natural producer of valuable secondary metabolites like alkaloids, glycosides, flavonoids, and terpenoids, which are known for their possible biological activi-**

ties. Environmental factors—such as moderate water alkalinity, the presence of minerals, and low nutrient levels—likely played a role in stimulating the production of these compounds. FTIR analysis confirmed the presence of functional groups typically associated with bioactive substances. Altogether, these findings suggest that this alga could serve as a sustainable and natural source of bioactive compounds with promising applications in medicine and environmental protection.

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