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In vitro Anti-Sickling Effect of Arthrospira Platensis Thallus Extract

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Abstract: *Arthrospira platensis thallus, commonly called spirulina, has a long history of traditional use in addressing iron deficiency and managing sickle cell anemia. There are established therapies for sickle cell disease, but many of these treatments can lead to undesirable side effects, prompting a search for more natural and holistic alternatives. This research specifically investigates the antisickling properties of Arthrospira platensis thallus and seeks to identify the bioactive compounds responsible for these beneficial effects. The phytochemicals discovered included flavonoids, saponins, tannins, terpenoids, alkaloids, and phenolic compounds, known for their potential health benefits. Statistical methods to assess significance were employed, with a threshold of $p<0.05$. The effects of different extracts, including n-hexane, ethyl acetate, and methanol, were examined on sickle blood cells, and microscopy was used for detailed observations. The analysis helps to visually assess any changes in cell morphology and behavior in response to treatment. The gas chromatography-mass spectroscopy (GC-MS) technique was utilized to provide a detailed composition of the extracts, revealing a wide range of beneficial compounds such as fatty acids, unsaturated fatty acids, ketones, aldehydes, terpenes, esters, alcohols, and alkanes. These compounds are known for their diverse roles in health, including anti-inflammatory, antioxidant, and immune-modulating effects.*

Keywords: *Bioactive Compounds, Genetic Mutation, Sickle blood cell, Arthrospira platenses, Therapeutic.*

Introduction

Due to its unique combination of high-quality proteins, fatty acid profile, antioxidants, vitamins, and minerals, *Arthrospira* is a great nutraceutical that has gained a lot of popularity (1). It can be used as a booster and, because it contains certain pigments and secondary metabolites, it also acts as an antiviral agent. It has been shown in numerous animal and human experiments to be significant enough to be commercialized and sold for therapeutic purposes (2). In vitro antioxidant and hepatoprotective activities of selenium-containing allophycocyanin (Se-APC) were examined using APC, reducing disease-related stress, where fast protein liquid chromatography was introduced from selenium-enriched *Arthrospira platensis* (3). A treatment of the blood sample repairs the red blood cells from selenium-containing allophycocyanin (Se-APC).

Sickle cell disease (SCD) is therefore a condition that requires early diagnosis to help reduce the mortality resulting from the disorders and control the illnesses. Several methods have been designed

to screen SCD and the intermediate states with good sensitivity and specificity. It was proposed that utilizing phytoconstituents for targeted therapeutic management of sickle cell anemia holds a great deal of promise for lowering the disease's pathophysiological symptoms (4). Strongly poisonous, ethidium bromide (EtBr) caused notable changes in hematobiochemical parameters, RBC morphonuclear properties, lipid peroxidation (LPO), and O. niloticus histological and histochemical features. In Nile tilapia (Oreochromis niloticus) fry, the possible protective properties of *Arthrospira platensis* (SP) against the effects of EtBr poisoning were studied (5).

It demonstrated how Cameroonian aqueous extracts of *Arthrospira platensis* had antisickling and antihemolytic properties, which could improve the disease's natural medicine approach (6). The 6 liters of *Arthrospira platensis* that was obtained in Nomayos, Yaounde, was freeze-dried after being dried, crushed, and macerated in distilled water for 24 hours. To manage sickle cell disease, some families in the Western Region of Cameroon frequently use black bean seeds (Phaseolus vulgarus L). In addition, the study was conducted to determine the phenolic compound profile of these seeds and assess their antioxidant, membrane stability, and in vitro antisickling qualities (7).

According to (8) studies which determined the prevalence of sickle cell disease in Sudan by haemoglobin electrophoresis among 400 patients. Among 14.8% of the disease the sickle cell trait patients were 11.3% and Sickle cell disease positive patients were 3.5%. Individuals with SCA have consistently low blood Hb concentration, normal MCV and high mean WBC's. Individuals with sickle cell trait had haematological parameters near to those of normal individuals. (8). Tchoulegheu et al. presented an antihemolytic property at the concentrations 800 ($\mu g \cdot mL^{-1}$ and 1600 ($\mu g \cdot mL^{-1}$ on various hemolysis inducers (9).

In vitro cold and hot extracts of the three Khaya species' stem barks were evaluated for Sickle Cell Disease management with standard anti-sickling inhibitory and reversal of sickled red blood cells process (10). In vitro evaluation of *D. microcarpum* stem bark was estimated for antisickling of blood samples obtained from thirty-five confirmed sickle cell disease patients (11). Sickle cell disease was managed using *Azadirachta indica* J. seed oil where, the plant's antioxidant properties, fatty acid, and vitamin E compositions were determined (12). Fung et al. associated the deficiency of plasma Zinc with the prevalence of sickle cell disease among children of age between 4-10 years (13).

X-RF analysis and *in vitro* antisickling properties of four ethnobotanical plants were studied for the management of sickle cell disease in Katsina (14). Tiloke et al. attributed the potential of *Moringa Oleifera* for the treatment of cancer due to the presence of bioactive components that are effective for biomedical application (15). *Spirulina* contained bioactive component and served as food (16) with various advantages such as preventing oxidative stress, modulate immune system, and support cardiovascular health (17). Saini et al. investigated the effect of pigments like chlorophyll and carotenoids present in cyanobacteria that have applications in biotechnology and genetic engineering (18). Beekrum et al. highlighted the potential of a marine diatom, *Amphora sp.* as a food supplement with therapeutic significance (19). Girotto et al. studied an earthware-based biofilter photobioreactor for Spirulina culture and organic nutrient sources (20).

This paper is classified into five section. Section 1 is the introduction. Section 2 is methodology. Section 3 is the result. Section 4 is the discussion. Section 5 is the conclusion.

Methodology

Plant sample collection and identification

Fresh samples of *Arthrospira plantesis* plants were collected in Kaduna North local government area of Kaduna State, Nigeria. The samples were identified and voucher numbers were obtained at the herbarium unit of the Biological Science Department at Nigerian Defence Academy Kaduna. Sand particles were removed from the plant material, the samples were rapidly rinsed under running tap water, and then air dried in the shade. When the sample was dried, it was ground and 500g of the powdered plant material was weighed and then loaded into a Soxhlex extractor.

Blood sample collection

Blood sample (HbSS) was collected in EDTA tubes from confirmed sickle cell disease patients who attended the sickle cell clinic by the personnel of the laboratory Unit of Barau Dikko Teaching Hospital Kaduna State Nigeria. The donors were informed by the ethical committee of the hospital on the need for the blood samples.

Soxhlet extraction

The extraction process was carried out using the Soxhlet extraction method. 100g each of the plant material was weighed into three different reflux apparatus set up, containing 500ml of three different solvents which include; n-hexane, methanol, and ethyl acetate. The electronic hot plate is set according to the boiling point of each solvent. The extraction process was carried out for 8-9 hours until the extraction was completed (the refluxing solvent became clear). The extract was collected by evaporating the solvent using rotary evaporators, which were poured into an air-tight container.

Qualitative Phytochemical screening of the plant sample

Test for Alkaloid

A small portion of the plant extract was added to 10% of Mayers reagent in a test tube. The formation of white precipitate indicates the presence of alkaloids in the sample.

Test for Saponins

A small portion of the plant extract was added to 5ml of distilled water in a test tube, the mixture was shaken vigorously and then allowed to stand for a few minutes observing the formation of stable foam indicating the presence of saponins.

Test for Flavonoids

A few drops of sodium hydroxide solution were added to the test tube containing the sample solution of the plant extract forming yellow coloration which indicates the presence of flavonoid.

Test for phenol

A small portion of the plant extract dissolved in distilled water, 10% of ferric chloride was added forming green, purple or red indicate the presence of phenol.

Test for Tannins

A small portion of the plant extract dissolved in distilled water; few drops of lead acetate was added forming white color precipitate indicate the presence of tannins.

Test for Steroid

A small portion of the plant extract dissolved in chloroform; a few drops of concentrated sulfuric acid were added to the solution. A red or pink color indicates the presence of steroids.

Test for Anthraquinone

A small portion of the plant extract dissolved in chloroform in a test tube and a few drops of sodium hydroxide (NaOH) were added to the solution. The formation of a fent pink color at the upper layer indicates the presence of Anthraquinone.

Test for Cardiac glycoside

A small portion of the plant extract was dissolved in acetic acid containing traces of ferric chloride, and concentrated sulfuric acid. The formation of a reddish-brown ring at the interphase indicates the presence of cardiac glycoside.

Quantitative Phytochemical screening of the plant sample

Test for polyphenols

The standard solution of 20µl of the extract was mixed with 150ml of Folin-Ciocalteu reagent and stood for 6 minutes. The mixture was allowed to react for 5 minutes at room temperature, 80µl of 7.5% sodium carbonate solution was added, and the mixture was kept in the dark at room temperature for 1hr. The absorbance was measured at 760nm using a spectrophotometer.

Test for Flavonoid

Standard solution of 0.5ml of the extract was mixed with 1.5ml of methanol, 0.1ml of aluminum chloride (10%), 0.1ml of potassium acetate (1M), and 2.8ml of distilled water. The mixture was incubated at room temperature for 30 minutes and the absorbance was taken at 415 nm using a spectrophotometer.

Test for Saponins

The standard solution was added to 20 µl of the samples into a clean test tube, 150 µl vanillin reagent was added to each test tube, 2.5 ml of 72% sulphuric acid was added, and 60% was incubated for 10 minutes. An ice block was broken into small pieces in a beaker and the test tubes were transferred into the ice to cool. The absorbance was taken at 544 nm.

Test for Tannins

A standard solution of 20µl was mixed with 1.5ml of distilled water, 100µl of Folin-Denis reagent, and 200µl of sodium carbonate solution (35%) was added and was diluted to 2ml with distilled water and mixed. The mixture was incubated for 30 minutes. Absorbance was measured at 700 nm using a spectrophotometer.

Test for Alkaloid

The filtrates were Concentrated to dryness using a water bath and the residue was dissolved in 2ml of 1% hydrochloric acid, 1ml of Dragendorff's reagent was added. Wagner's reagent and Mayer's reagent were added to separate test tubes containing 1 ml of the acidic extract. The formation of precipitates was observed and noted. The intensity of the precipitates as a comparative measure was used for quantification.

Column Chromatography

Different extracts of *A. platensis*, (7 g)) was separated into its component fractions using column chromatography. Silica gel 60 g served as the stationary phase, while solvent combinations of

increasing polarity were used as the mobile phase. To set up the column chromatography, the lower portion of the glass column was filled with glass wool using a glass rod. A slurry made of 150 g of silica gel and 350 ml of n-hexane was carefully poured into the column. The top of the glass column was left open, allowing the solvent to flow freely into a conical flask below. The setup was confirmed to be correct when the solvent drained freely without carrying any silica gel or glass wool into the tap. About 50 fractions were collected in a sample bottle which was subjected to further analyses (TLC).

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a useful analytical technique for separating and identifying compounds in a mixture, such as plant extracts. Thin layer chromatography (TLC) is a chromatographic technique used to separate and analyze different compounds based on their interaction with a thin layer of adsorbent material present on a plate. This technique is commonly utilized for the separation of low molecular-weight compounds. Various types of adsorbents, such as silica gel and alumina, were employed to separate a wide range of compounds through TLC. This method was adopted by (21).

Preparation of TLC Plate

The TLC plate was cut 5cm long, and a straight line was drawn using a pencil across the plate which was above the bottom edge (baseline). A small equal space was marked on the baseline where samples were applied which was 1cm apart.

Application of Plant Extract

The fractions of the plant extract solution that was collected during the column chromatography, a small drop was applied using a capillary tube to one of the marked spots on the baseline. The spot was allowed to dry. This application was repeated for the rest of the samples.

Preparation of Mobile Phase

An appropriate solvent mixture was chosen as the mobile phase. The solvent was poured into the TLC chamber to a depth of about 0.5-1cm. The solvent level was arranged below the baseline on the TLC plate to prevent direct dissolution of the samples.

Development of the TLC Plate

The TLC plate was carefully placed in the chamber using forceps, making sure it stands upright and the baseline was above the solvent level. The chamber was close with a lid and allowed the solvent to saturate the chamber atmosphere and rise up the plate by capillary action. The solvent was allowed to travel up the plate until it was about 1-2cm from the top edge. Once the solvent front travelled to the desired distance, the plate was removed from the chamber. The solvent was marked immediately with a pencil before it evaporated and the plate was allowed to dry in a well-ventilated area. The plate was examined under a UV lamp and visualized any spots that fluoresce.

Identification of Bioactive Components in *Arthrospira platensis* Leave Extracts

The bioactive compounds were analyzed by GC–MS system (Perkin Elmer USA) makes GC-MS instrument, Model: Clarus 680 GC & Clarus 600C MS comprising a liquid auto-sampler). The identified compounds with their retention time (RT), molecular formula, molecular weight (MW), and

concentration (peak area %) for ethyl acetate leave extract of *Arthrospira platensis* and methanolic extract of *Arthrospira platensis.*

Results

The research findings were presented below using tables, charts, and figures depending on the research outcome.

Percentage Yield of extract

The percentage yield of the extract was calculated as follows. Table 1 is the percentage yield of *Arthrospira platensis.*

$$\%\ Yield = \frac{Weight\ of\ extract}{Initial\ weight\ of\ sample} \times 100$$

Qualitative Phytochemical Screening of *Arthrospira platensis* Extracts of three different solvents

The phytochemical screening was performed to discover the compound present in the extract. The following extracts were considered flavonoids, saponins, tannins, terpenoids, alkaloids, and phenolic compounds, known for their potential health-promoting effects. Table 2 is the Qualitative Phytochemical Screening of *Arthrospira platensis* extracts.

Quantitative phytochemical analysis of the plant extracts

Quantitative phytochemical results are presented in Table 3 and Figure 1. The values were expressed as mean standard error ± SEM (n=3). Statistical significance mean difference was considered at $p<0.05$ and LSD comparison test was used for post hoc analysis. Values bearing the same superscripts under the same column are significantly different.

Thin Layer Chromatography (TLC) analyses

In TLC, the retention factor is calculated as follows:

$$\mathrm{Re}tention\ \ factor(Rf) = \frac{Dis\tan ce\ travel\ by\ compound(Dc)}{Dis\tan ce\ travel\ by\ solvent(Ds)}$$

The *Rf* value is a dimensionless number and typically ranges from 0 to 1. It is the characteristic of the compound and the specific conditions used (such as the type of solvent and the type of TLC plate). The *Rf* values indicate how far each compound traveled relative to the solvent front. Compounds in *S. plantesis*1 with *Rf* value of 0.8 is likely more polar than the compound in *A. platensis* 2 as shown in Table 4.

4.5 Anti-sickling activity of the plant extracts

The sickle blood was used to demonstrate the effect of the *Arthrospira Platensis* Thallus. Figure 2 is sickle blood cells sample without the application of the extract. Figure 3 shows the action of *A. Platensis* extract on sickle blood.

4.8 Identification of Bioactive Components in *Arthrospira platensis* Leave Extracts (GC-MS analysis)

The bioactive compounds were analyzed by GC–MS system (Perkin Elmer (USA) make GC-MS instrument, Model: Clarus 680 GC & Clarus 600C MS comprising a liquid auto-sampler). The identified compounds with their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area %) for methanolic leave extract of *Arthrospira platensis* were presented in Tables 5. While a total of 13 bioactive compounds were identified in *Arthrospira platensis* methanolic leave extract fraction; (Z)-9-(E)-12-Tetradecadien-1-ol acetate, Methyl 11-octadecenoate, Methyl 2-oxooctadecanoate, Methyl tridecanoate, Hexadecanoic acid, Methyl (13E,16E)-13,16-octadecadienoate, 14-Methyl-14-(3-oxobutyryloxy)-hexadec-15-enoic acid, Methyl petroselinate, 2-Methyl-Z,Z-3,13-octadecadienol, Methyl stearolate, Brassidic acid, Erucic acid, and Squalene (Table 4). The bioactive compound identification is presented in Table 5.

Table 1: Percentage yield of *Arthrospira platensis* Plant

S.platensis	Initial weight (g)	Final weight of extract (g)	% Yield	Solvent used
Sample 1	55	2.03	3.69	n-hexane
Sample 2	55	7.16	13.02	Ethyl acetate
Sample 3	55	2.43	4.42	Methanol

Table 2: Qualitative Phytochemical Screening of *Arthrospira platensis* extracts

Compounds	***n*-hexane**	**ethyl acetate**	**Methanol**
Alkaloids	+	+	+
Phenols	-	-	+
Tannins	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Anthraquinone	-	-	+
Steroid	+	+	+
Terpenoid	+	+	+
Cardiac glycoside	+	+	+

Keys: Present = +, Absent = -

Table 3: Quantitative phytochemical analysis of the extracts of *Arthrospira platensis*

Extracts	**TTC Mean ± SD**	**TSC Mean ± SD**	**TPC Mean ± SD**	**TFC Mean ± SD**	**TAC Mean ± SD**
Arthrospira Hexane	2.12 ± 0.01 ^{b}	0.03 ± 0.00 ^{e}	0.81 ± 0.06 ^{f}	0.90 ± 0.32 ^{d}	269.47 ± 16.49 ^{b}
Arthrospira Ethyl acetate	1.88 ± 0.11 ^{cd}	0.17± 0.00 ^{d}	0.96 ± 0.01 ^{e}	1.13 ± 0.00 ^{cd}	88.67 ± 6.11 ^{e}
Arthrospira Methanol	2.15 ± 0.01 ^{b}	0.22± 0.00 ^{b}	1.19± 0.00 ^{b}	1.28 ± 0.01 ^{bc}	332.93 ± 6.11 ^{a}

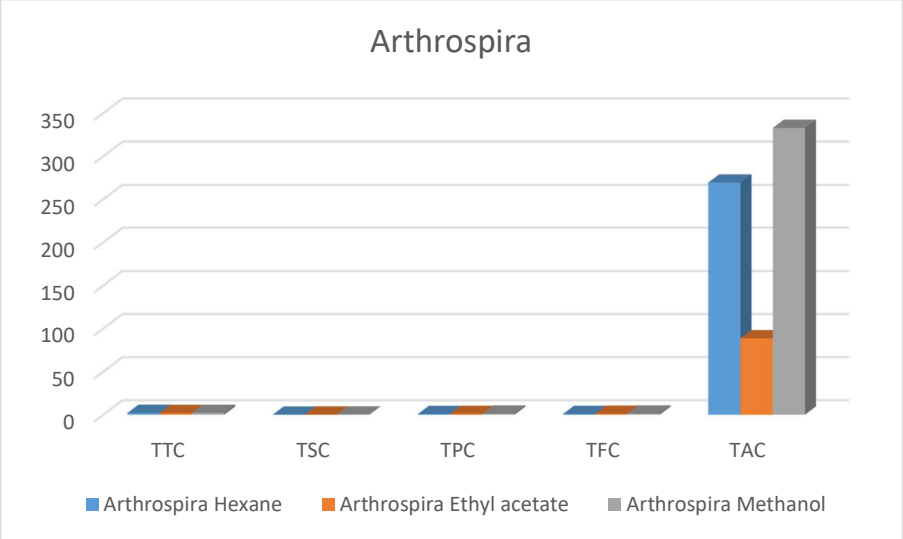

Figure 1: Phytochemical Content of Algae extract

Table 4: Retention factor

Compound	Dc	Ds	Rf
A. platensis 1	3.5	4.3	0.8
A. platensis 2	4.0	4.5	0.9

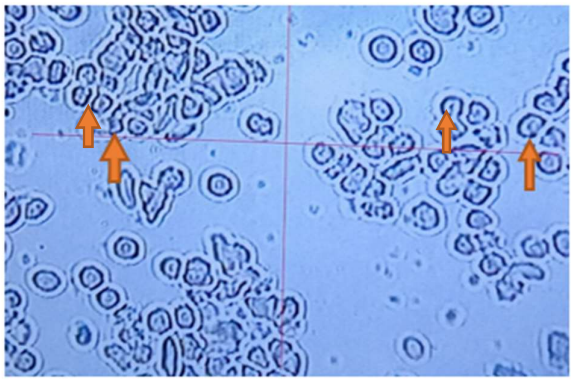

Figure 2: Sickle cells

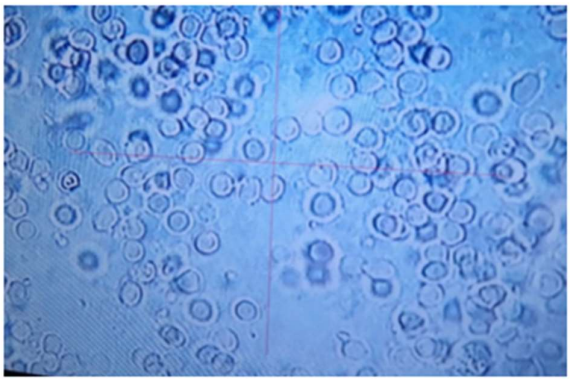

Figure 3: Effect of adding *A. platensis* extract

Table 5: Bioactive Compounds Identified in *Arthrospira platensis* Methanolic Leave Extract Fraction using GCMS techniques

Peak No.	Identified Compound	RT (Min)	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)	Functional Groups
1	(Z)-9-(E)-12-Tetradecadien-1-ol acetate	12.721	$C_{16}H_{28}O_2$	252	3.23	Unsaturated Fatty acid
2	Methyl 11-octadecenoate	13.484	$C_{19}H_{36}O_2$	296	7.87	Unsaturated Fatty acid
3	Methyl 2-oxooctadecanoate	16.245	$C_{19}H_{36}O_3$	312	5.31	Ketone
4	Methyl tridecanoate	16.751	$C_{14}H_{28}O_2$	228	5.36	Ester
5	Hexadecanoic acid	17.765	$C_{16}H_{32}O_2$	256	7.60	Fatty acid
6	Methyl (13E,16E)-13,16-octadecadienoate	18.325	$C_{19}H_{34}O_2$	294	5.15	Unsaturated Fatty acid
7	14-Methyl-14-(3-oxobutyryloxy)-hexadec-15-enoic acid	19.047	$C_{22}H_{38}O_5$	382	4.95	Ketone
8	Methyl petroselinate	19.885	$C_{19}H_{36}O_2$	296	2.29	Ester
9	2-Methyl-Z,Z-3,13-octadecadienol	20.646	$C_{19}H_{36}O$	280	34.44	Unsaturated Fatty acid
10	Methyl stearolate	21.180	$C_{19}H_{34}O_2$	294	17.96	Ester
11	Brassidic acid	23.744	$C_{22}H_{42}O_2$	338	2.97	Fatty acid
12	Erucic acid	26.010	$C_{22}H_{42}O_2$	338	1.15	Fatty acid
13	Squalene	26.954	$C_{30}H_{50}$	410	1.12	Terpene

Discussion

The application of phytomedicines as a substitute therapeutic modality has demonstrated encouraging results. These include the observable decrease in the number of crisis events, the reversal of sickling cells, and the stability of the membranes surrounding red blood cells to stop hemolysis. These findings show that natural treatments can effectively treat sickle cell disease and enhance the lives of those

who are afflicted with it. According to (6) *S. platensis* is a rich source of various macronutrients, including protein, lipids, carbohydrates, and fibers. Besides, the HPLC profile of the extract revealed the presence of different classes of polyphenols, such as flavonoids and phenolic acids, including caffeic and coumaric acids. Notably, the extract was found to contain a high concentration of iron, while copper, manganese, zinc, and selenium were also detected in significant amounts. To identify the bioactive compound that causes the sickle cells to revert to their normal disc shape, the plants are subjected to various analyses using three different solvents: n-hexane, ethyl acetate, and methanol. These analyses include column chromatography, which aids in extract separation into different fractions, thin layer chromatography (TLC), which aids in identifying fractions of the same compound that are subjected to gas chromatography/mass spectroscopy (GC-MS) analyses for compound identification. From GCMS analysis, it was identified that the compound contained fatty acids, unsaturated fatty acids, esters, alcohols, and alkanes. Suffice it to say, secondary metabolites are perhaps the most critical component of medicinal plants in managing sickle cell disease. Because of the existence of several sorts of secondary metabolites within the plants, the studies had revealed the possible of preventing sickling. The phytochemical screening analysis conducted in this study revealed the presence of flavonoids, anthocyanin, alkaloids, saponin, phytosterols, phenolic compounds, flavonoids, terpenoids among others.

Conclusion

Sickle cell disease is a congenital hemoglobin-related illness that results in sickle-shaped blood cells and associated problems. It results from the reduction of oxygen binding efficiency to hemoglobin induced by the substitution of valine for glutamic acid at the sixth position of the β chain of hemoglobin. Nevertheless, there are inefficient and adverse therapy options for sickle cell disease. The use of plant extracts to treat sickle cell disease was suggested in this study. Because plant extract has anti-sickling properties and can mitigate the consequences of this genetic condition, it is incredibly beneficial. Investigating the precise Antisickling properties in the *Arthrospira plantesis* extracts and identifying the phytochemical component causing these effects have been the main goals of the endeavor. The research has successfully recognized and characterized bioactive compounds' presence in *Arthrospira*, showcasing significant antisickling activity. The particular compounds include; (Z)-9-(E)-12-Tetradecadien-1-ol acetate, Methyl 11-octadecenoate, Methyl 2-oxooctadecanoate, Methyl tridecanoate, Hexadecanoic acid, Methyl (13E,16E)-13, and 16-octadecadienoate**,** have displayed the remarkable capability to hinder the sickling of red blood cells. This suggests a possible use of medication to treat sickle cell disease. These results offer fresh prospects for creating all-natural, plant-based sickle cell disease therapeutics while also deepening our knowledge of *Arthrospira's* therapeutic benefits.

The phytochemicals discovered included flavonoids, known for their antioxidant properties; saponins, which have immune-boosting effects; tannins, recognized for their astringent qualities; terpenoids, which are often linked to anti-inflammatory responses; alkaloids, celebrated for various health benefits; and phenolic compounds, known for their potential health-promoting effects.

Conflict of interest

No conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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