JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

EXTRACTION, CHARACTERIZATION OF PHYCOCYANINS AND PREPARATION OF LATHER FROM SPIRULINA

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Abstract:

In modern days, most of the research studies are focusing on naturalized commercial antibacterial soap products from the plant source and animal source, to avoid the tissue damaging many other skin diseases. spirulina is a cyanobacterium (blue green algae) ,it has the anti-inflammatory properties, anticancerous activities and to the prevent the tissue damaging hence it can be used in the lather soap production .spirulina was initially analyzed for the phytochemical constitutes, in this study we estimated the valuable properties of secondary metabolites such as alkaloids, terpenoids, flavonoids, coumarins, quinones, amino acids, tannins, steroids, saponins, which have been involved in pharmaceutical industries and food industries. The phycocyanin were extracted from the spirulina powder by the enzymatic method with the help of the lysozyme enzyme for lysis the cell wall of spirulina, while cell lysis the natural components are exposed. After the extraction of phycocyanin, it was treated with another step of partial purification of phycocyanin with the method of ammonium sulphate precipitation. In this process the phycocyanin were binds to the ammonium sulphate precipitation on centrifugation. The purified phycocyanin was characterized by the UV Visible spectroscopy for their chemical compounds and structure of molecules at the range from 0 to 800nm and it shows the maximum peak value at 652 nm of absorbance. FT-IR is the main source of technical analysis of chemical structure and formation bonded molecules and it has been with the beam of spectrum, it plays important role to identify the molecules by IR and KBr spectrum analysis. The purified compounds were proceeded to the production of lather by the process of saponification with different oil sample such as Coconut oil, Olive oil, Palm Oil and Peanut oil, the soap was produced and checked for the Foam ability and Moisture contents. This results in the optimum range of the foam and moisture in Coconut oil soap sample and Peanut oil soap sample. It can be used as added product and it can be commercialized by this analysis.

Keywords: Spirulina, Phytochemical analysis, Extraction and Partial Purification, Lather Soap Production.

1. Introduction:

Spirulina is the type of the microalgae ,it also refers to the dried biomass of arthospira platensis ,an oxygenic photosynthesis bacterium found worldwide in fresh and marine waters[1].They falls under the family pharmidiaceae it aggregates as the trichomes with the helicoid structure[3].They are more commonly called as the blue green algae also it inhibits the rich protein content with the composition of Vitamin B12, Provitamin A(β-carotene) and mineral ion [2].It has many remarkable involvement in the pharmaceutical industry and food industry on the past 50 years .Spirulina are naturally photosynthetic organism and are prokaryotic .In addition to the protein content they composed of the phenolic acids such as tocophenols and γ-linolenic acids .Spirulina is made up rigid peptidoglycan layers hence they are non heterocystous and non-nitrogen fixers. Habitually spirulina (microalgae) consume the large amount of CO_2 and that leads to the conversion of the solar energy into the chemical energy.

Spirulina has different types of the phycobilin protein with phytochemical activity, It has very promising role in the medical industry for the treatment of TB, cancer and inflammatory reactions in the immune system and also in food industry it is being used as the supplements on the food products due to their enormous nutrient content[4].It is a water soluble protein and the phytochemicals are classified based on the spectral properties of the pigmented leaves that influences its photosynthetic nature. They are classified into phycoerythrin(red), Phycocyanin(blue) and Allo- Phycocyanin(cyano) pigments [6]. They can be screened by the qualitatively and quantitatively considering the parameters such as temperature, pH, nutrient content, composition of the metal ions and other elements. As a result, Bioactive compounds can be identified [11].

The bioactive mixture from the phycocyanin is mainly used in the application of the treatment of healthy complications as they are abundant in their antioxidant, anticancer and anti-viral characteristics. Phycocyanin protein pigment can be treated to eliminate the hypocholesterolemia and hypoglycemic potentially by the sulfated polysaccharides and *γ*-linolenic acid. These bioactive mixtures are the secondary metabolites and are obtained only from the plant materials . These are attributing their activity in the manufacturing of the drugs, dyes, pesticides and as food additives [12]. It is one of the important properties of the phycobilin protein and it is thus studied in this report to analysis the role of the phycocyanin in the manufacturing of the Lather in soap. Lather gives foaming consistency to maintain the nature of the soap, shampoos and also in some surfactants. Soap is one of the important commodities that is used frequently and it is usually made from the animal's fat and wood ash and the process of manufacturing soap is known as saponification. It is a mixture of the oil and certain base like sodium hydroxide and potassium hydroxide. The basic soap may be synthetic or supplemented with natural bioactive compounds. To overcome the harmful effects of the synthetic soap they are manufactured from the medicinal products thus it paved the path for the production of the soap from the phytochemical pigment like phycocyanin. Alkaloids are ready to dissolve in solvent since they are the cyclic compounds and it is used in skin therapeutics. The terpenoids are the lipids that has five carbon atoms, function as are great detoxifiers also maintaining sebum range in normal range to gain healthy skin. The steroids are the natural key hormone that guard skin with its anti-inflammatory properties on the skin. Tannins are the polyphenol compound that is water soluble complement the skin by removing excess oil. The saponins are composed of sugar and are water soluble. It has defense mechanism against the pathogenic organism by their antioxidant properties. It is the widely used cleansing agent in removing the sludge dirt from the skin. Flavonoids are antioxidant phenols with 15 carbon compounds that can neutralize the free radicals and protecting the skin from the UV radiations due to their antioxidant properties. Coumarins are the secondary products that are used to enhance the fragrance of the soap. Quinones are mainly used as the anti-inflammatory agent. Amino acids are the compounds which acts as a natural skin moisturizer because they are rich in omega 3 fatty acids. The carbohydrates are the sugar it can provide the energetic compounds to the skin's epidermis layer will react with the glucose and it will efficient in providing the enough amount of the energy to maintain the skin natural texture [13][14][15].

All these compounds in the Phycocyanin have the excellent performance in the skin by controlling the oil secretion and maintaining the pH of the skin thus it helps to protect the texture of the skin. It reveals that the photosynthetic pigment will provide the skin with cleansing agent so it will maintain the skin with natural glow

and smoothness. To establish the role of the lather from the phycocyanin pigment has been lead us to form the perfect skin moisturizer, it symbolizes the phytochemicals also retain the outstanding performance as the value-added products. With the help of the saponification process certain oil such as coconut oil and crude oil were added for the manufacture of soap [2]. In this study, the phycocyanin are extracted and purified by ammonium sulphate and they subjected to the phytochemical analysis test. Finally, they are characterized under the UV Visible spectroscopy and FT-IR Spectroscopy and it was used in the manufacture of Lather soap.

2.Materials and Methods:

2.1 Sample collection:

The powdery form of the Spirulina (Fig :1) was obtained commercially and placed under the room temperature for the phytochemical analysis, purification and preparation of Lather.

Figure :1 Spirulina powder

2.2. Phytochemical Analysis:

The phytochemical analysis is performed to analyze the nature of the photosynthetic pigment and the activity of the secondary metabolites in the photosynthetic pigment can be experimentally noted for the further use of the Phycocyanin in the preparation of the Lather .It can be analyzed by both qualitative method and quantitative method .

2.2.1 Qualitative Analysis and Quantitative Analysis:

It is to examine source of the bioactive constitute with the visible colour change in the reaction by the phytochemical compounds with the intake of basic aqueous solution and the amount of the bioactive substance can be determined from this tests .It include the test of alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, and phenols, coumarins, quinones and glycosides .The collected sample of powdery form of Spirulina was taken in the separate test tube to perform the phytochemical analysis[24][25].

a) Analysis of Alkaloids:

With 0.5 g of Spirulina extract, the 1% of the 5ml of the HCl was mixed and placed in the water bath. After few minutes Dragondroff's reagent was added to 1ml of the extract filtrate and observed for the results.

b) Analysis of Terpenoids

Around 5 ml of extract was mixed with 2ml of the chloroform and 3ml of Sulphuric acid and observed for the results.

c) Analysis of Steroids

The Spirulina extract of 1ml was saturated in the 10ml of Chloroform and added with few drops of concentrated Sulphuric acid and results were analyzed.

d) Analysis of Tannins

To the Spirulina extract equal amount of 1% of the gelatin solution was added along with 10% of the NaCl.

e) Analysis of Saponins

The Spirulina extract of 3ml was added to the 10ml of the distilled water and stirred vigorously for 5 minutes and set in rest for next 30 Minutes.

f) Analysis of Flavonoids

Spirulina extract of 2 ml was added to the 1 ml of 2N of sodium hydroxide solution.

g) Analysis of Coumarins

Spirulina extract of 2ml was added to the few drops of 10% of the sodium hydroxide.

h) Analysis of Quinones

To the 1ml of the Spirulina extract, the 1ml of the concentrated sulphuric acid was added and the reaction was observed.

i) Analysis of Amino Acid

The 1ml of the Spirulina extract was mixed with 0.5 ml of 40% sodium hydroxide in addition with this 1% of the copper sulphate was mixed.

j) Analysis of Carbohydrates

By the addition of the 1ml of the Benedict ‘s reagent in 1ml of the Spirulina extract and kept in the water bath for 2 minutes [24].

2.3 Extraction of Phycocyanin:

The enzymatic method for the extraction of the Phycocyanin from the powdery form of the Spirulina has been processed by two methods as follows,

1.Lysozyme extraction method –The sample of 200ml was treated with the 0.1mM of the sodium phosphate buffer at neutral pH of 7 and mixed with the 10mM of sodium EDTA and the 100 µg of the lysozyme enzyme. This mixture was incubated for 24hrs at 30°C.

2. Fractionation centrifugation method- After the incubation of the lysozyme extract, they were centrifuged at 4°C for 20 minutes at 8000 rpm. From the centrifuged sample, the supernatant was extracted as photosynthetic containing particles and collected in separate tube and the pellet was discarded [24][25].

2.4 Purification of Phycocyanin by Ammoinum Sulphate Precipitation Method:

The purification of the extracted sample was done in two steps process ,to induce the pure form of the extracted sample.

- The Precipitation of the Solid Ammonium Sulfate was performed by the addition of the crude extract of 50% phycocyanin sample and stirred gently and shaken vigorously for the saturation of the extract for 30 minutes .
- The mixture was refrigerated at the 4°C for the overnight. After incubation, that was centrifuged at 10000 rpm for 30 minutes in cooling centrifugation at 4°C.
- Precipitated phycocyanin wasollected by discarding the supernatant.
- The pellet extract was then fractionated into the 50% of 0.025M sodium phosphate buffer and incubated in the dark brown bottle at 4°C.

2.5 Partial Purification of Phycocyanin by Dialysis:

The dialysis is carried out to eliminate the presence of the other solid materials present in the extracted compound. The precipitated pellet extract was dialyzed further by the same phosphate buffer and fractioned by the same concentration and dialyzed for 24 hrs [25].

2.6 Analysis of Phycocyanin by UV Visible Spectroscopy:

The UV visible spectroscopy was performed to analyze the quality and identify the presence of the phenolic compounds in the pure form. It also used to analyze the quantitative amount of the aromatic compound. It plays a vital role in the photosynthetic fluorescent pigment which can be detected in different UV ranges whereas phenol is optimum for the 280nm range of spectrum, the flavonoids are visible under optimum range of 320nm and the overall alkaloids are optimum to the UV ranges from 520nm. The purified form of the sample extract was performed under these different spectral properties to analyze the presence of the pigmented compounds [2][11][16][22].

2.7 Analysis of Phycocyanin By Fourier Transfer Infrared Spectroscopy (FT-IR)

The Fourier Transform Infrared Spectrophotometry is to analyze the chemical bonding of the certain functional group and molecular arrangement of the specific compounds. In this study, the FTIR was used to analyze the functional groups of the Phycocyanin pigment presented in the extracted compounds.

In first step, the precipitated extract of 100mg KBr pellet was mixed with 10mg of the dried powder of the Spirulina, this mixture has been made to translucent sample disc to check the homogenicity of the sample. They are detected under the spectral range of 700-4000 cm-1 of the resolution with 1cm-1[2][11][16][22].

2.8 Preparation of Lather from Spirulina:

2.8.1 Materials

The process of the lather preparation was performed by the saponification process.The lather soap is prepared by the addition of the palm oil ,peanut oil ,olive oil and coconut oil were mixed with the caustic soda and water to form the standard solution of the lye solution with the each sample of the oil.The concentration of the oil and water was tabulated below (Table :1).

Table :1 Concentration of 20% NaOH solution in oil samples

Oil samples	Concentration of NaOH(g)	Amount of water for 20% of the NaOH solution
Coconut oil	20.6	86.8
Olive oil	20.2	86.6
Palm oil	18.2	94.6
Peanut oil	17.2	91.6

2.8.2 Methods:

- The pure extract was taken in separate beaker and the 250ml of water was gently added to the 200ml of each sample of oil with 20% of the caustic soda (sodium hydroxide 20%) was stirred well.
- The mixture was subjected to heat treatment by the Bunsen burner and blend well for the whitish paste formation with the help of glass rod and it has to be cooldown to room temperature in the 1:3 ratio of the caustic soda and distilled water.
- The different sample of the oil were fused with that mixture and it has been kept under undisturbed condition for 30 minutes in ambient temperature and filtered by the filter funnel and transported to the filter paper.
- After few times of filtration, it can be molded to a desired shape for the soapy texture with the help of the filter paper and knife as the solid rectangular soap bars. It has been stored in the non-contaminated environment for the prefect formation of the soap for 30 days. [17].

2.8.2 Determination of Foamability:

The soap of 2.0g was added with 100 cm^3 of distilled water and shaken vigorously in the shaker for 2 minutes.

2.8.3 Determination of Moisture content:

The dried sample of 10g of the soap was weighted according to AOAC (2000). It was allowed to cool and reweighted.

3. Result :

3.1. Phytochemical Analysis:

The phytochemical analysis of spirulina was performed by standard protocols. Extraction of aqueous extraction of spirulina was examined for the presence of secondary metabolites such as alkaloids, terpenoids, steroids, flavonoids, coumarins, amino acids and saponins. It shows that the secondary metabolites of Spirulina have the beneficial effects of alkaloids on skin protection and the amino acids has property to prevent ageing and tissue breakdown, tannins act as precursor in resistance against bacterial and fungal infections, steroids have the property to treat the skin rashes and itchiness, then basically terpenoids, flavonoids and coumarins are the anti-inflammatory agent and saponins act as a foaming agent [4][6][17].

In this analysis, the positive results were observed by alkaloids, terpenoids, Flavonoids, coumarins, Amino acid, steroids and saponins expect quinones and Tannins from the aqueous extraction of Spirulina.
The results are given below (table 2)

TABLE :2 Phytochemical analysis

S. No.	**PHYTO CONSTITUENTS**	**QUALITATIVE ANALYSIS**
1.	Alkaloids	+
2.	Terpenoids	+
3.	Flavonoids	+
4.	Coumarins	+
5.	Quinones	-
6.	Amino acids	+
7.	Taninns	-
8.	Steroids	+

9.	Saponins	+

3.2. UV-Visible Spectroscopy:

The UV-Visible spectroscopic studied were performed with the extracted phycocyanin. It showed the maximum peak at 652nm of absorbance[2][11][16][22]. The peak result was given below (Fig:2).

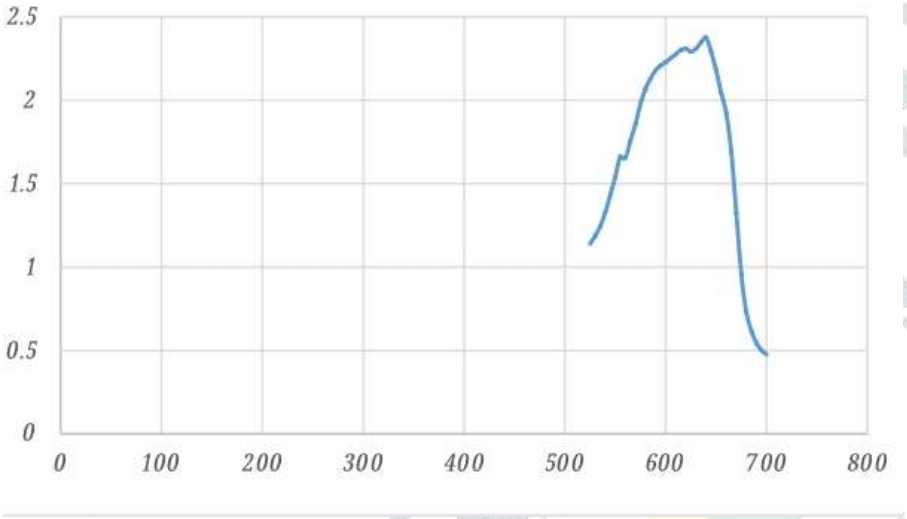

Figure 2 UV Visible spectroscopy of Phycocyanin

3.3. FT-IR Spectroscopy:

The FT-IR analysis reveal that the presence of functional group of phycocyanin with the spectrum characterization. The absorbance bands which were observed at 1643.98, 2350.17, 2984.89, 3270.80, 3296.53, 3330.84, and 3362.29 corresponds to c=c in alkene groups, C-H in aldehydic groups, C-H stretch, O-H in alcohol and N-H stretch [2][11][16][22].
The results are given below (Fig:3).

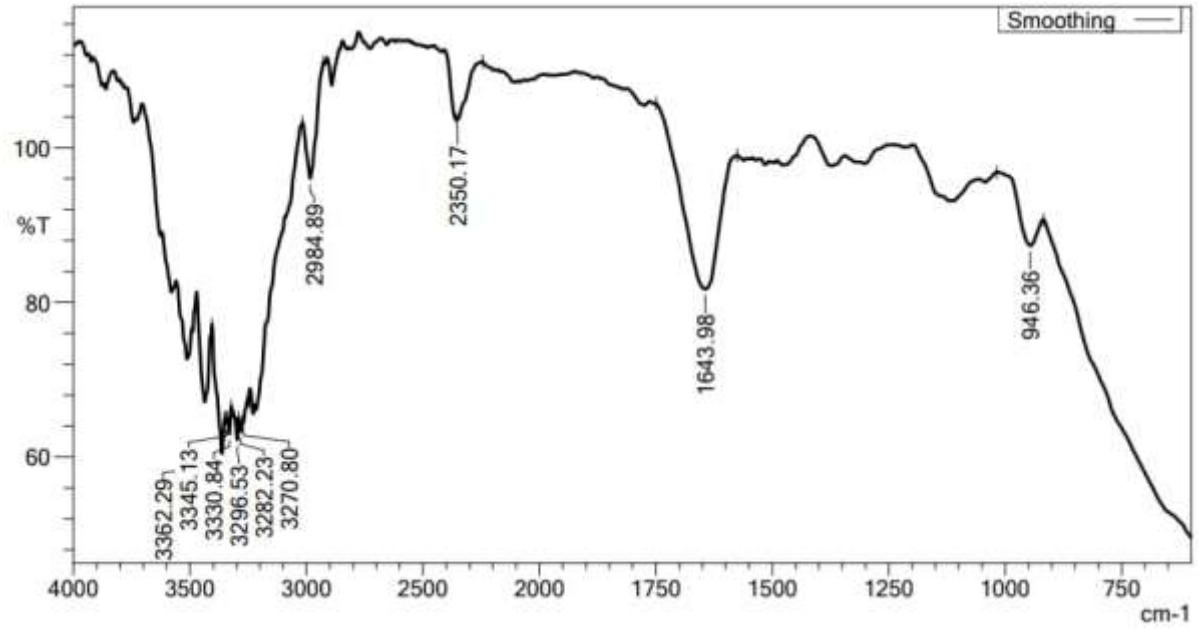

Figure 3 FT- IR Spectroscopy

3.4. Lather Production

3.4.1 Determination of Foamability test:

The soap of different oil samples was noted for the height of the foam. The height of the foam was increases with amount for each oil sample. The maximum height of the foam was obtained and recorded from the coconut oil amongst the other oil sample. The obtained values were plotted as a bar diagram (fig 4) with moisture content.

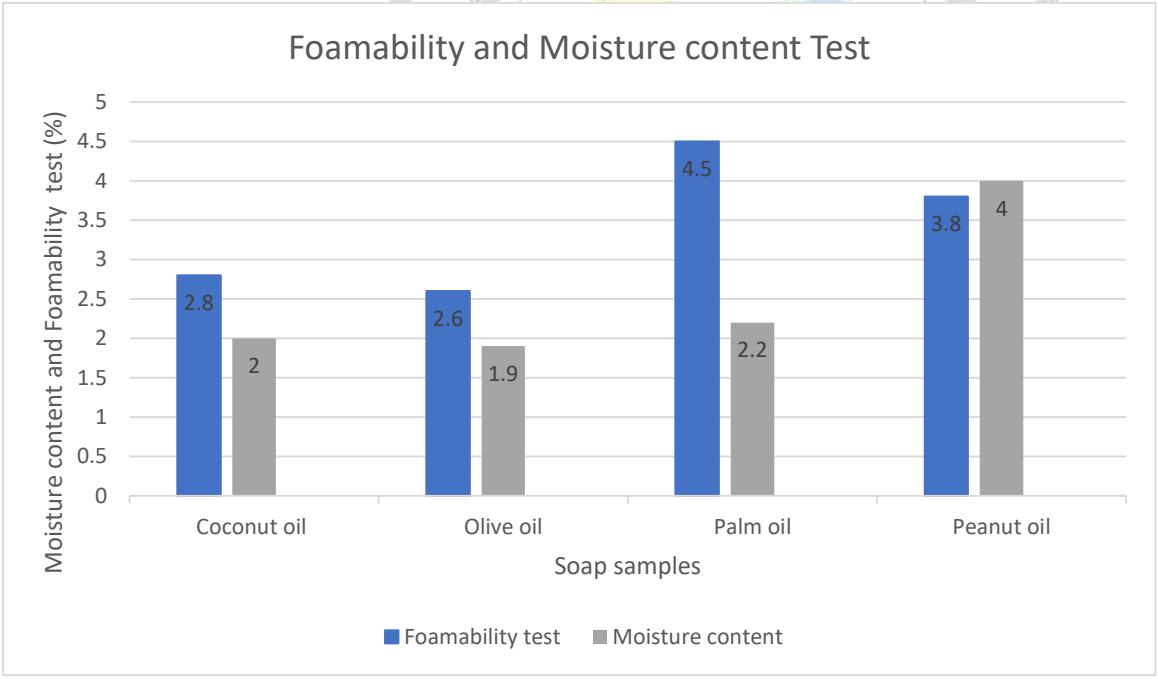

FIGURE :4 Bar graphs of Foamability test and Moisture content

3.4.2 Determination of Moisture content:

The moisture content of the soap was determined after the two weeks of soap production. The moisture content values were recorded in the above graphs (Fig 5) with the foamability test.

The prepared soap was finally produced on further analysis for reliability and consistency this can be commercialized [1][11]. The lather soap formed was depicted in.(Fig:5)

Figure 5 Lather Production

4.Discussion

The spirulina were collected commercially as the dried form. The powdery spirulina were tested by the phytochemical analysis, usually the photosynthesis plant source have the ability to produce metabolites and bioactive compounds, this phytochemical method is used to analyze the photosynthesis pigment and there are two types of metabolites present in the cyanobacterium (blue green algae) such as primary and secondary metabolites. The primary metabolites have the ability to promote the significant effects in growth as in the form of lipid consistency and which can be performed by using photosynthesis and the secondary metabolites are used for the food industries and pharmaceutical industries as a form of a drug, cosmetics etc. Spirulina extract was subjected to phytochemical analysis to examine the ability of secondary metabolites and bioactive compounds it was done with the standard procedure of phytochemical analysis, finally observed the positive results in alkaloids, terpenoids, flavonoids, coumarins, amino acid, steroids, saponins and negative results were observed in tannins, quinones, when compared to other compounds saponins had maximum quantity. It was performed with lysozyme enzyme for breaking of the cell wall of spirulina and the extracted samples were purified by the ammonium sulphate precipitation, while the process of centrifugation, the purified phycocyanin were bind to ammonium as the supernatant and were transferred into another tube and sedimented pellet particles were discarded. Partial purification was carried out by method of dialysis to get the purified form of the Phycocyanin [24][25]. After the extraction of phycocyanin were further characterized by analysis of the functional group and chemical bond between the bioactive compounds using UV-Visible spectroscopy. It was absorbed with spectral range from 0 to 800 and it observed at a absorbance maximum peak at 652nm.FT-IR is a disperse method and it also done for analyze the chemical structure and molecular bonds with the suitable blended with KBr and IR spectral analysis, the maximum value noted 1643.98,2350.17,2984.89,3270.80, 3330.84 and 3362.29 it corresponded to c=c in alkene group value and it shown partially modified results of extracted phycocyanin compounds. The purified spirulina was used to preparation of lather soap with different concentration of oil samples by the process of

saponification and the foamability and the moisture content of the soap was determined. It reveals that the Coconut oil gives the optimum range of 2.8 value of foamability test and it is very high for other oil samples. The moisture content values shows that the peanut oil soap sample was recorded as the optimum range but these values can be vary from one sample from another with their time period, quality of the distilled water and mainly the soap will produce the better moisture content according to their testing time intervals [26]. Finally, this lather soap has been analyzed for the further use before commercialization.

5.Conclusion

Spirulina is a cyanobacterium (blue green algae) this alga represents an important staple diet in humans and has been used as a source of protein and vitamin supplement in humans without any significant side effects. It protects the cell wall and prevent the tissue damaging ,it acts like anticancerous properties and antibacterial properties [3][4].From this study, the role of the Phycocyanin which is present as the photosynthetic pigment in the spirulina can be used in production of value added products as it also involved in the pharmaceutical and food industry .It also has the potential in expanding its role in the value added product and further its role can be applied in cosmetic products like soap ,shampoos and also as surfactants.

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