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Quality of Spirulina Platensis Produced from Different Local Resources

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Jerusalem/ Palestine

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To my grandmother's soul

Declaration

I hereby declare that this thesis has been completely composed by me, it was the result of my own research, except where otherwise acknowledged, and this research has not been submitted for any other degree or university. It will be submitted only to Al-Quds University.

Signed: 

Date:21/5/2022

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ABSTRACT:

This study is very important for introducing a new and very important economical product for the benefits of the Palestinians. Spirulina platensis is a type of cyanobacteria (blue-green algae). The present study was designed to test the quality of Spirulina Platensis that were grown in five different local cheap resources. The quality of the product is an indication for the possibility of producing Spirulina platensis using cheap local resources, and thus introducing a new product industry for the Palestinian market. The quality of the algae was compared to the quality of Spirulina platensis microalgae that was grown on the expensive media reported in the literature. The quality was reported by measuring and comparing the total phenolic content TPC, total flavonoid content TFC, HPLC analysis of polyphenolic compounds, antioxidant and antibacterial activities of S.Platensis crude extract of the five samples and the standard.

The different media that was tested include: 1. Zarrouk media (ZM) 2. fish waste water pond (FW), 3. plant ash solution 3% (PAS), 4. Brackish water (BW) 1:2 dilution with d.water. 5. mix solution (MS) from (fish wastewater pond+ plant ash solution 3%+ brackish water dilution 1:2 as 1:1:1, respectively). The Quality of the media was tested by measuring antioxidant activity, total phenolic and flavonoids content, as well as antibacterial and anticancer activities for the five local media. HPLC was used also to determine the phenolic compounds in the extracts of S.platensis. The result was compared with standard S.platensis that was grown in Zarrouk media. Results showed that TPC of S.platensis from ethanolic extract was found to be 42±2 mg gallic acid /g, 33±0.9 mg gallic acid /g, 32±1 mg gallic acid /g, 40±1 mg gallic/g, 22±0.7 mg gallic acid /g and 5±0.9 mg gallic acid /g, for S.platensis cultivated with media (ZM, MS, FW, BW, PAS, and standard S.platensis, respectively). For TFC S.platensis from ethanolic extract was found to be 3.3±0.6 mg quercetin /g, 2±0.4 mg quercetin/g, 1±0.8 mg quercetin /g, 2.6±0.5 mg quercetin/g, 1.4±1 mg quercetin /g, and 1.2 ±0.2 mg quercetin/g for S.platensis cultivated with ZM, PAS, BW, MS, FW, and standard S.platensis, respectively. HPLC analysis showed that all extracts contained major phenolic compounds like chlorogenic acid, gallic acid, catechin and caffeic acid. The highest phenolic compounds in all extracts were found to be chlorogenic acid followed by gallic acid. Results also showed an increase in the % of these phenolic compounds in spirulina with the new culture media (MS, ZM, FW, BW, PAS) compared to the reference *S.*Platensis. Antioxidant activity as % inhibition of S. platensis extract was found to be 77%±0.6, 47%±0.9, ±, 46%±0.3, 17.1%±0.3, 8.3%±0.5, and 54%±0.6, for S.platensis cultivated with new culture media MS, ZM,BW, FW, PAS, and standard S.platensis, respectively. Antibacterial activity was performed by disc diffusion method, and

the inhibition zone was found to be 11 mm, 15 mm, 8mm, for Standard S.platensis, 11mm, 18mm, 13mm for BW media, 10mm, 11mm,11mm, for PAS media against Staphylococcus, E.coli, and Marsa, respectively. While no effect on bacterial growth for S.platensis cultivated in ZM, FW, and MS media. Anticancer activity was performed against two cancer cell lines (breast cancer cells MCF7 along with colon cancer cells from the cell line HT29) cultured in RPMI media. No anticancer activities were observed for S.platensis cultivated with MS, ZM,BW, FW, PAS, and standard S.platensis, against the two cancer cell lines.

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List of Abbreviations:

Abbreviation	**Description**
AA	Antioxidant activity
AA	Arachidonic acid
ALA	Gamma-linolenic
BW	brackish water
DHA	Docosahexaenoic acid
DMSO	Dimethyl Sulfoxide
DPPH	2,2-diphenyl1-1-picrylhydrazyl
DW	Deionized water
EFSA	European Food Safety
EPA	Eicosapentaenoic acid
Escherichia coli	E.coli
EtOH	Ethanol
FDA	Food drug Administration
FW	fish wastewater
GAE	Gallic acid equivalent
HDL	High density lipoprotein
HT29	colon cancer cells
ITS	internal transcribed spacer
IZ	Inhibition Zone.

LDL	Low density lipoprotein
MCF7	breast cancerc cells
Methicillin-resistant Staphylococcus aureus	MARSA
mg/ml	Milligram/milliliter.
Ml	Millilitre
Mm	Millimeters
MS	mix solution from(fish wastewater +plant ash solution +brackish water
MS	Zarrouk medium
Ml	Microlitre
NASA	National Aeronautics and Space Administration.
OD	Optical Density
PAS	plant ash solution
PH	Power of hydrogen
Ppt	Part per thousand
S. platensis	Spirulina platensis
S.S.platensis	Standard Spirulina platensis
SDA	Steari-donic acid
SOT	Society of Toxicology
Staphylococcus aureus	S.aureus
TFC	Total flavoniodscontent
TPC	Total phenolic content
WHO	World Health Organization
ZM	Zarrouk media

Chapter one: Introduction

1. INTRODUCTION:

1.1Cyanobacteria:

Cyanobacteria, are known to be a blue green algae. The dense growth of the cyanobacteria often turn water green or blue green or brownish green. Cyanobacteria are found in water bodies, desert crust, or even in symbiosis with other animals. Cyanobacteria live in large varieties of environmental conditions, such as low or high temperature, highlight intensities, pH and salinity (BarsantiL,Coltelli P, 2008). Cyanobacteria are a very old screening method of cyanobacteria for antibiotics, and other pharmacologically active compounds have recently received considerable attention (Borowitzka, 1995). Cyanobacteria are a good source of pigments such as (chlorophyll a, carotenoids and phycobiliproteins, vitamins, polysaturated, proteins, and other biologically active compounds of high commercial value (Thajuddin and Subramanian, 2005). Cyanobacteria act as food for mollusks, fish and crustaceans. Cyanobacteria can maintain O_2 and CO_2 balance of the water body thus, facilitating aquaculture (Al-Badri,2010). Cyanobacteria help in removing phosphorus and nitrogen from polluted water with simultaneous production of biomass (Kuritz and wolk, 1994). Cyanobacteria are promising tool for removal of heavy metals from single as well as from multi-metal containing waste waters (Mehta and Gaur, 2005). In the last decade, an increase attention has been directed to ward the possibilities of the growing algae commercially, due to that, some strains of microalgae and cyanobacteria have demonstrated the ability to produce a variety of bioactive products (Elena and Carlos, 2018).

Cyanobacteria are exploited as food for human and animals. It is also incorporated in food supplement and animals feed by manufacturing it through single cell protein due to high protein content. There are many examples of cyanobacteria as shown in Figure 1.

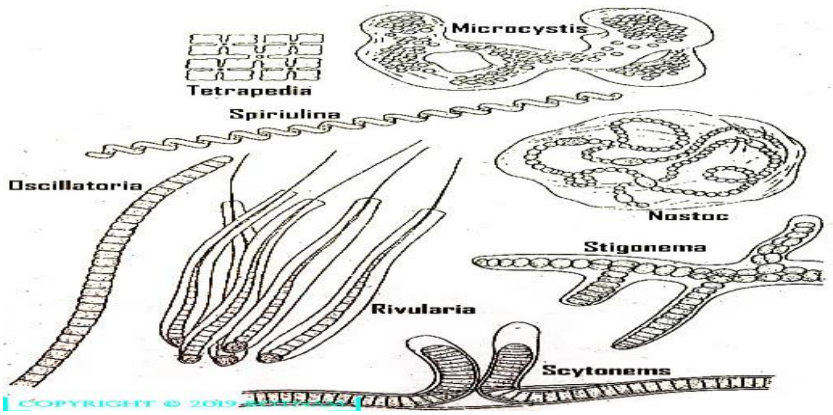


Figure (1): Examples of Cyanobacteria (Hassan Kurd, 2021)

1.2 Polyphenols:

Polyphenols are secondary metabolites of plants' tissues, found in fruits, vegetables, cereals and beverages. More than 8,000 polyphenolic compounds have been identified in various plant species. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid. Primarily, they occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of sugar (polysaccharide or monosaccharide) to an aromatic carbon also exists. Association with other compounds, like carboxylic and organic acids, amines, lipids and linkage with other phenol is also common (Kondratyukand Pezzuto 2004). Polyphenols may be classified into different groups as number of phenol rings they contain, and based on structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes and lignans(Spencer and Abd El Mohsen, 2008).

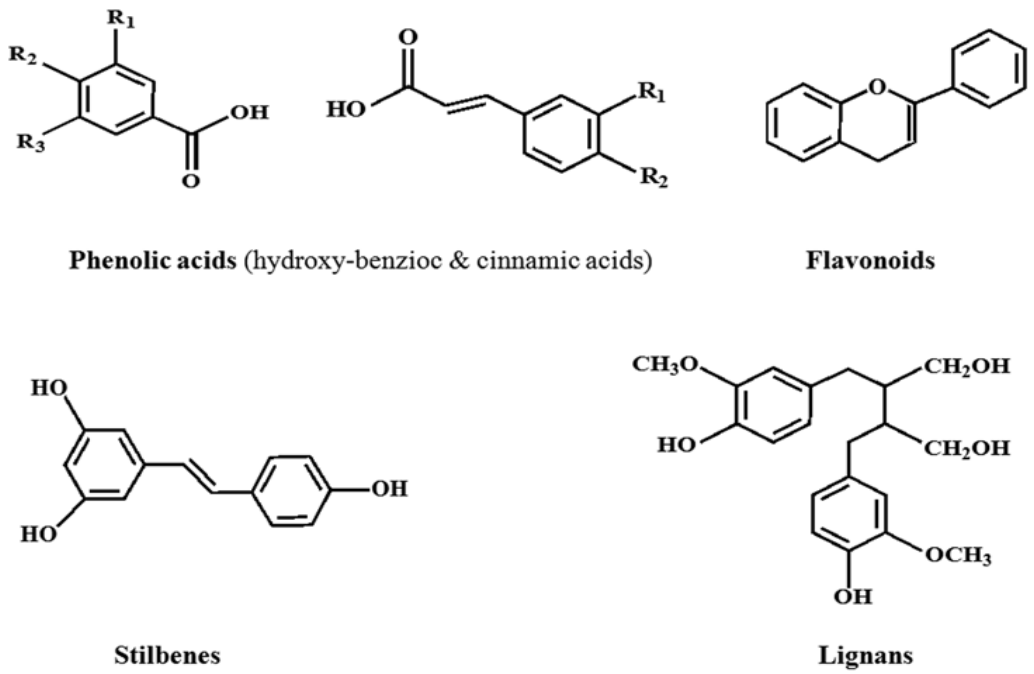


Figure (2): chemical structure of the different classes of polyphenols, where R1,R2and R3 are H,OH or OCH3,respectively.

1.2.1 Phenolic acids:

They are found in a variety plant-based foods viz, seeds, skins of fruits and leaves of vegetables contain them in highest concentrations. Typically, they are present in bound form such as amides, esters, or glycosides and rarely in free form (Pereira and Valentão, 2009). They possess one or more aromatic rings with one or more hydroxyl groups. It can be split into two classes: derivatives of benzoic acid such as gallic acid and cinnamic acid such as coumaric, caffeic (is the most rich acid in many fruit and vegetables) and ferulic acid.

1.2.2 Flavonoids:

Flavonoids are a group with a basic structure that consists of two aromatic rings bound together with three carbon atoms forming an oxygenated heterocycle, (Figure 3). Over 4,000 varieties of them have been identified. A various of flavonoids are responsible for the flowers, fruits and leaves colours (agaroot et al, 1998). Flavonidsare divided into six subclasses in accordance with the degree of oxidation of the centralring, the position and the number of OH groups. Flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones, Quercetin, catechins, myricetin etc. Here are some most common flavonoids shown in Figure3.

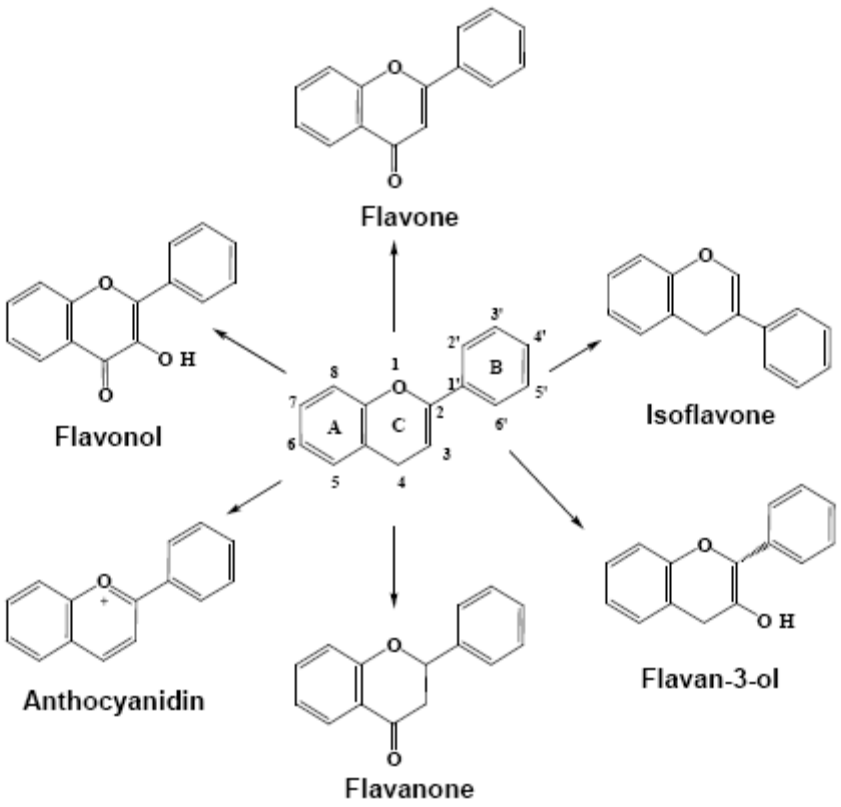


Figure (3): Chemical structure of the six main classes of flavonoids.

1.3 Antioxidant Activity(AA):

Antioxidant is defined as any substance which delays or inhibits oxidative damage to target molecules (Yamagishi S et al., 2011). Antioxidant's main characteristicis its ability to trap free radicals. Antioxidant exists in food and medical plants, such as fruits, vegetables, cereals, mushrooms, beverages, flower, spices and traditional medical herbs. Antioxidant exhibits a wide range of biological effects including anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer.

The natural antioxidant from plant materials are mainly polyphenols (phenolic acid, flavonids, anthocyanins, lignins and stilbenes, carotonides (Xanthophylls and carotenes), and vitamins (vitamin E and C).

1.4 Antioxidant Activity of Spirulina platensis:

Phenolic compounds are studied for their antioxidant properties in fruits, vegetables, and cyanobacteria (Colla et al., 2007). Phycocyanin, β-carotene, tocopherol, γ-linolenic acid and phenolic compounds exist in spirulina provide it with the antioxidant activity, anti-viral, anti-bacterial, anti-cancer, anti-diabetic, anti-inflammatory, and immunomodulatory. Since Spirulina has high content of phenolic compound, it has higher antioxidant activity than other commercial algae, like Chlorella. The natural antioxidants exist in S.platensis are vitamins (B1, B5, B6, and E6), minerals (zinc, manganese, and copper), amino acid (methionine), and beta-carotene. Spirulina protective effects against oxidative-stress induced by lead acetate in the liver and kidneys of rats (Ponce-Canchihuaman et al. 2010). Spirulina platensis feeding reduces hepatoxicity induced by cadmium in rats. This effect is suggested to be mediatezd through its antioxidant properties (Karadeniz et al. 2009). Due to oxidative damage induced by gentamicin, Spirulina is has protective effects against nephrotoxicity (Karadeniz .A et al. 2008).

1.5 Antimicrobial Activity:

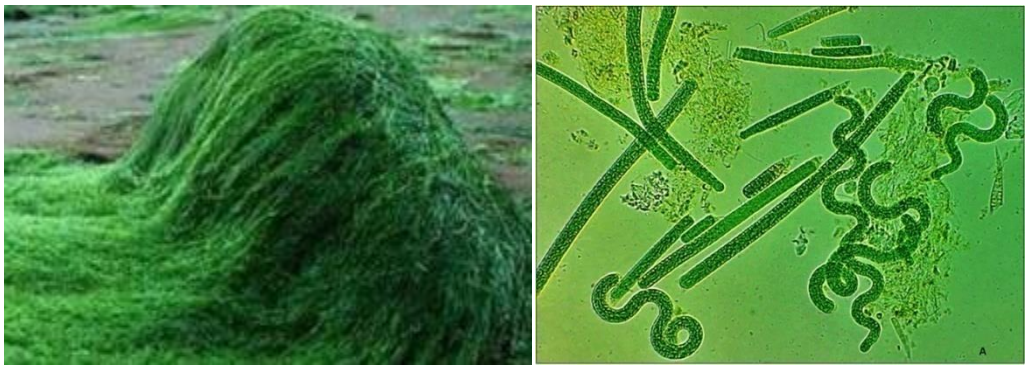
People have recently become more interested in the search for natural compounds with antimicrobial activity. This is because of the worldwide growing concern about alarming increase in the rate of infection by Antibiotic resistant microorganisms (Kaushik and Chauhan, 2008). The cyanobacteriaum “Spirulina platensis” is becoming one of the most promising agents to synthesize potentially new therapeutic compounds.

Spirulina Gram-negative bacteria, as most cyanobacteria species, can be able to produce intracellular and extracellular metabolites with diverse biological activity such as antialgal, antibacterial, antifungal and antiviral activity (Noman et al., 2004; kumar et al, 2011;Al-Wathnani et al., 2012). The cyanobacteria S.platensis has become one of the most promising agents to synthesize potentially new therapeutic compounds. The antibacterial activity was determined by disc diffusion method by Okigbo et al., 2005.

1.6 SPIRULINA PLATENSIS:

S.platensis belonging to cyanobacteria class, green-blue microalga, photosynthetic, multicellular, autotropic, can make their food by themselves. Furthermore, S.platensis has several applications in food, feeding, nutraceuticals, pharmaceuticals, and cosmeceuticals. It grows in salty and fresh water lakes and bonds. Its name is derived from filaments, multicellular trichomes in an open left-handed helix with 50-500µm length and 3-4µm width. Spirulina may be spiral-shaped, containing essential amino acids, proteins, fatty acids, antioxidant pigments, carotenoids, beta-carotene, and phycocyanin.

In the last twenty years or so, millions of people worldwide have taken Spirulina as a safe food supplement. S.platensis is being commercially cultivated as a human food supplement and an animal feeding ingredient. Pharmaceutical uses it because of its ability to produce compounds such as carotene, omega 3 and 6 polyunsaturated fatty acids (Alonso and Maroto, 2000).



A. B.

Figure (4): A microscopic view of microalgae S.platensis (NilaySeyidoglu et al, 2017), B.Spirulina platensis in natural shape (Nasreen Abdulrhman, 2012).

S.Platensis is consists of two species S.Platensis and S.maxima. Geitler et al. Spirulina species being without septa and Arthrospira species with septa. The edible forms generally referred to as Spirulina platensis. The difference between them based on results from the complete sequence of the 16S ribosomal RNA gene and the internal transcribed spacer (ITS) betweenthe 16S and 23S rRNA genes determined for two Arthrospira strains and one Spirulina strain. (Nelissen B et al. (1992)'s study shows that the two Arthrospira strains formed a close cluster distant from the Spirulina strain. The biochemical composition of S.Platensis may vary according to the growing

condition mostly for response to the salinity of the growing medium. S.platensis is grows in freshwater at (pH 7). In addition, it is grown naturally in tropical and subtropical lakes, with highly alkaline levels pH 8.3 to 11, and a temperature of 35C^{o} to 38C^{o}, with high concentration of carbonate and bicarbonate levels. Algae collected from the natural environment differs from that produced under laboratory conditions.

S.platensis or its extracts have been used for skin health in recent times. Since it contains Collagen fibrils, it has positive effects on wound closure during the healing process. In addition, it is used in creams and ointments. Rabadiya et al. 2010 suggested that the antibiotic effects of S. platensis had inhibitive effects of bacteria and promoted skin healing, during the scarring process. Syarina p et al. 2015 suggested that aqueous extract of S. platensis has a healing activity and it is an economical method for promoting skin, especially for diabetic wounds.

S.Platensis has a many effects for its high nutritional value. It is an excellent source of good quality protein (60-70%), fatty acids, amino acids, minerals, vitamins (especially B_{12}), pro-vitamin A (β-carotene), mineral (copper, manganese, magnesium, selenium, and zinc.), lip soluble antioxidants (vitamin E and carotenoids), antioxidant pigments (phycobiliproteins and carotenoids) and polysaccharids (Belay et al., 1993, Vonshak. 1997).

Its also has a Polyunsaturated fatty acids, including the gamma-linolenic (ALA), and linoleic acids (Devinameri et al,. 2007). Steari-donic (SDA), eicosapentaenoic acid (EPA),

docosahexaenoic acid (DHA), and arachidonic acid (AA). It has many therapeutic properties, such as hypocholesterolemic, immunological, antiviral and antiglutagenic effects (McCarty, 2007). The spirulina is free from cholesterol. It reduces the level of serum LDL (bad cholesterol) and raises HDL (good cholesterol).

Cheng-wu 1994, first described the immunmodulatory function of spirulina in mice. The study of the University of Missisippi, School of Pharmacy, also showed that the extraction of a polysaccharide from Spirulina called Immulina. Pugh et al., 2001 demonstrated the powerful immunostimulating activity of this polysaccharide due to activation of this monocytes and macrophages.

(Phycocyanin) is a main photosynthetic pigment in S.Plantensis, It gives the S.Platensis the blue color and contains chlorophyll and carotenoids. The chlorophyll in S.Platensis acts as cleansing

and detoxifying factor against toxic substances. It is also used as food additive to improve the coloration in ornamental fish (james et al., 2006) and a probiotic agent (Ramakrishnan et al. 2008). The Phycocyanin is a water-soluble antioxidant. It is able to scavenge the very dangerous hydroxyl radical and inhibit the oxidation of lipids in the liver and kidneys. A study demonstrated 71 % antioxidant capacity for the group taking the S.Platensis extract and 54% for the group that did not. It indicates the strong antioxidant protection (Miranda., 1998., Brazilian Journal of Medical and Biological Research).

1.6 Spirulina as food supplements:

S.platensis is now becoming a healthy food worldwide for its high nutritional value and pharmaceutical properties. Its development of potential pharmaceuticals lacking toxicity and having corrective properties against anemia,tumor growth and malnutrition. More than 70 per cent of the Spiraling consumption for human use, mainly as health food. It is a potential food item for people suffering from coronary illness and obesity (Richmond, 1992). Studies showed that S.Platensisand its extract could prevent or inhibit cancer in human and animals. Furthermore, it is a powerful tonic for immune system, as shown in feeding studies since a small amount of Spirulina can build up both humoral and cellular mechanisms of the immune system. S.Platensis cyanobacteria produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity.S.platensisis used in aquaculture as food additive to improve growth, feed efficiency, car-cass quality, and physiological response to disease in several species of fish (Mustafa et al,. 1994). NASA recommended it as a food source for long-term outer-space missions, using it as palatable diet. The International Association of Applied Microbiology also reported that the S.Platensis is a wonderful future food source.

There is no risk in taking S.Platensis because it is a good food supplement for health reported by The World Health Organization (WHO). The Food and Drug Administration (FDA) suggested that the S.Platensis is a safe dietary supplement. They recommended the dose 3-10 g of daily of these microalgae for human health. S.platensis helps to control the blood sugar level for glycemic health as shown by the European Food Safety Authority (EFSA). The S.platensis supplements are available in various forms, mainly powder, tablets, straw, capsules and liquids, or incorporated to other foods, such as pasta, gums, and beverages.

Figure (5): Various forms of Spirulina platensis (Henrikson, 2021).

The authors study the toxicity of spirulina during animal experimentations. Authors Sharma et al. 2012 have found that Spirulina did not demonstrate any harmfulness even with raised doses of human consumption (Zhu et al. 2014). Spirulina is also a good supplement for iron deficiencies during pregnancy and Lactation. Furthermore, it has no risk and does not have side effects on animals or human.

The experiments on Spirulina human feeding showed that when the adults take the protein in Spirulina, it is adequately absorbed, and results in low level of uric acid in the serum and a moderate increase in fecal nitrogen. Spirulina seems to be better than soy and as good as cow milk and human milk. It is also good with third-degree malnutrition-stricken children.

The light is the most important factor during microalgae and cyanobacteria cultivation, so the energy is captured by the pigments to carry out the photosynthesis process (Atta et al., 2013). Artificial light is more commonly used in the cultivations, with efficient and standardized control of photosynthetic photon flux density, resulting in high productivitied (Blanken et al., 2013).

S.Platensis requires high costs to make suitable media for high biomass production. S.Platensis was cultured mostly in Zarrouk's or modified Zarrouk's medium or Society of Toxicology (SOT) medium, which are expensive, as they require increased amounts of $NaHCO_3$, Na_2CO_3, $NaNO_3$, and trace metals. (Lamela and Rocha, 2000; Tredici et al., 1986).

S.Platensis can be cultivated under laboratory conditions as well as outdoor for large-scalesystem. The outdoor culture systems rely mainly on zarrouk medium (Belay, 2008;Madkour et al 2012; tarko et al 2012) though it is highly expensive. Thus, efforts have been made to develop a more

convenient and less expensive culture media (Raoof et al 2006;Chen,2011;Gami etal 2011;Madkour et al 2012), which can produce high-quality S.platensis biomass comparable to the standard culture media.

(Cho and slinger, 1979) has shown that the presence of low-cost food is one of the important factors in aquaculture. In addition, that study corresponds with our study to presence S.pirulina with low cost media.

The aims of this research were designed to cultivate S.Platensisin five different media in a less expensive local resources for culture media, then compared the (TPC,TFC), antioxidant and antibacterial activity of Standard S.Platensis crude extract with S.Platensis cultured with new media.

1.7 Research questions:

1. Does the quality of the S.Platensis from different local resources (as culture media) have the same characteristics compared to the standard S.Platensis from literature ideal resource (standard culture media)?

2. Does S.Platensis cultured in the new developed media have anti-oxidant activity, total phenolic and total flavonoid compounds comparable to the standard S.Platensis?

3. Does S.Platensis have antibacterial and anticancer activities comparable to the standard S.Platensis?

1.8 OBJECTIVES AND AIMS:

1.To test the quality of the produced S.Platensis from different local resources compared to the standard .S.Platensis from literature ideal resources**.**

2.To evaluate the total phenolic and flavonoids contents of S.Platensis and standard S.Platensis extract, and analyze their phenolic compounds by using HPLC.

3. To evaluate the in-vitro antibacterial,antioxidant, and anticancer activities of standard S.Platensis from literature ideal resources and produced S.Platensis from different local resources.

1.9 HYPOTHESIS:

The quality of the S.Platensis produced from different local resource have the same characteristics compared to the standard S.Platensis from literature ideal resource.

Chapter two:

2.1Literature Review:

In Ashgan A et al. 2018 study, There was a focus on studying biomass concentrations (g/lDW), optical density (OD), and phytochemical screening (Total phenolic, total flavonoids and Antioxidant activity) of crude extracts of Spirulina platensis of all media with commercial Spirulina (dry product). There are three different media mentioned, (modified commercial low cost Zarrouk medium (MS), Khul medium and Sea Water enriched medium). The possible potentials of Spirulina against hepatic intoxication induced by CCl_4 in albino male mice were also examined. The results showed that, methanolic extract recorded the highest values in modified Zarrouk's medium (88.98 mg gallic acid equivalent/ml, 78.57 mg Rutin equivalent/ml and 82.04%) for Total phenolic, total flavonoids and antioxidant activity respectively. Results also showed that, the Spirulina has an ameliorating effect of CCl_4 induced chromosomal aberrations of bone marrow cells, which prove the protective role of it against the chromosomal damage.

Abeer.abu zaid at el. 2015, studied antioxidant activity of the Spirulina platensis water extract and their cytotoxicity against cell lines (colon carcinoma cells (HCT116) and hepatocellular carcinoma cells (HEPG2). She found out that, spirulina platensis water extract has a highest antioxidants percentage (81.1%) for DPPH inhibition, and total phenolic compounds (40.45 mg g-1). In cytotoxicity assay, the IC50 concentrations (the concentration of spirulina platensis that is required to cause 50% inhibition in cell lines viability) were 18.8 and 22.3 µg ml-1 for HCT116 and HEPG2 cell lines, respectively. In addition, the correlation between HCT116 and HEPG2 and spirulina platensis water extracts was negative and significant.

In a study by (M. Kannan1 et al., 2014) has examined the appraisal of the phytochemicals, and the antioxidant activity of marine algae Gracilariacorticata (G.corticata) and Spirulina platensis (S.platensis). To achieve the objective of this paper, the authors screened the phytochemicals of S.platensis and marine algae G.corticata, and tested the antioxidant activities of the two-selected spirulina.They found out that there is a variety of chemical constituents, such as saponins, phenols, glycosides, flavonoids and alkaloids , the antioxidant activities of the marine algae was stablished by Fentons method and DPPH assay. The authors proved that the selected algae have antioxidant potential and G. corticata and S. platensis are the prospective sources of bioactive compounds.

In a study, antioxidant potential of selected Spirulina platensis preparations by Beter et al., 2008, the authors examined the four selected Spirulina platensis preparations : (1) Biospirulina, (2) Spiru Complex, a preparation with naturally bound selenium, chromium and zinc, (3) Spiru Zink, a preparation with naturally bound zinc, and (4) Zinkspirulina + Acerola, a preparation with naturally bound zinc and acerola powder.The results demonstrated that the tested Spirulina preparations have a high antioxidant and anti-inflammatory potential. Especially Spiru Zink and Zinkspirulina + Acerola might be useful as a supportive therapeutic approach for reducing oxidative stress and/or the generation of oxygen radicals in the course of inflammatory processes.

Wagih Abd El-Fattah Elshouny et al. (2017), attempted to find out the effect of the various extracts of Spirulina platensis, Chlorella vulgaris, Saragassumwightii and Saragassumlatifolium using different solvents (methanol, ethanol, ethyl acetate and chloroform) as antimicrobial agents against five bacterial pathogens; S. aureus, E. coli, P. aeruginosa, Salmonella sp, Shigella sp. The results indicated that among the various extracts used, methanol extracts of tested cyanobacterial and algal species appeared to be the most effective ones showing maximum antibacterial activity against the selected bacteria pathogens. Therefore, the Spirulina platensisitswas the most effective against all the pathogens studied. The antibacterial substance was purified using column chromatography. The nature of the purified active fractions was detected using different chemical analyses (UV, FT-IR, 1H NMR and GC-MS) which indicated that it is an aliphatic compound and has different active groups (-OH,-C=O,-CH2 and – CH3). In addition, results of this investigation proved that the tested cyanobacterium could be a good source for the production of promising antimicrobial agents.

Rajaa et al. 2019, has characterized the nutraceutical properties and the antimicrobial effect of Moroccan Spirulina (Arthrospira platensis). Seghiri has studied microbiological and antioxidant activity of the Moroccan Spirulina. Results showed that Spirulina contained large amount of protein (76.65±0.15%), carbohydrates (6.46±0.32%), minerals (20.91±0.88%), crude fiber (4.07±1.42%), lipids (2.45±0.82%), and ash (14.56±0.74%), as well as twenty phenolic acids have been identified and quantified. Moreover, flavonoid and phenolic contents were present at 15.60±2.74 mg RE/g dw, 4.19±0.21 mg GAE/g dw, respectively. The antioxidant activity was higher in the methanolic fraction (23mg TE/g dw). The study also indicated that this product is safe to be consumed as a human food product, by using the minimum inhibitory concentration method on bacteria and fungi to test the antimicrobial effect of Moroccan Spirulina.

Rohit Shankar et al 2018, demonstrated that the phytochemical screening of the Spirulina platensis revealed that metabolites with higher medicinal activities such as alkaloids, terpenoids, steroids, saponins, phenols and flavonoids were present in all the five extracts, while Tannins, Coumarins, Quinones and glycosides were absent in few extracts of Spirulina platensis.

Another research by (M.kavisri et al. 2021), investigated the phytochemical, antibacterial and antioxidant activities of mass cultured Spirulina platensis and the structural characterization of the methanolic extracts. Results indicated that seven phytochemicals were quantified by standard procedure, and the phenolic compound was found maximum 96.7 µg/mg followed by alkaloid 89.5 µg/mg, Antibacterial activity was performed by disc diffusion method, and the -maximum activity was noted against Salmonella typhi (19 mm) followed by Escherichia coil (12 mm). The antioxidant effect was recorded in higher concentration level including as standards FT-IR spectral analysis, the active functional groups alkyl halide and amine were recorded simultaneously in ^{1}H NMR analysis, 11 chemical shifts were recorded, and it revealed the stretching of alkane, allylic, benzylic, ketone, alkyne and esters. It concluded that S. platensis is an alternate source of pharmaceutical industry due to its phytochemical profile and bioactive potential.

Angelina Michael et al. 2018 studied the content of antioxidants (total phenols, total flavonoids, Beta-carotene, and lycopene, and activity of A.fusiform produced hosing low-cost culture media (LCMA) and standard culture (Zarrouk) media. The result showed that A.fusiform has high antioxidant activity, high scavenging and chelating activities. The LCMA has higher amount of antioxidant and lower EC50 values than Zarrouk medium. It concluded that production of natural antioxidant could be increased through use of cost-saving, inorganic culture media.

Chapter three: Materials and methods

3.1Chemical and equipment:

The materials and equipment used in the study are mentioned below;

99.9% ethanol, sodium hydroxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, aluminum chloride, ammonium acetate, sodium bicarbonate, sodium acetate solution, methanol (MeOH) and water (H2O). 95%Ethanol (EtOH) solvent. Phenolic and flavonoids standards: Gallic acid, Quercetin, antibiotic (Gentamicin, pencilin) all from sigma company, bacterial strains, as gram-negative bacteria: *Escherichiacoli (ATCC 25992),* and gram-positive, *Staphylococcus aureus (ATCC 25923), Methicillin-resistant staphylococcus aureus (MARSA).* UV.VIS spectrum (Jenway 6850 spectrophotometer operatinh manual), Rotary evaporator, sonication, analytical HPLC (Water Alliance (e2695 separations module), with 2998 Photo diode Array (PDA). Plant count agar, plates, sterilized discs of filter paper, micropipette, analytical balance are available at Al-Quds University Laboratories.

3.2 Methodology:

3.2.1 Algal source:

The S.Platensis used in this study are standard S.Platensis, and Produced S.Platensis (Five different local materials were used for growing S.Platensis in a low-cost local media) obtained from Aquatic Environmental Research Center at Al-Quds University.

3.2.2 Preparation of algal materials:

Five types of local media were used to grow the Spirulina in the lab to prepare a cheap local media:

1.Zarroukmedia(ZM):

Zarrouk media was prepared from the following components from Sigma Aldrich. All components were dissolved together in distilled water to form Zarrouk medium.

Table 1: components that require to make Zarrouk media.

Components	Weight

$NaNO_3$	2.5g
K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.2g
$CaCl_2$	0.04g
$FeSO_4.7H_2O$	0.01g
Na-EDTA	0.08g
$NaHC0_3$	16.8g
NaCL	1g
K_2SO_4	1g
H_3BO_3	0.62g
$MnCl_2.4H_2O$	0.012g
$ZnSO_4.4H_2O$	0.044g
Na_2MoO_4	0.012
$CuSO_4.5H_2O$	0.02g

2. Fish wastewater (FW).

3. Plant ash solution to a final concentration of 3% (PAS3%).

4. Brackish Brine water (BW) was diluted with distilled water as 1:2 dilution factor)

5. Mix solution from (MS) (fish wastewater pond+ plant ash solution 3%+ brackish water dilution 1:2 has a ratio 1:1:1 solution, respectively)

Table 2: The PH value and salinity (part per thousand) of the growth mediums of new five culture media.

Chemical parameters	ZM	FW	BW 1:2	PAS 3%	MIX
PH	9.35	9.65	9.55	9.75	9.79
Salinity(ppt)	11.35	7.72	8.69	9.60	8.79

the light energy is the most important parameters for growth the spirulina platensis, so for All 5 new cultur media (ZM, ,MS, FW, PAS, BW) White LED Light (1500 lux) was used as a light source to supports the cultivation of spirulina platensis, and the Measurements for all were analyzed using single beam UV/Vis spectrophotometer (Jenway 7305) at 880nm.

3.3.3 Extraction of algal materials:

All S.S.Platensis and S.Platensis (cultured with the new media) were grinded with a blender. then, 10g of dried powder of S.Platensis materials were macerated with 75ml of EtOH 99% and extracted by sonication for 90min (Sung-Ho oh et al. 2011). Each of the S.Platensis extracts were subjected to rotary evaporator to evaporate the solvent. Then the crude extracts were stored in refrigerator at 4C^{o} until analysis.

3.3.3 Total Phenolics Content (Folin-Ciocalteu assay).

Total phenolic contents were determined using Folin-Ciocalteureagents(singleton et al. 1999). S.platensis extracts (20µl) were mixed with 0.1ml of Folin-Ciocalteu reagent (pre-diluted 10-fold with D.water),then they were allowed to stay at room temperature for 5 min, next, 100µl of sodium carbonate (7.5%) was added to the mixture. After staying for 30min at room temperature, absorbance was measured at 765nm. Solution of known gallic acid concentration in the range (20-50ppm) were used for calibration. Results were expressed as mg gallic acid equivalents (gallic)/g sample.

3.3.4 Total Flavonids Content (TFC).

The determination of flavonids was performed according to the aluminum chloride methods (Djeridane et al., 2006). 100 µl of all S.platensis extracts was added to 0.3 ml of methanol. Then 0.02 ml of 1M of sodium acetate solution was added to the solution, followed by 0.3 ml of Aluminum chloride (10%) solution, then 0.6 ml of D.water was added to the solution, the test tube were incubated at 25Cº for 30 min, Absorbance was measured at 415nm. The standard calibration curve was used in the range 1-20µg/ml using quercetin as standard.

3.3.5 Antioxidant Activity by DPPH Radical Scavenging Assay.

Free radical scavenging activity of spirulina extract were measured by 1,1 diphenyl-2-picryl hydrazyl (DPPH) by (Batool et al. 2010). 0.1mM solution of DPPH in methanol was prepared. 0.3ml was added to 0.1ml of different S.platensis extract. The mixture was shaken vigorously, allowing it to stay at room temperature foe 30 min, and then absorbance was measured at 517 nm by using UV-VIS spectrophotometer. Lower absorbance of the reaction indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using the following equation:

DPPH scavenging effect (%) or percent inhibition= (A0-A1/A1)*100%

Where A0 is the absorbance of control reaction (DPPH diluted with methanol), and A1 is the absorbance in presence of test or standard sample.

HPLC analysis of phenolic compounds

1. Chromatographic conditions.

The HPLC analytical experiments of the crude extracts were run on ODS column of Waters (XBridge, 4.6 ID x 150 mm, 5 µm) with guard column of Xbridge ODS, 20 mm x 4.6mm ID, 5 µm. The mobile phase is a mixture of 0.5% acetic acid solution (A) and acetonitrile (B) ran in a ratio of 60%:40% (V/V ratio). All the samples were filtered with a 0.45 µm PTFE filter. The PDA wavelengths range was from 210-500. The flow rate was 1 ml/min. Injection volume was 20 µl and the column temperature was set at 25Cº.

2. Sample preparation for HPLC analysis

The plant extracts were filtered using suction filtration, and then the solvents were evaporated under reduced pressure at 40Cº using rotary evaporator. The resulting crude extracts were dissolved in 80% ethanol at a concentration of 5 mg/mL, and 20 µL were injected into the HPLC chromatograph, and analyzed for their phenolic and Flavonoids. Different phenolic and flavonoid standards were injected and separated simultaneouslyto identify the presence of any of these compounds in the crude extracts.

3.3.6 Antimicrobial Activity:

The antibacterial effect of ethanolic extract of S.Platensis and produced S.Platensis against bacteria and fungi were performed by disk/well diffusion test. Three bacterial strain was tested as gram-negative bacteria: *Escherichia coil (ATCC 25922), and gram positive Staphylococcus aureus (ATCC 25923), Methicillin-resistant Staphylococcus aureus (MARSA).*

The disc diffusion method (Okigbo et al., 2005) was used to determinate the zone of inhibition growth of bacteria. Plate count agar was poured in plates. The plates were allowed to harden, sterilized discs of filter paper (whatman,uk)were soaked in 60µl of extract for 1 min and then screened on the plate count agar plated . Disk with d.water were added as a negative control, antibiotic as a positive control, the plates were incubated at 35-37Cº, at ambient air for 24 hours. After incubation, the diameters of inhibition zone were measured using a scale to the nearest mm including the disk diameter, this test was performed three times.

3.3.7 Testing for Anticancer Activity:

The two cancer cell lines to be tested were transforme breast cancer cells from the cell line MCF7 along with the transformed colon cancer cells from the cell line HT29. The two cell lines were cultured in RPMI media and incubated for 24 hours prior to the experimentation. A control dish was prepared containing the breast cancer cell line MCF7 alone, the same was done for the HT29 cells; these samples served as the negative control. Different plates were prepared for each extract (s. platensis cultivated with MS, ZM, BW, FW, PAS, and standard S. platensis). This process was repeated for both cancer cell lines. The cells were then incubated and observed 48 hours later.

3.3.7 Statistical analysis

To examine the relation between the different studied parameters, SPSS.v.20 was used and correlation coefficient was found, also the graphs were formed using Excel.

Chapter four: RESULTS AND DISSCUSSION

The standard and produced samples of S.platensis were collected, dried at room temperature, milled, extracted in ethanol and then filtrated. Samples of crude extract were analyzed for TPC, TFC, AA and antibacterial activity in additions to HPLCanalysis.

4.1 Total Phenolic Content(TPC)

Total phenolic content of the S.SPlatensis and S.platensiscultured with new different media was determined by using the folin-ciocalteu reagents, and were expressed as mg gallic acid equivalents (GAE) per gram of S.platensis extract. The TPC of the tested fraction were calculated using the standard curve of gallic acid. Linear equation was generated y= (0.021x-0.009) with high coefficient of determination R^2 = 0.997 (**Figure (6**).

In the present study, results showed that, the total phenoleic for six different algal extract of Spirulina (standard S.Platensis, ZM, MS, PAS, BW, FW), have highly significant quantities of phenolic contents as gallic acid. Results revealed that the highest content of phenolic compound (42±2 mg gallic equivalent/g) was determined in the ZM media whereas; the lowest value was shown in standard S.platensis (5±1 mg gallic equivalent/g) as seen in the **table 3**. Ashgan et al,. (2018) found that the highest phenolic content was determined in modified zarrouk media (88.98±0.12mg Gallic equivalent/ml) as compared to the commercial spirulina (48.93±0.07mg Gallic equivalent/ml). Angelina et al. (2018) also showed that the total phenolics of spirulina extract grown in the low cost local resources have higher level (409.28±28.78) as compared to other extract media.

On the other hand, this study showed that the S.splatensis has lowest TPC compared to other Spirulina cultured with the new media, it indicates that when the spirulina cultivated with modified media can give a high TPC content as shown in **table 3**.

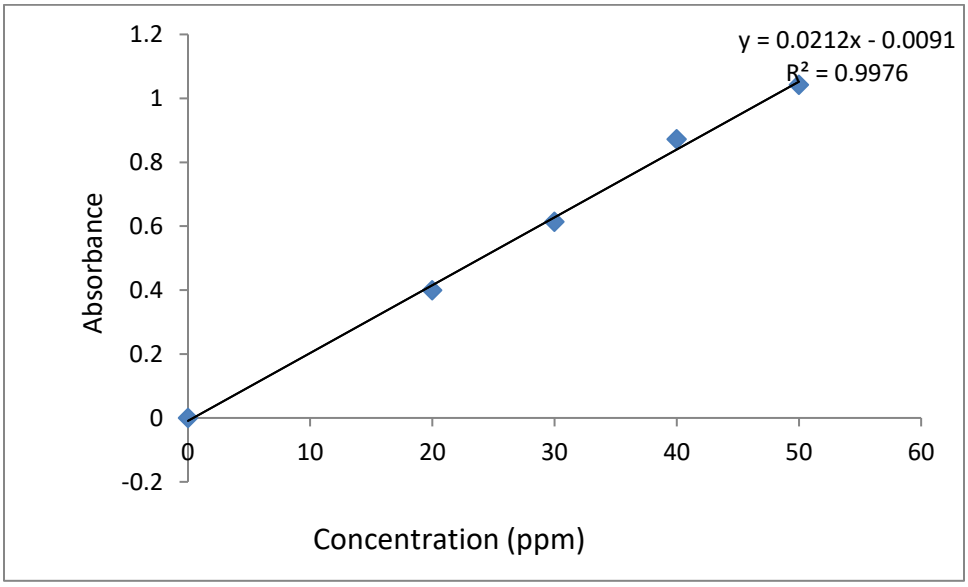


Figure (6):Calibration curve of Gallic acid Standard.

Table3:TPC in (mg Gallic acid/ g sample) of standard S.platensis and produced S.platensis as ± standard deviation.

S.SPlatensis and S.Platensis cultured in new media	mg Gallic acid/g sample
S.Spirulina	5±0.9
ZM	42±2
FW	32±1
BW	40±1
PAS	22±0.7
MS	33±0.9

4.2 Total Flavonoid Content (TFC)

TFC was calculated based on standard curve of quercetin by aluminum chloride method. Linear equation was generated y= (0.009x+0.003) with high coefficient of determination R^2=0.995 **Figure (7**).

Results showed that spirulina cultivated with ZM has high total flavonids (3.3±0.6 mg quersiten /g) followed by the MS (2.6 ±0.5 mg quercetin/g) as compared to standard S.platensis (1.2±0.2 mg quercetin/g), while other spirulina cultivated media have different TFC as shown in **table 4**.

In a previous study by (Ashgan et al,. 2018), results showed that modified zarrouk media have highest value (78.57+1.40 mg Rutin equivalent/ml) compared to the commercial S.platensis, which have the lowest value (41.92+0.45 mg Rutin equivalent/ml). Angelina et al,. (2018) stated that the total flavonoids in low cost media contain higher total flavonids (13.25+0.5 mg Rutin equivalent/g), and that correspond to our result that when cultivating Spirulina with modified media the TFC content increased.

In general, the total phenols have higher content in spirulina than total flavonids for all extract. El-Baky et al. 2009 recorded lower amount of flavonids compared to total phenols, due to large part of phenolic compound present in spirulina instead of flavonoids. In this study, results showed that the S.splatensis has lowest value compared to the other media. They also indicated that when cultivating Spirulina with modified media the TFC content increased, as we discussed earlier.

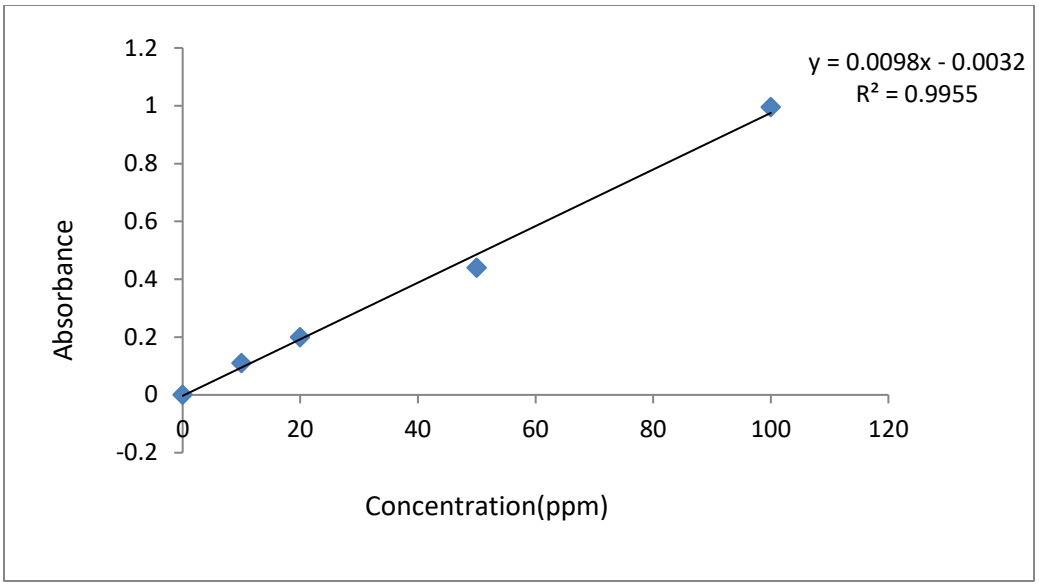


Figure (7):Calibrationcurve Quercetin standard.

Table4: TFC in (mg quercetin/g sample) of standard S.platensis and produced S.platensis as ± standard deviation.

S.SPlatensis and S.Platensis cultured in new media	mg quercetin /g sample
S.platensis	1.2±0.2
MS	2.6±0.5
BW	1±0.8
ZM	3.3±0.6
FW	1.4±1
PAS	2±0.4

4.3 DPPH Scavenging Activity:

DPPH is the most popular and frequent method used in antioxidant assays. The free radical in vitro scavenging activity of different Spirulina media were evaluated by examining the ability to reduce DPPH*. DPPH* stable free radical which possesses a purple color,with maximum absorption wavelength of 517 nm, the purple color fades away because DPPH radical converts to more stable DPPH molecular product by donating an electron or a hydrogen atom, resulting in a decrease in absorbance. A lower in absorbance indicates high radical scavenging activity of the extract (Barros et al 2007). Linear equation was generated y = (0.008x + 0.022) with high coefficient of determination R^2= 0.999 **(Figure 8).**

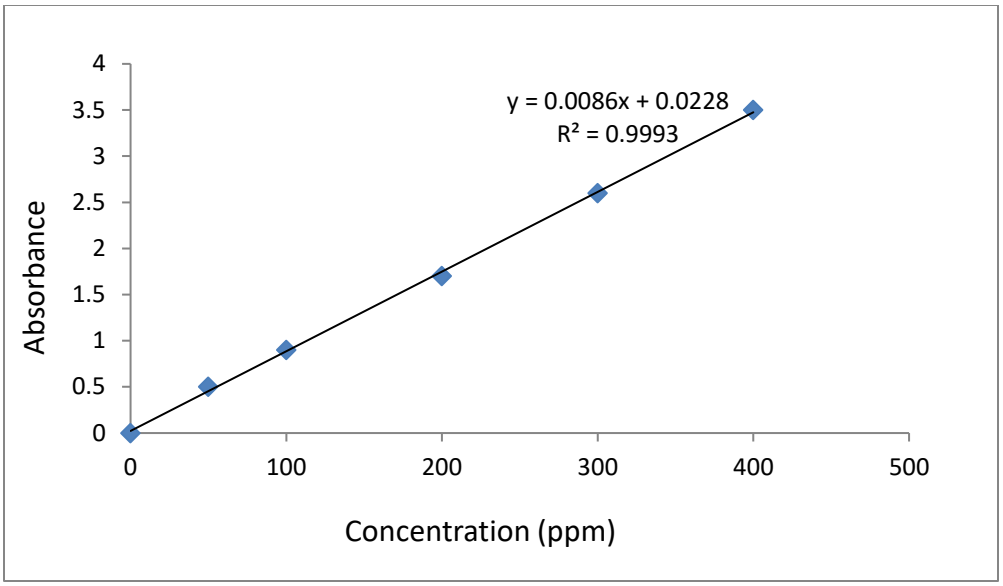


Figure (8): Calibration curve for DPPH (Abs Vs Conc. of Ascorbic acid).

All S.platensis extracts showed good scavenging activities with some variation in percent inhibition. The result showed highest antioxidant activity obtained from MS medium exhibited (77%±0.6) inhibition effect of DPPH, compared to the standard S.platensis exhibited (54%±0.6).

In a previous study by Ashgan et al. 2018, the result showed that the highest antioxidant activity was obtained from modified zarrouk media exhibited (82.04%) inhibition, compared to commercial spirulina (52.68%). For all algal extract study, the commercial spirulina medium have the lowest antioxidant activity. Angelina et al. 2018, showed that the DPPH inhibition of spirulina in low cost media have higher antioxidant activity 95%.

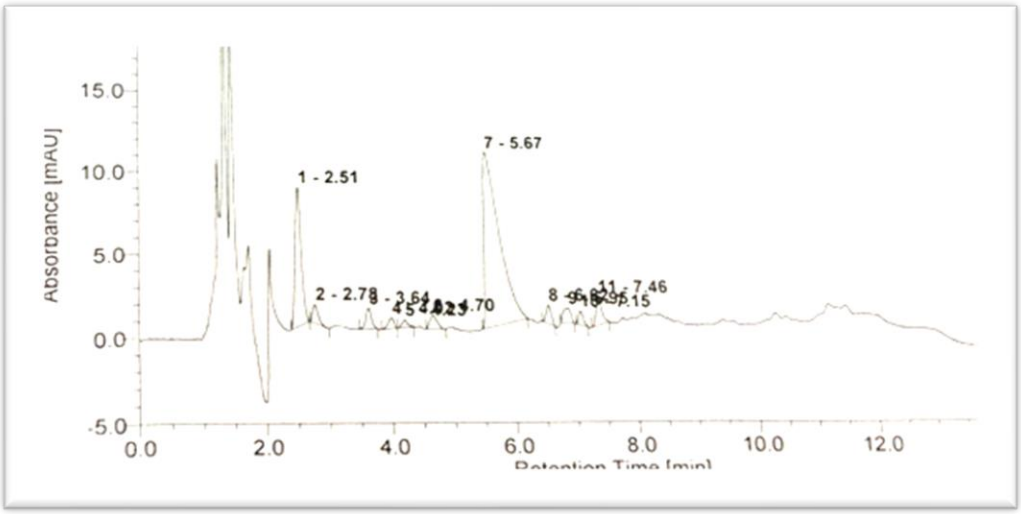
In this study, there is a significant difference between S.Splatensis and produced S.platensis as shown in table3, which indicates that the modified media can give high antioxidant activity.

Table 5: DPPH (% inhibition) of standard S.Platensis and produced S.Platensis extracts as± standard deviation.

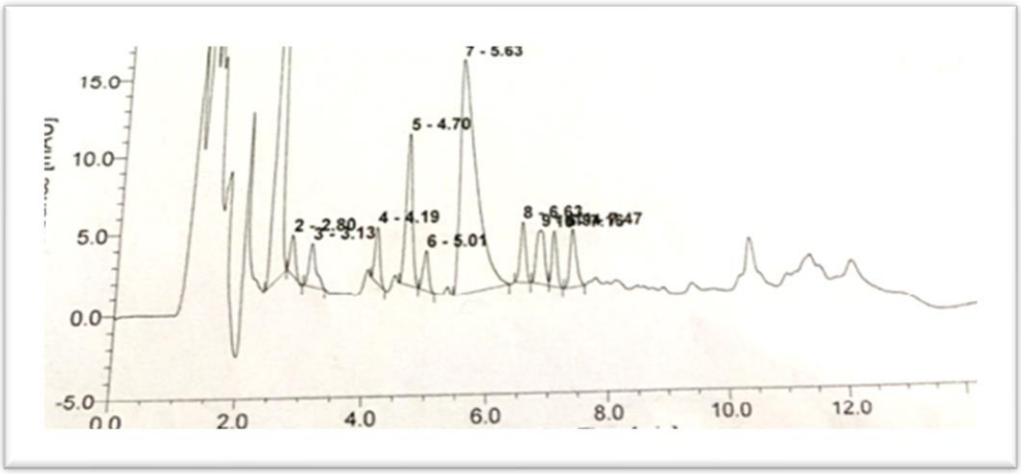
S.SPlatensis and produced Splatensis	Inhibition %
S.S.Platensis	54%±0.6
BW	47%±0.9
ZM	46%%±0.3
PAS	8.3%±0.5
FW	17.1%±0.3
MS	77%±0.6

4.4.1 HPLC analysis of the extracts

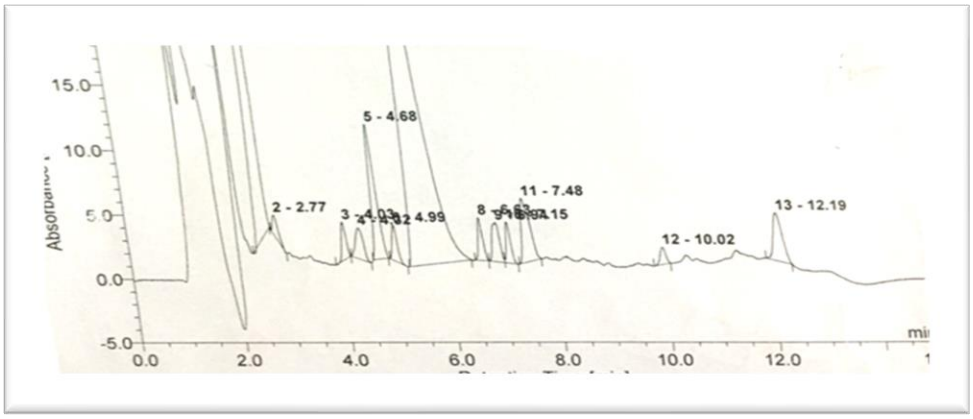
Figure (9 a-f), shows the chromatogram of the crude ethanolic extracts of standard *S.*Platensis (with standard culture media) and S. Platensis extracts with 5 new culture media (BW, ZM, PAS, FW, and MS)with a concentration of 5mg/ml at 250 nm at 20µl injection. A (250 nm) wavelength was selected since the main peaks showed a maximum absorption close to it. Standard of five phenolic compounds wereused(gallic acid, caffeic acid, chlorogenic acid, catechin, and rutin).Standard solutions concentration for the five phenolic compounds were 30ppm.See (**Figure 9**) below.


Absorbance [mAU]
15.0
10.0
5.0
0.0
-5.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
1 - 2.51
7 - 5.67
2 - 2.78
11 - 7.46

a.


15.0
10.0
5.0
0.0
-5.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
7 - 5.63
5 - 4.70
2 - 2.80
4 - 4.19
6 - 5.01

b.


15.0
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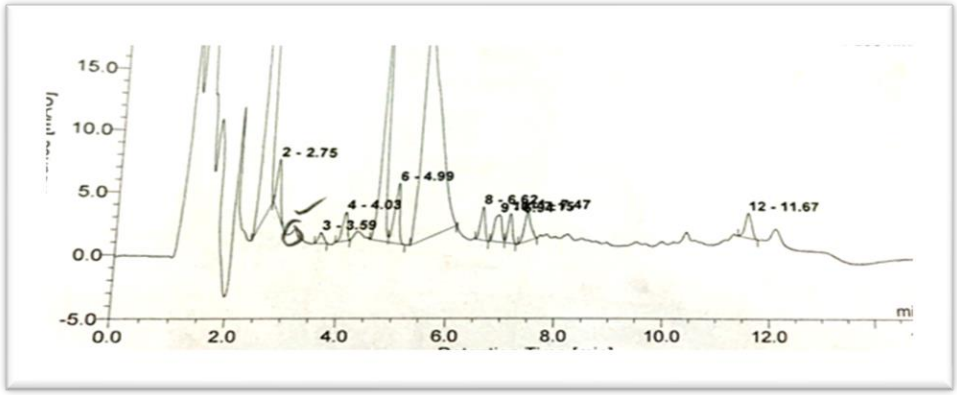
0.0
-5.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
5 - 4.68
2 - 2.77
11 - 7.48
13 - 12.19
12 - 10.02
4.99

c.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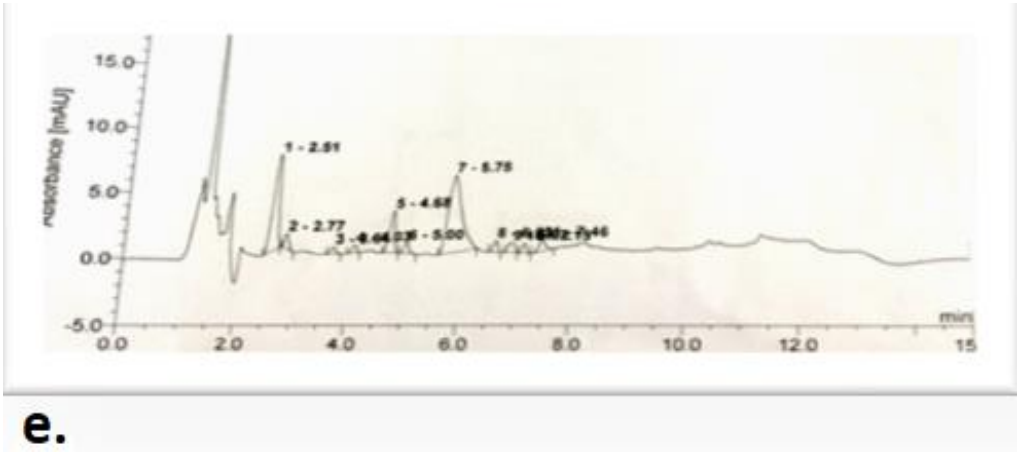
10.0
12.0
2 - 2.75
6 - 4.99
4 - 4.03
3 - 3.59
12 - 11.67

d.


15.0
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8.0
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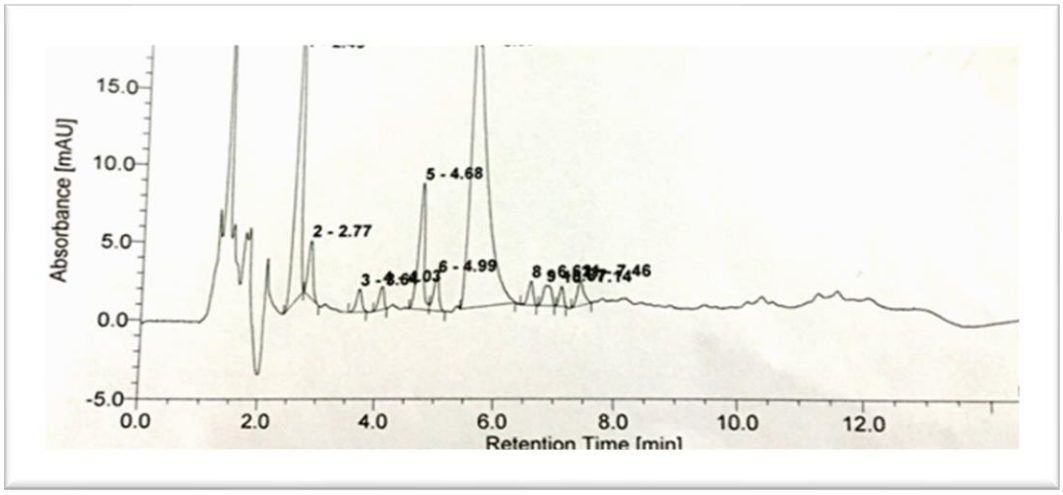
12.0
1 - 2.91
7 - 8.75
5 - 4.68
2 - 2.77

e.



f.

Figure (9): HPLC Chromatogram for ethanolic extracts of standard *S.*Platensis (with standard culture media) and S. Platensis extracts with 5 new culture media (BW, ZM, PAS, FW, and MS), respectively (a to f).

Table (6) shows the main peaks detected at this wavelength. The major phenolic constituents determined by HPLC analyses of the ethanol extracts includedsome of the previous phenolic standard and other compounds, whichcould not be identified because some of the standard phenolic compounds were not available (see Figure10a-f). As seen from these chromatograms, different phenolic compounds were detected in the range of 2-12 minutes. From the UV-Vis spectra, the compounds showed an absorption of about 268 nm and 317-357 nm. These absorptions are a typical indication of flavonoid and phenolic compounds presence.Gallic acid; catechin; chlorogenic acid; and caffeic acid were detected in all extracts of *S.*Platensis (with standard culture media), and S. Platensis extracts with 5 new culture media (BW, ZM, PAS, FW, and MS), **Table 6.**

Table 6: Main peaks detected for ethanolic extracts of standard *S.*Platensis (with standard culture media) and S. Platensis extracts with 5 new culture media (BW, ZM, PAS, FW, and MS).

A. Standard *S.* Platensis (with standard culture media)

#	Retention time	Peak area	Relative area	Identity
1	2.5	57143	18.9	Gallic acid
2	2.7	6284	2.0	
3	3.6	7885	2.6	
4	4.0	4224	1.4	
5	4.2	3037	1.0	
6	4.7	6496	2.1	Catechin
7	5.6	187535	61.9	Chlorogenic acid
8	6.6	6528	2.1	caffeic acid
9	6.9	7988	2.6	
10	7.1	4491	1.4	
11	7.4	11198	3.7	

B. *S.* Platensis (with FW culture media)

#	Retention time	Peak area	Relative area	Identity
1	2.5	54673	24.3	Gallic acid
2	2.7	6753	3.0	
3	3.6	3804	1.7	
4	4.0	3766	1.6	
5	4.6	22491	10.0	Catechin
6	5.0	5525	2.4	
7	5.7	104130	46.4	Chlorogenic acid
8	6.6	5180	2.3	caffeic acid
9	6.9	6488	2.9	
10	7.1	4115	1.8	
11	7.4	7215	3.2	

C. *S.* Platensis (with BW culture media)

#	Retention time	Peak area	Relative area	Identity
1	2.5	230112	30.6	Gallic acid
2	2.8	14322	1.9	
3	3.1	21134	2.8	
4	4.1	19323	2.5	
5	4.7	68843	9.1	Catechin
6	5.0	15532	2.0	
7	5.6	280525	37.3	Chlorogenic acid
8	6.6	23362	3.1	caffeic acid
9	6.9	31936	4.2	
10	7.1	20047	2.6	
11	7.4	26535	3.5	

D. *S.* Platensis (with MS culture media)

#	Retention time	Peak area	Relative area	Identity
1	2.4	132091	19.6	Gallic acid
2	2.7	21224	3.1	
3	3.6	9743	1.4	
4	4.0	9480	1.4	
5	4.6	58156	8.6	Catechin
6	4.9	15510	2.3	
7	5.5	384109	57.2	Chlorogenic acid
8	6.6	9192	1.3	caffeic acid
9	6.8	12569	1.8	
10	7.1	6324	0.9	
11	7.4	12480	1.8	

E. *S.* Platensis (with PAS culture media)

#	Retention time	Peak area	Relative area	Identity
1	2.4	323822	30.2	Gallic acid
2	2.7	24384	2.2	
3	3.5	5892	0.5	
4	4.0	15486	1.4	
5	4.6	130756	12.2	Catechin
6	4.9	34765	3.2	
7	5.4	444684	41.5	Chlorogenic acid
8	6.6	16464	1.5	caffeic acid
9	6.9	21820	2.0	
10	7.1	13844	1.2	
11	7.4	20704	1.9	
12	11.6	17767	1.6	

F. *S.* Platensis (with ZM culture media)

#	Retention time	Peak area	Relative area	Identity
1	2.4	345006	25.5	Gallic acid
2	2.7	10730	0.8	
3	4.0	17016	1.2	
4	4.3	24628	1.8	
5	4.6	72543	5.3	Catechin
6	4.9	18294	1.3	
7	5.4	689943	51.0	Chlorogenic acid
8	6.6	19559	1.4	caffeic acid
9	6.9	28799	2.1	
10	7.1	18520	1.3	
11	7.4	53408	3.9	
12	10.0	9817	0.7	
13	12.1	43303	3.2	

As shown in the figure and table above, the highest phenolic compounds in all extracts are chlorogenic acid followed by gallic acid.

It is interesting to find the content of the main phenolic compounds detected in the standard S.Platensis extracts and to compare it with those from the new culture media of *S.*Platensis. To this end, the percentage peak area of these phenolic compounds were calculated and summarized in Table 7. Gallic acid came out to be the highest in S. Platensis extracts (PAS) followed by BW, MS, while the lowest value was found to be for ZM, FW and standard *S.*Platensis (with standard culture media). This shows an increase in the % of gallic acid with the new culture media for *S.*Platensis, especially when using PAS, BW, and MS culture media. It was found that Catechin was in the following order: PAS>ZM>BW>MS>FW>standard *S.*Platensis. This also shows an increase in the % of catechin with the new culture media for *S.*Platensis.Chlorogenic acid was found to be inthe following order: ZM>PAS>MS>BW> standard *S.*Platensis and FW,which also shows an increase in the % of chlorogenic acid with the new culture media for *S.*Platensis. Caffeic acid was found to be in the following order: BW>ZM>PAS>MS> standard *S.*Platensis and FW, which also shows an increase in the % of caffeic acid with the new culture media for *S.*Platensis.

Table 7: known peaks with their peak areas and the percentage peak area for ethanolic extracts of standard *S.*Platensis (with standard culture media) and S. Platensis extracts with five new culture media (BW, ZM, PAS, FW, and MS).

Item	Gallic acid	Catechin	Chlorogenic acid	Caffeic acid
Standard *S.*Platensis	57143 %: 5	6496 %: 1.6	187535 %: 8.9	6528 %:8.1
FW	54673 %: 4.7	22491 %:6.1	104130 %: 5	5180 %: 6.3
BW	230112 %: 20.1	688431 %:18.8	280525 %:13.3	23362 %: 29.1
MS	132091 %: 11.5	58156 %:16.1	384109 %:18.3	9192 %: 11.4
PAS	323822 %: 28.3	130756 %: 36.1	444684 %: 21.2	16464 %: 20.5
ZM	345006 %: 5	72543 %: 20	689943 %: 33	19559 %: 24.3
Total peak areas	1142847 %: 100	359285 %: 100	2090926 %: 100	80285 %: 100

4.5 Anti-microbial Activity

Evaluation of antibacterial activity of different spirulina extract was performed by the inhibition zone(IZ)of S.Platensis. Results obtained from the disk diffusion test showed different effect between the S.SPlatensis and produced S.platensis on the growth inhibition of bacteria. Some of produced S.Platensis shows no effect of growth inhibition of bacteria.

For Standard S.Platensis, the inhibition zone were 11mm, 15mm, 8mm, for *Staphylococcus aureus, E.coli and MARSA* respectively. On the other hand, the inhibition zone for the PAS were 10mm, 11mm, 11mmfor *Staphylococcus aureus, E.coli and MARSA* respectively. The inhibition zone for the BW were 11mm, 18mm, 13mmfor *Staphylococcus aureus, E.coli and MARS*A respectively. While there was no effect of bacterial growth revealed for S.platensis (MS,ZM and FW). The positive control of Penicillin for *staphylococcus* was (12mm) and so did for *E.coli*. The inhibition zone for Gentamicin was (15mm), while there were no effect of penicillin on Marsa. G. Usharani et al. (2015) showed that the zone of inhibition of Spirulina platensis extract against

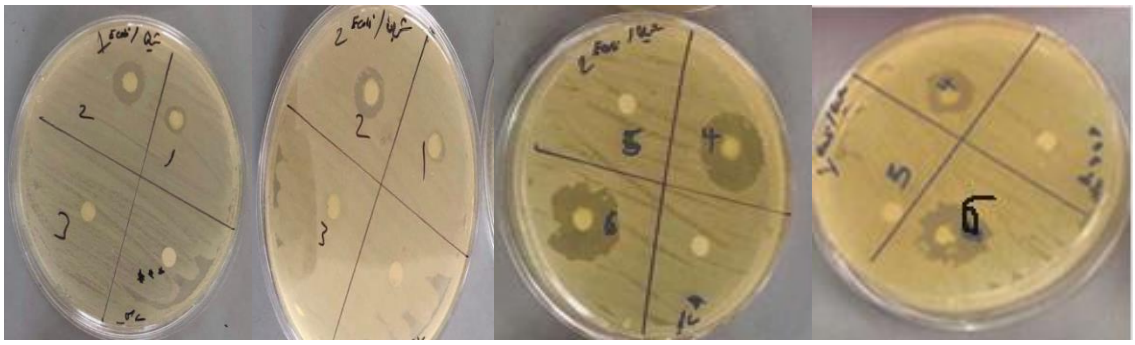
bacteria ranged between 10 mm to 20 mm. In addition, Kaushik and Chauhan reported that extract of spirulina platensis inhibited the growth of *Staphylococcus aureus*, *Escherichia coli.*

Table 8: inhibition zone mm of S.SPlatensis and produced SPlatensis against *Staphylococcus, E.coli and MARSA* with ± standard deviation.

S.SPlatensis and S.Platensis cultured in new media	Inhibiton zone of *S.aureus*	Inhibition zone of *E.coli*	Inhibition zone of *MARSA*
S.Splatensis	11mm±1.5	15mm±2	8mm±1.5
Bw	11mm±1	18mm±2.8	13mm±2.5
PAS	10mm±1	11mm±1	11mm±0.6
FW	00	00	00
ZM	00	00	00
MS	00	00	00

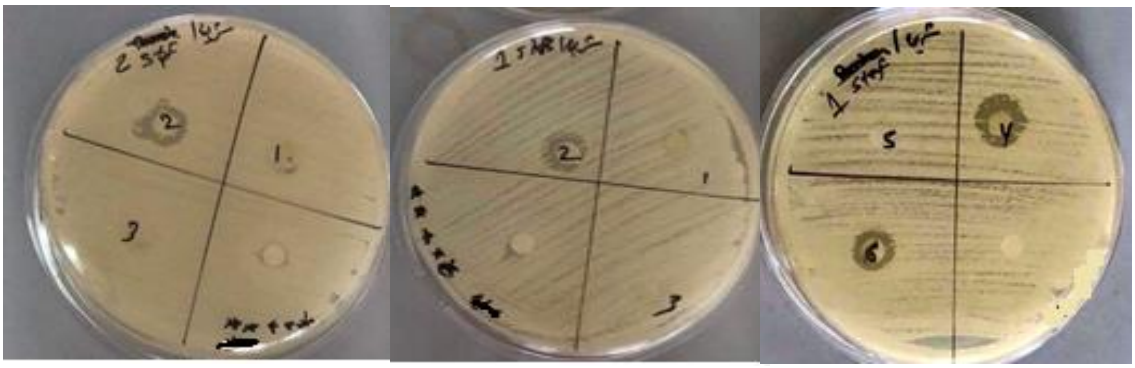
Table 9: dick diffusion assay of two antibiotics against *MARSA B.E.coli .C.S.aureus*

Bacteria	Antbiotics	
	Gentamicin	Pencillin
	Inhibition Zone(mm)	
MARSA		0.0
E.coli	15mm	
S.aureus		12mm



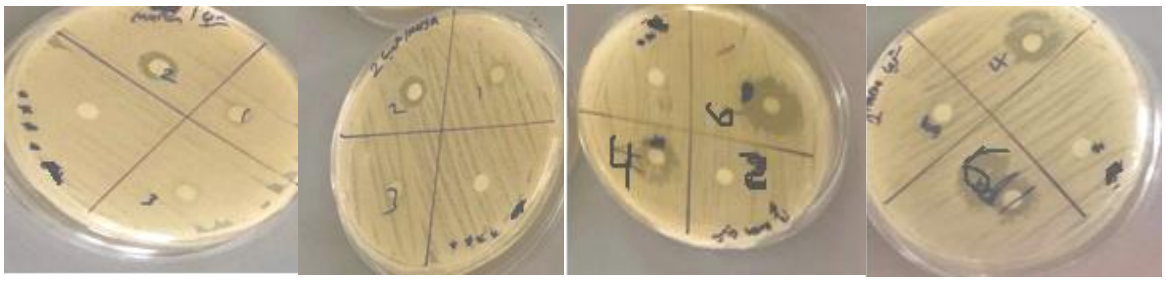
A. B. C. D.

Figure (10 A,B,C,D): Disc dif fusion assay of S.SPlatensis and produced SPlatensis against *E.coli*. While 1. FW, 2. PAS, 3.ZM, 4.BW, 5.MS, 6.S.platensis.



A. B. C.

Figure (11 A,B,C) :Disc diffusion assay of S.SPlatensis and produced SPlatensis against *Staphylococcus*. 1. FW, 2. PAS, 3.ZM, 4.BW, 5.MS, 6.S.platensis



A. B. C. D.

Figure (12 A,B,C,D):Disc diffusion assay of S.SPlatensis and R.SPlatensis against *MARSA*. 1. FW, 2. PAS, 3.ZM, 4.BW, 5.MS, 6.S.platensis.

A. B. C.

Figure (13): disc diffusion assay of two antibiotic against *E.coli, B.MARSA and C.S.aureus*.

4.6 Anticancer activity:

The MCF7 control was observed to contain all living cells 48 hours later upon observation (Figure 16). The anticancer activity of the extracts of s. platensis standard cultivated with MS, ZM, BW, FW, PAS, and standard S. platensiswere tested for the MCF7 breast cancer cell line as well as the HT29 colon cancer cell line. Results showed no anticancer activities of all extracts against both types of cancer cell lines, as mostly adherent living cells of these cancer cell lines (MCF7, and HT29) were observed (figure 16). Figure 16, shows the living cells of MCF7 and HT29 cell lines (Untreated, Figure a and c), and these cells treated withstandard S. platensis(Figure b and d).The S. platensiscultivated with MS, ZM, BW, FW, PAS also did not show anticancer activities against MCF7 and HT29 cell lines (data not shown). The reason is that there is no effect is unknown.

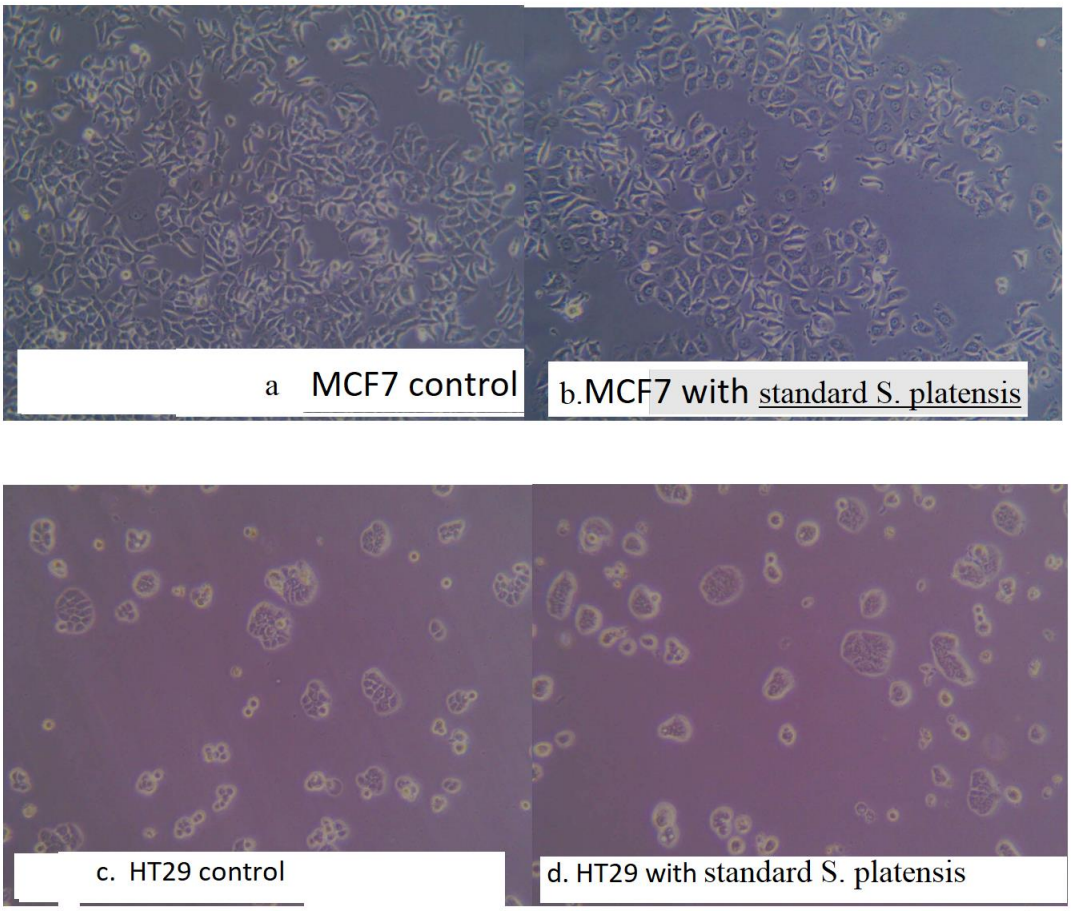


Figure 14: The control samples of the MCF7 and HT29 cell lines 48 hours **a.** Untreated MCF7 cells **b.** MCF7 cultured with 100µL of extract of standard S. platensis**c.** Untreated HT29 cells **d.** HT29 cultured with 100µL of extract of standard S. platensis.

4.6 statistical analysis:

4.6.1 Correlations between the studied parameters:

In this section, the researcher studies the relation between parameters in S.platensis sample and five media (ZM, PAS, BW, MS, and FW).

4.6.1.1The relation between the parameters in S.platensis sample:

According to the results in **table (10)** see appendix , there is a positive relationship between TPC and DPPH(r=1.000**). Sahu et al. (2013) observed a significant correlation between phenolic content and the scavenging radical of DPPH radical in all examined leafy vegetables. This explains the relationship between TPC and DPPH. However, there is no correlation between the TPC and MARSA, E.coli, S.aureus, TFC pairwise.

4.6.6.2 The relation between the parameter in ZM media:

The relations in **table (11)** see appendix, indicates that there is a negative relationship between the TPC and DPPH(r=-1.000**). While, there is no correlation between other parameters (TPC and MARSA, E.coli, S.aureus, TFC).

4.6.6.3The relation between the parameters in PASmedia :

As shown in **table (12)** see appendix**,** there is a negative relationship between the TPC and S.aureus (r=-1.000**) in PAS media. There is no correlation between the other parameters (TPC, MARSA, E.coli, S.aureus and TFC).

4.6.6.4The relation between parameters in MsMedia:

Table (13) see appendix**,** indicates that there is no correlation between the parameters (TPC, MARSA, E.coli, S.aureus, TFC, and DPPH).

4.6.6.5The relation between parameters in BW media

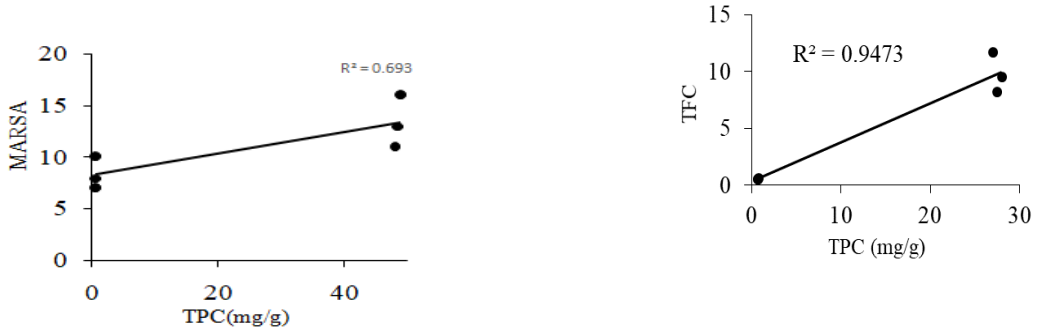
The result showed that, there is a negative relationship between TPC and DPPH(r=1.000**). While there is no correlation observed between the rest of parameter (TPC, MARSA,S.aureus,TFC). As shown in **Table (14)** see appendix.

4.6.6.6The relation between parameter in FW media :

The results in **table (15)** see appendix**,** showed that there is a negative relationship between the TPC and DPPH(r=-1.000**). In addition, there is no correlation between the other parameter (TPC, MARSA, E.coli, S.aureus, and TFC).

4.6.6.7 The relation between S.platensis parameters and ZM parameters:

Table (16) see appendix**,** indicates that there is a positive relationship between the TPC and TFC (r=.986**), while there is no correlation between the rest of parameters (TPC and MARSA, E.coli, S.aureus, TFC).

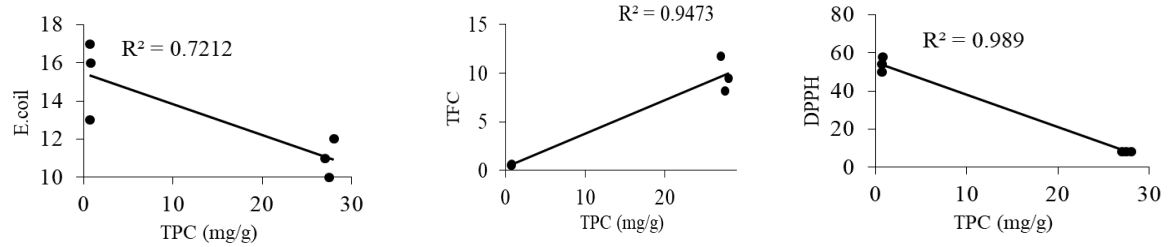


A. **B.**

Figure (15): Relation between TPC and A.MARSA, B.TFC.

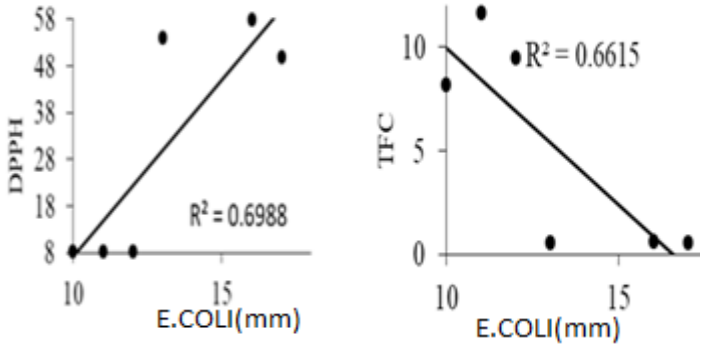
4.6.6.8 The relation between S.platensis parameters and PAS parameters:

Table (17) see appendix**,** indicates that there is a negative relationship between the TPC and E.coli, DPPH (r=-.849*, r=-.994**, respectively). In addition, there is a positive relationship between TPC and TFC(r=.973**). While, E.coli has a negative relationship with TFC (r=-.813*), while it has a positive relation with DPPH (r=.836*).Furthermore, there is a negative relationship between TFC and DPPH(r=-.971**).



A. B. C.

Figure (16) :Relation between TPC and A. E.coli, B.TFC, D. DPPH, respectively.



A. **B.**

Figure(17): Relationbetween E.coli and A.TFC , B.DPPH, respectively.

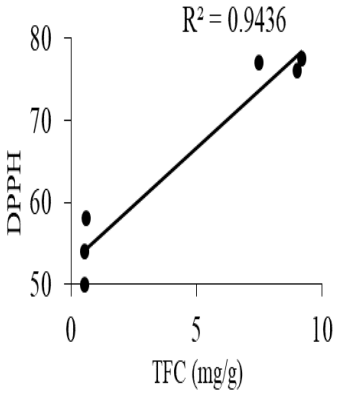


Figure (18):Relation between TPC and DPPH activity.

4.6.6.9 The relation between the S.platensis and MS parameters:

The result between the S.platensis and MS media, showed that there is a negative relationship between the TPC and MARSA, E.coli, S.aureus (r=-.978**,r=-.988**,r=-.988). While there is a positive relationship between TPC and TFC(r=.973**). There is also a negative relationship between TPC and DPPH(r=-.991**). In addition, there is a positive relationship between the TPC and DPPH (r=.979**).

There is a negative relationshipbetween MARSA and E,coil(r=.965**) and between the MARSA and S.aureus,TFC,DPPH (r=-.937**,r=-.969**,r=-,918**,respectively). In addition, there is a

positive relationship between the Ecoli and S.aureus (r=.980**). While there is a negative relationship between E.coli and TFC, DPPH(r=-.979**,r=.975**).

There is also a significant positive relation between the TFC and DPPH. As shown in **table (18)** see appendix**.**

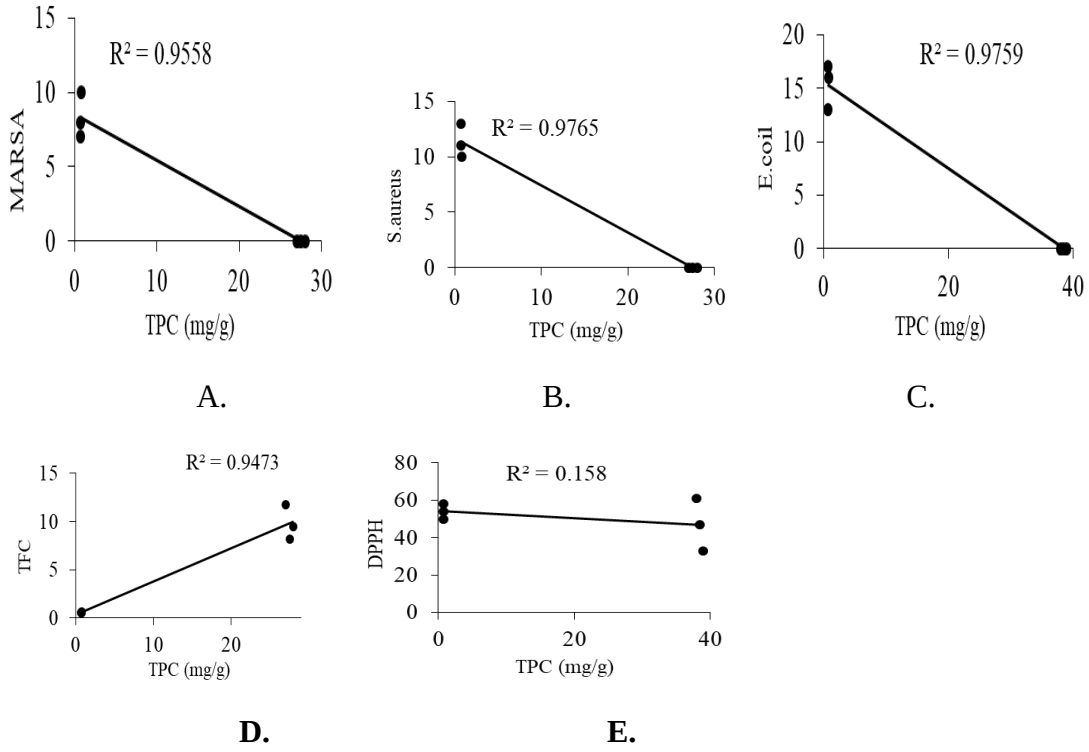


Figure (19): Relation between the TPC and A.MARSA, B.E.coli ,C.S.aureus, D.TFC, E.DPPH, respectively.

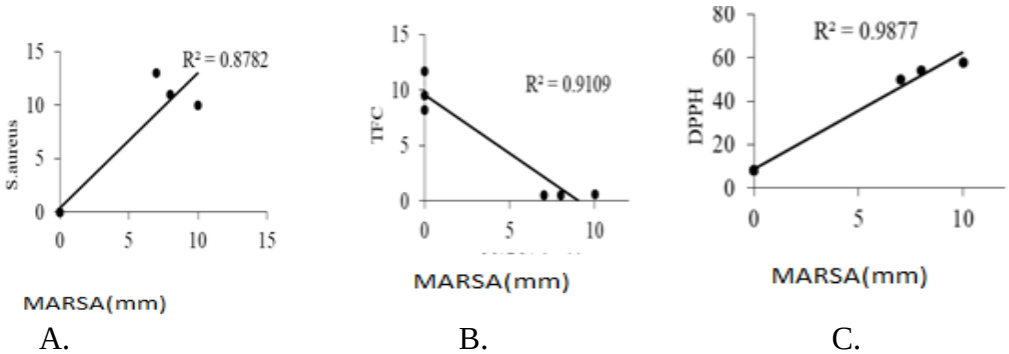
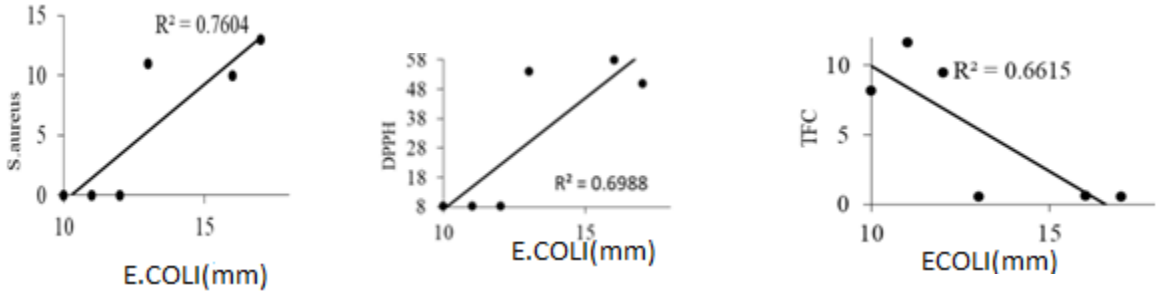
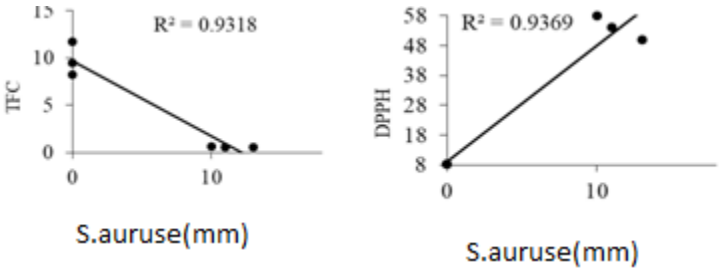


Figure (20): Relation between the Inhibition zone of MARSA and A.S.aureus, B.TFC, C.DPPH.6



A. **B.** **C.**

Figure (21): Relation between the E.coli and S.aureus, TFC, DPPH, respectively.



A. **B.**

Figure (22): Relation between S.aureus and A.TFC, B.DPPH, respectively.

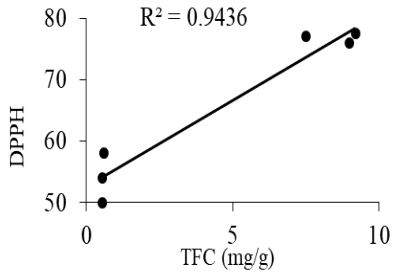
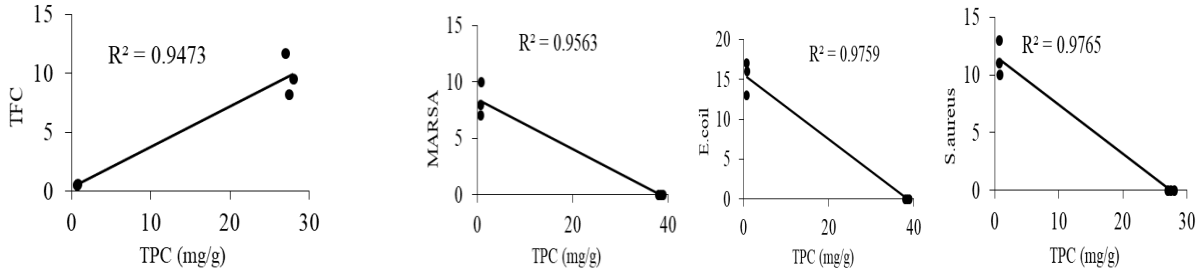


Figure (23): Relation between TFC and DPPH.

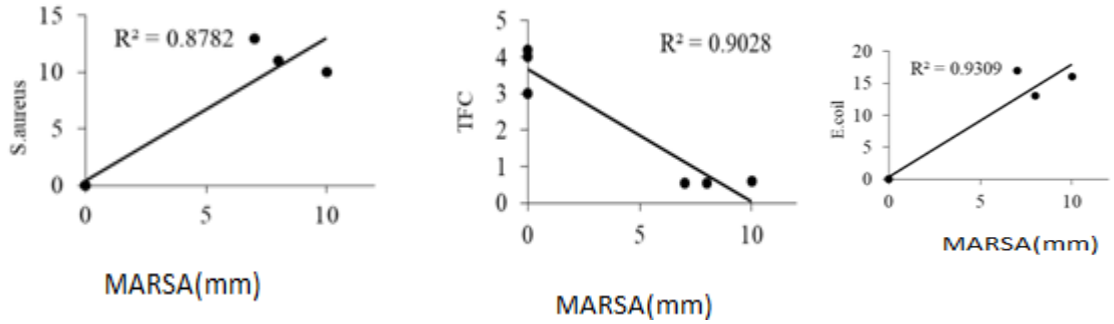
4.4.10: The relation between the S.platensis parameters and BW parameters:

Table(19) see appendix, indicates that there is a negative relationship between the TPC and MARSA, Ecoli, S.aureus, DPPH(r=-.978**,r=- .988**,r=-.988**,respectively). In addition, there is a positive relationship between TPC and TFC(r=.974**). On the other hand, there is a negative relationship between MARSA and Ecoil(r=.965**) and between the Marsa and E.coli,S.aureus,TFC(r=-.965**, r=-.937**, r=-.950**). A positive relationshipappearswith Ecoli and S.aureus (r=.986**),whilea negative relationship appears between Ecoli and TFC(r=-.962**) and between the S.aureus and TFC (r=-.963**).



A. **B.** **C.** **D.**

Figure (24) : Relation between the TPC and A. TFC, B.MARSA, C.E.coli ,D.S.aureus.



A. B. C.

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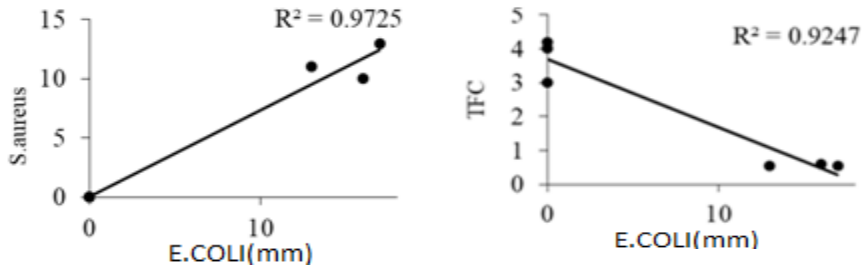
Figure (25) : Relation between the MARSA and A. S.aureus, B. TFC, C.E.coli, respectively.



A. B.

Figure (26): Relatione between E.coli andA.S.aureus, B.TFC, respectively.

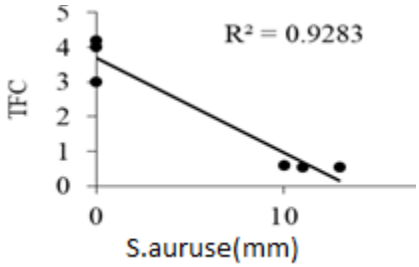
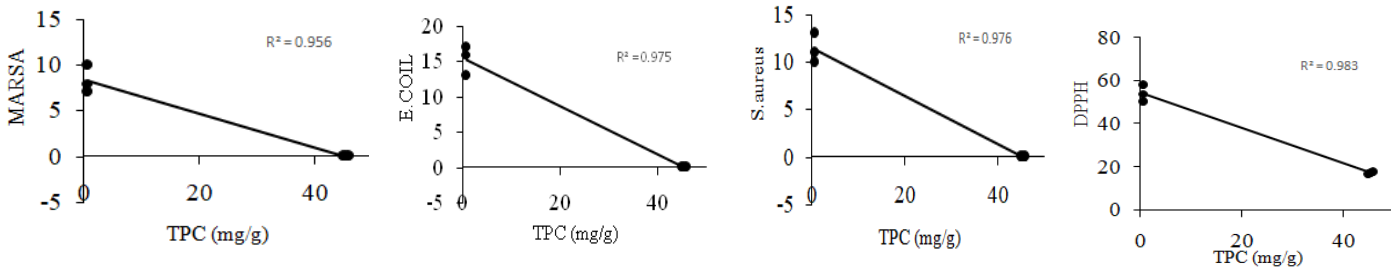


Figure (27): Relation between S.aureus and TFC.

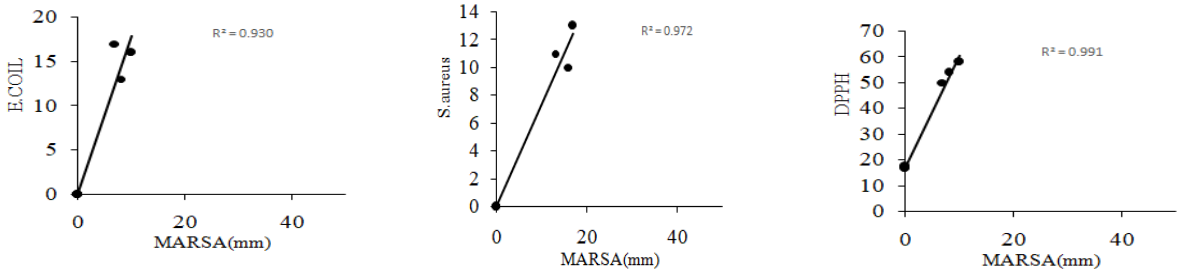
4.6.6.12 The relation between the S.platensis and FW parameters:

In **table (20)** see appendix**,** results showed that there is a negative relationship between the TPC and MARSA, Ecoli,S.aureus,DPPH,(r=-.978**,r=-968**,-.975**,-.992**).There is also a negative relation ship between MARSA and Ecoli (r=.965**). inaddition, there is a positive relationship between MARSA and S.aureus, DPPH (r=.937**,r=-.996**),and a positive relationship with DPPH(r=-.962**),and between the E.coil and S.auruse (r=.986**),while there is a negative relation with DPPH (r=-.976**).There is also a positive relationshipbetweenS.aureus and DPPH(r=.962**).



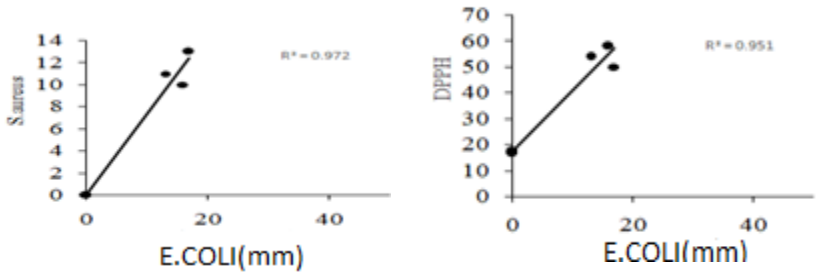
A. B. C. D.

Figure (28): relation between TPC with A.MARSA, B.E.coli, C.S.aureus, D. DPPH, respectively.



A. **B.** **C.**

Figure (29): Relation between the MARSA and A, E.coli B.S.aurues, C.DPPH, respectively.



A. B.

Figure (30): Relation between E.coli and A.S.aureus, B.DPPH, respectively.

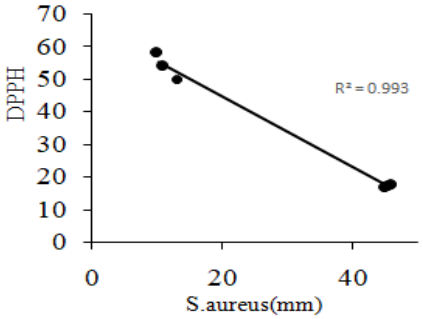


Figure (31): Relation between S.auerus and DPPH.

Chapter five: Conclusion

Microalgae in general are excellent sources for food and supplements for humans and animals. They are environmental filters for organic maters and pollutants. Spirulina platensis in particular have high growth rate and very high nutritional value.S.platensisis rich in phenolic compounds, flavonoids, essential amino acids, and mineral, which can help in curing and preventing diseases. S.platensisis also a very good protein sources.

The current study showed that the Spirulina platensis that was grown in different cheap local resources are rich in antioxidant activity, with a substantial amount of total phenols, and flavonoids. Also, it can inhibit the growth of bacteria, as compared to the standard spirulina.

It has been concluded that the spirulina can grow in low-cost local resources, and that these resources have no negative impacts on the quality of the algae.Our results had showed that the impact of low-cost local resources is in fact positive as compared to Spirulina platensis that was grown on the expensive standard media. Local cheap resourceshad increased the nutritional value and productivity of produced algae.

Also**,** the results showed that the extract contained major phenolic compounds like chlorogenic acid,gallic acid, catechin and caffeic acid, which are valuable sources of antimicrobials. Phenolic compounds act by inhibiting microbial cellwall outgrowth, perturbing, degrading, impairing biogenesis, suppressing deoxyribonucleic acid replication and transcription, impairing ATP production, suppressing bacterial toxins, and generating reactive oxygen species.

Recommendations:

This work was mainly focused on the chemical characterization of spirulina platensis, We suggest focusing on the nutritional value of spirulina platensis include crude protein, carbohydrates, vitamins , lipids, amino acids, minerals. Also, determine the level of phycobiliproteins.

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AppendiX:

Table 10: Correlation between the parameters in S.platensis sample.

Parameters	TPC	MARSA	E.COLI	S.aureus	TFC	DPPH
TPC	r=1					

MARSA	r=.982	1				
	p=.121					
E.coli	r= -.240	r=-.052	1			
	p=.846	P=.967				
S.aureus	r=-.982	r=-.929	r=.419	1		
	P=.121	P=.242	P=.725			
TFC	r=.928	r=.981	r=.140	r=-.840	1	
	P=.244	P=.123	P=.911	P=.365		
DPPH	r=1.000**	r=.982	r=-.240	r=-.982	r=.928	1
	P=.000	P=.121	P=.846	P=.121	P=.244	

Table 11: Correlation between the parameters in ZM media.

parameters	TPC	MARSA	E.COLI	S.aureus	TFC	DPPH
TPC	1					
MARSA	r=.993	1				
	P=.073					
E.coli	r=-.866	r=-.918	1			
	P=.333	P=.260				
S.aureus	r=.500	r=-.596	r=-.866	1		
	P=.667	P=.593	P=.333			
TFC	r=-.924	r=-.875	r=.610	r=-.132	1	
	P=.249	P=.322	P=.582	P=.916		
DPPH	r=-1.000**	r=-.993	r=.866	r=-.500	r=.924	1
	P=.000	P=.073	P=.333	P=.667	P=.249	

Table 12: Correlation between the parameters in PAS media.

parameters	TPC	MARSA	E.COLI	S.aureus	TFC	DPPH
TPC	r=1					
MARSA	r=.000	1				
	P=1.000					
E.coli	r=.500	r=.866	1			
	P=.667	P=.333				
S.aureus	r=-1.000**	r=.000	r=-.500	1		
	P=.000	P=1.000	P=.667			
TFC	r=-.622	r=.783	r=.367	r=.622	1	
	P=.573	P=.427	P=.760	P=.573		
DPPH	-	-	-			1

Table 13: Correlation between the parameters in MS media.

parameters	TPC	TFC	DPPH
TPC	1		

TFC	r=-.108 P=.931	1	
DPPH	r=-.990 P=.091	r=-.036 P=.977	1

Table 14: Correlation between the parameters in BW media

parameters	TPC	TFC	DPPH
TPC	1		
TFC	r=.156 P=.901	1	
DPPH	r=-1.000** P=.000	r=-.158 P=.901	1

Table 15: Correlation between the parameters in FW media

parameters	TPC	TFC	DPPH
TPC	1		
TFC	r=.345 P=.776	1	
DPPH	r=1.000** P=.000	r=.345 P=.776	1

Table 16: Correlation between the parameters inS.platensis sample and ZM parameter.

Parameters	TPC	MARSA	E.COLI	S.aureus	TFC	DPPH
TPC	r=1					
MARSA	r=.833* p=.039	1				
E.COLI	r= .583 p=.225	r=.191 P=.761	1			
S.aureus	r=-.154 P=.771	r=-.198 P=.707	r=-.235 P=.654	1		
TFC	r=.986** P=.000	r=.756 P=.082	r=.140 P=.645	r=-.174 P=.742	1	
DPPH	r=-.319 P=.537	r=-.650 P=.162	r=.328 P=.525	r=-.349 P=.498	r=-.170 P=.748	1

Table 17: Correlation between the parameters inS.platensis sample and PAS parameter.

Parameters	TPC	MARSA	E.COLI	S.aureus	TFC	DPPH
TPC	r=1					
MARSA	r=.779	1				

	p=.068					
E.coli	r= -.849*	r=-.633	1			
	p=.032	P=.177				
S.aureus	r=-.546	r=-.802	r=.543	1		
	P=.263	P=.055	P=.266			
TFC	r=.973**	r=.799	r=-.813*	r=-.462	1	
	P=.001	P=.056	P=.049	P=.365		
DPPH	r=-.994**	r=-.716	r=.836*	r=.462	r=-.971**	1
	P=.000	P=.110	P=.038	P=.356	P=.001	

Table 18: Correlation between the parameters in S.platensis sample and MS parameter.

parameters	TPC	MARSA	E.COLI	S.aureus	TFC	DPPH
TPC	r=1					
MARSA	r=-.978**	1				
	p=.001					
E.coli	r= -.988**	r=.965**	1			
	p=.000	P=.002				
S.aureus	r=-.988**	r=-.937**	r=.986**	1		
	P=.000	P=.006	P=.000			
TFC	r=.991**	r=-.969**	r=-.979**	r=.980**	1	
	P=.000	P=.001	P=.001	P=.001		
DPPH	r=.979**	r=-.918**	r=-.975**	r=-.998*	r=.971**	1
	P=.001	P=.010	P=.001	P=.000	P=.001	

Table 19: Correlation between the parameters inS.platensis sample and BW parameter

Parameters	TPC	MARSA	E.COLI	S.aureus	TFC	DPPH
TPC	r=1					
MARSA	r=-.978**	1				
	p=.001					
E.coli	r= -.988**	r=-.965**	1			
	p=.000	P=.002				
S.aureus	r=-.988**	r=-.937**	r=.986**	1		
	P=.000	P=.006	P=.000			
TFC	r=.974**	r=-950**	r=-962**	r=-.963*	1	
	P=.001	P=.004	P=.002	P=.002		
DPPH	r=-.398	r=.428	r=.370	r=.341	r=-.403	1
	P=.435	P=.398	P=.470	P=.508	P=.428	

Table 20: Correlation between the parameters inS.platensis sample and FW parameter.

parameters	TPC	MARSA	E.COLI	S.aureus	TFC	DPPH
TPC	r=1					
MARSA	r=-.978** p=.001	1				
E.coli	r= -.988** p=.000	r=-.965** P=.002	1			
S.aureus	r=-.988** P=.00	r=.937** P=.006	r=.986** P=.000	1		
TFC	r=.730 P=.100	r=-.707 P=.116	r=-.718 P=.108	r=-.721 P=.106	1	
DPPH	r=-.992** P=.000	r=.996** P=.000	r=-.976** P=.001	r=.962** P=.002	r=-.716 P=.110	1

جودة سبيرولينا بلاتنسيس المنتجة من مصادر محلية مختلفة

اسم الطالب: شيرين زياد محمد جعفر

المشرف: دكتور فؤاد الريماوي

المشرف الثاني: دكتور معتز القطب

الملخص:

سبيرولينا بلاتنسيس(فيكوسيانين) هي نوع من أنواع الطحالب الخضراء المزرقة التي تعرف أيضا بأسم البكتيريا الزرقاء، وتستخدم بشكل فعال كمكمل غذائي.

تمتلك السبيرولينا خصائص مضادة للبكتيريا ومضادات أكسدة، و لها العديد من الفوائد الصحية والطبية ضد العديد من الأمراض، مثلا السرطان، إرتفاع ضغط الدم، فرط كوليسترول الدم، السكري، والأنيما ، وتتميز أيضا بالكميات العالية من البروتين التي تحتويها هذه الطحالب،

سيحقق إدخال هذا النوع من الطحالب الى عجلة الإقتصاد الفلسطيني فائدة كبير لمجالات الزراعة والصناعة في فلسطين، غير أن نجاح إدخال هذا الطحلب الغني بكافة العناصر الغذائية والبروتين لن ينجح إلا بخفض تكلفة زراعته، ولن يتم خفض هذه التكلفة إلا إذا نجحنا في تربية وإكثار هذا الطحلب بإستخدام مصادر مخلفات محلية متوفرة ورخيصة الثمن، تكمن أهمية هذه الدراسة بتقديمها تحليل لجودة الطحالب المنتجة من مصادر محلية وتقارنها بجودة الطحالب المنتجة بإستخدام الوسط المثالي المعروف عالميا، تم في هذا الدراسة مقارنة جودة المنتج من خمس بدائل محلية ومقارنته مع المنتج المزروع بالطرق العالمية . تم دراسة النشاط المضاد للأكسدة وأيضا تم الكشف عن إجمالي محتوى الفينول ومحتوى الفلافونويد الكلي من المستخلصات الخمسة والسبيرولينا بلاتينسس الاصلية. حيث أظهرت النتائج أن المستخلص الإيثانولي للمستخلصات الخمسة بالإضافة للسبيرولينا بلاتينسس الأصليه هي كالتالي:

42±2 mg gallic/g, 33±0.9 mg gallic/g, 32±1 mg gallic/g, 40±1 mg gallic/g, 22±0.7 mg gallic/g and 5±0.9 mg gallic/g, for ZM, MS, FW, BW, PAS, and standard S.platensis.

,ومحتوى الفلافونيد كالتالي:

3.3±0.6mg quercetin/g, 2±0.4mg quercetin /g, 1±0.8mg quercetin /g, 2.6±0.5mg quercetin /g, 1.4±1mg quercetin /g, and 1.2 ±0.2mg quercetin /g for ZM, PAS, BW, MS, FW, and standard S.platensis.

,وكانت نتائج دراسة النشاط المضاد للأكسدة باستخدام فحص (DPPH) كالتالي: ,77%±0.6 47%±0.9, ±, 46%±0.3, 17.1%±0.3, 8.3%±0.5, and54%±0.6, for MS, ZM,BW, FW, PAS, and Standard S.platensis)

ولتحديد إمكانية استخدام المستخلصات الخمسة في الحد من نمو البكتيريا تم إجراء إختبار الفعالية ضد ثلاثة أنواع من البكتيريا وكانت النتائج كالتالي :(E.COLI, MARSA) اثنتان موجبة الجرام(S.aureus) , واحدة سالبة الجرام

11 mm, 15 mm, 8mm, for Standard S.platensis, 11mm, 18mm, 13mm for BW, 10mm, 11mm,11mm, for PAS against Staphylococcus, E.coli, and Marsa, على التوالي. حيث لا يوجد أي تأثير للحد من نمو البكتيريا في هذه المستخلصات (ZM, FW, MS.).

أكدت النتائج أن سبيرولينا بلاتينسس تسطيع العيش بأوساط مختلفة وتحافظ وتزيد من محتوى الفينول والفلافونيد والنشاط المضاد للأكسدة والبكتيريا وبذلك يمكن إدخالها إلى فلسطين وتوفير مصدر جديد يدعم عجلة الإنتاج في السوق الفلسطيني وتوفير فرص عمل جديدة.