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PHYSICOCHEMICAL CHARACTERIZATION AND DRUG STANDARDIZATION OF *NANNOCHLOROPSIS OCULATA*

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ABSTRACT

Algal biomass has been explored as nutritional supplements for their rich content in secondary metabolites. This study was carried out to assess the primary and secondary metabolite content in the methanolic extract of *Nannochloropsis oculata.* Drug standardisation was also done to prove the nontoxic nature of the extract. The phytochemical analysis revealed the presence of phenolics, pigments and saponin predominantly among the different extracts of *Nannochloropsis oculata.* The marine microalgae were found to be rich in carbohydrate and protein. Thus, the micro algal species can be explored for nutraceutical and pharmacological potential.

KEYWORDS: *Nannochloropsis oculata*, Primary metabolites, Physiochemical parameters, Organoleptic features.

INTRODUCTION

Algal culture is an important process in the seed production of finfish and shellfish. They have either an initial link with the food chain or an indirect effect on the rearing process. They are cultured to feed the rotifers and to serve as water conditioner of the fish larval rearing system. Among numerous species of microalgae *Nannochloropsis oculata* has received priority attention in recent years in view of its demand for rearing operations in many hatcheries .The investigations revealed the nutritive value of this species as it contains required amount of Eicosapentaenoic acid (EPA,20:5n-3).[1,2,3] *Nannochloropsis oculata* is a marine unicellular microalga classified into the *Eustigmatophyceae* class, and is frequently used in fish seedling production and rearing firms. It has been reported that *N.oculata* has a superior nutritional value with relatively high levels of vitamin E.[4,5,6] Marine microalgae have been an unique source of chemical compounds of pharmaceuticals, aquaculture, cosmetics, anticancer agents, enzymes, pigments, antioxidants, polyunsaturated fatty acids, dietary supplements, agrochemicals and biofuel.[7-13] The aims of this study Phytochemical, quantitative determination of primary metabolites drug standardisation of methanol extracted from marine microalga *Nannochloropsis Oculata*.

MATERIALS AND METHODS

Preparation of Extract

10 mg of powered *Nannochloropsis oculata* dissolved in 10 ml of methanol, aqueous, isopropanol, pyridine and acetone, the mixed well keep in 24 hours. After filter it collected in clean bottle which stored at 15˚c for further studies.

Phytochemical Analysis

Phytochemical analysis was done to investigate the Phytochemical constitutes present in different extract of *Nannochloropsis oculata*, which is carried out by the standard method.[14]

Quantitative Determination of Primary Metabolites[15]

Determination of carbohydrate

100 mg of sample was hydrolysed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 1.0 ml of phenol reagent was added followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30°C for 20 min. The absorbance was read at 490 nm.

Estimation of total chlorophyll content

100 mg leaf tissues were soaked in 10 ml of DMSO: acetone mixture (1:1) for overnight incubation (in the dark) and absorbance read at 663 and 645 nm and total chlorophyll content was calculated using the following equations.

Chlorophyll a (Ca) = (12.25 × OD at 663) - (2.79× OD at 645) ×10/ (1000×wt)

Chlorophyll b (Cb) = (21.50 × OD at 645) - (5.10× OD at 663) ×10/ (1000×wt)
Total Chlorophyll (C) = (7.15 × OD at 663) + (18.71× OD at 645) ×10/ (1000×wt)

Determination of protein
The dried and powdered samples was extracted by stirring with 50 ml of 50% methanol (1:5 w/v) at 25 °C for 24 h and centrifuged at 7,000 rpm for 10 min .0.2 ml of extract was pipette out and the volume was made to 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to all the tubes and allowed it to stand for 10 min. Then 0.5 ml of Folin's Ciocalteau reagent was added and incubated in dark for 30 min. The intensity of the colour developed was read at 660 nm.

Estimation of total lipid content
10 gm sample was used to extract lipids with 150 ml of petroleum ether for 16 hr, at a solvent condensation rate of 2–3 drops/sec according to AACC Approved Method 30-25 with minor modifications of sample size and extraction time. The obtained extract was concentrated and evaporated at room temperature to dryness. The weight of extract gives the total lipid content which was expressed as mg/g dry matter.

Estimation of Vitamin C
The assay mixture for vitamin C consisted of 0.1 ml of brominated sample extract, 2.9 ml of distilled water, 1 ml of 2% DNPH reagent and 1-2 drops of thiourea. After incubation at 37ºC for 3 h, the orange-red osazone crystals formed were dissolved by the addition of 7 ml of 80% sulphuric acid and absorbance was read at 540 nm after 30 minutes. Vitamin C concentration was expressed in terms of µg/mg plant tissue.

Determination of Vitamin E
Vitamin E was evaluated in the methanol extract following the method given by [15]. An aliquot of 0.1 ml of extract (10 mg/ 2 ml) was mixed with 1 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 37°C for 90 min with vigorous shaking. Absorbance of the aqueous phase at 695 nm was measured against the appropriate blank. A typical blank contained 1 ml of reagent solution and 0.1 ml of the respective solvent, incubated under the same conditions as the samples. The analysis was performed in triplicate and the vitamin E content was expressed as α-tocopherol equivalents.

Drug Standardization[17,18]

1. Study of Organoleptic Characters
The organoleptic characters like color, odor, taste, size, texture, appearance and size using the sensory organs of our body.

2. Physico-Chemical Analysis

Determination of foreign matter
Weigh 10 g to 50 g of the substance to be examined, or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. Examine for foreign matter by inspection with the unaided eye or by use of a lens (6 ×).Separate foreign matter and weigh it and calculate the percentage present.

Solubility
The presence of adulterant in a drug could be indicated by solubility studies with various solvents.

i. Alcohol
5 gm of powdered material along with 100 ml of alcohol are shaken well occasionally for the first 6 hours and kept undisturbed for 18 hours. The liquefied extract thus obtained was concentrated in an vacuum oven and the percentage was calculated with the weight of the drug powder taken.

ii. Water
The procedure adopted for solubility percentage of alcohol is used with water instead of alcohol to get the water solubility.

Determination of Moisture content
The moisture content of the samples was determined by AOAC method. 5 g of the sample (without preliminary drying) was weighed and placed in the petri dish. It was dried at 105˚C for 2 hours. The dish and dried samples were transferred to a desiccator to cool at room temperature. The experiments were repeated until constant weight was obtained.

Determination of Total ash
The ash content was determined by AOAC method. About 2 to 3 g of sample was accurately weighed in a pre-weighed petri dish at a temperature 450˚C for 5 to 6 hours until it was free from carbon. Then it was cooled and weighed. The percentage of total ash was calculated with reference to the air dried drug.

Determination of Acid insoluble ash
The total ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter obtained was collected on an ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Determination of Water-soluble Ash
The ash obtained in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited for 15 minutes at a temperature not exceeding 450˚C. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as the water- soluble ash was calculated with reference to the air dried drug.

3. Determination of extractive values

Water-soluble extractive

4 g of test sample was weighed and macerated with 100 ml of 5% chloroform water in a closed flask for 24 hours, shaking frequently during and allowed to stand 6 hours. It was filtered rapidly, taking precautions against the loss of solvent. 25 ml of the filtrate was taken and evaporated to dryness in a tarred flat bottomed shallow dish at 105˚C for 1 hour, to constant weight and weighed the percentage of water soluble extractive was calculated with reference to the air dried sample.

$$\% \text{ of water soluble extractive values} = \frac{B\text{-}A \times 4 \times 100}{W}$$

Where, A = empty wt. of the dish (g)
B = wt. of dish + residue (g)
W = wt. of sample taken (g)

Alcohol-soluble extractive

Procedure for water soluble extractive was followed for the determination of alcohol soluble extractive but 100 ml of 90% ethanol was used instead of chloroform water. The percentage of alcohol soluble extractive was calculated using the formula mentioned for water soluble extractive value.

Ether soluble extractive

1 gram of dried samples was mixed with 100 ml of ether in closed flask for 24 hours with frequent shaking. It was filtered rapidly, taking precautions against the loss of ether. 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100°C and weighed. The percentage of ether soluble extractive was calculated using the formula mentioned for water soluble extractive value.

4. Determination of Physical Characteristics

Bulk density

It is the ratio of given mass of powder and its bulk volume. It is determined by transferring an accurately weighed amount of powder sample to the graduated cylinder with the aid of a funnel. The initial volume was noted. The ratio of weight of the volume it occupied was calculated.

Bulk density=W/V0 g/ml

Where, W = mass of the powder, V0 = untapped volume.

Tapped density

It is measured by transferring a known quantity (25g) of powder into a graduated cylinder and tapping it for a specific number of times. The initial volume was noted. The graduated cylinder was tapped continuously for a period of 10-15 min. The density can be determined as the ratio of mass of the powder to the tapped volume.

Tapped volume= W/Vf g/ml

Where, W = mass of the powder, Vf = tapped volume.

Compressibility index

It is the propensity of the powder to be compressed. Based on the apparent bulk density and tapped density the percentage compressibility of the powder can be determined using the following formula.

Compressibility index = [(v0-vf)/v0] x 100,

(Or)

% Compressibility = [(tapped density – bulk density)] / tapped density] x 100

Hausner ratio

It indicates the flow properties of the powder. The ratio of tapped density to the bulk density of the powder is called Hausner ratio.

Hausner ratio= Tapped density/bulk density

Where, h=height of the pile, r = radius of the pile

Determination of pH range

The powder sample was weighed to about 5g and immersed in 100 ml of water in a beaker. The beaker was closed with aluminium foil and left behind for 24 hours in room temperature. Later the supernatant solution was decanted into another beaker and the pH of the formulation was determined using a calibrated pH meter.

RESULT AND DISCUSSION

Different extracts of *Nannochloropsis oculata* were subjected to phytochemical analysis and founded that alkaloids were present in pyridine extract alone and absent in all other samples. All the different extract shows the absent of carbohydrates and the presence of steroids, phytosterols, triterpenoids, phenols and flavonoids. Saponins was founded to be present in aqueous, acetone, isopropanol and methanol extract but absent in pyridine extract. Aqueous, pyridine, isopropanol extract of *Nannochloropsis oculata* shows that both protein and glycosides were founded to be absent and acetone, methanol extract of *Nannochloropsis oculata* showed the presence of protein but the phytochemical compound glycosides were absent (**Table 1**).

Table 1: Phytochemical analysis of *Nannochloropsis oculata* extract.

Phytochemical constituents	Aqueous	Acetone	Pyridine	Isopropyl alcohol	Methanol
Alkaloids	-	-	+	-	-
Carbohydrates	-	-	-	-	-
Steroids	+	+	+	+	+
Saponin	+	+	-	+	+
Phytosterols	+	+	+	+	+
Triterpenoids	+	+	+	+	+
Phenols	+	+	+	+	+
Tannins	-	+	+	-	+
Flavanoids	+	+	+	+	+
Proteins	-	+	-	-	+
Glycosides	-	-	-	-	-

Table 2: Determination of primary metabolites.

S. No.	Primary metabolites	Weight(mg/g dw)
1	Carbohydrates	120.63 ± 2.73
2	Chlorophyll	3.04 ± 0.02
3	Protein	150.63 ± 0.83
4	Lipids	1.08 ± 0.16
5	Vitamin C	0.27 ± 0.26
6	Vitamin E	0.18 ± 0.01

Table 2 represents the primary metabolites founded in *Nannochloropsis oculata* powder. The quantity of carbohydrates, chlorophyll, protein and lipids were founded to be 120.63 ± 2.73, 3.041 ± 0.02, 150.63 ± 0.83 and 1.08 ± 0.16 mg/g dw. Vitamin content such as Vitamin C and Vitamin E were also present with 0.27 ± 0.26 and 0.18 ± 0.01 mg/g dw.

Table 3: Pharmacognostic study.

S. No	Properties	Methanolic extract of *Nannochloropsis oculata*
1	Colour	Dark green
2	Odor	Algal
3	Taste	Bitter
4	Texture	Smooth
5	Appearance	Semi-solid
6	Size	2-5µm

Pharmacognostic study of methanolic extract of *Nannochloropsis oculata* weas analysed and the color of the extract was detected as dark green with algal odour which was found to be bitter in taste. It has smooth texture, semisolid appearance and the size varies in the range form 2-5 µm (**Table 3**).

Table 4: Physicochemical Parameters of *Nannochloropsis oculata.*

S. No	Content	%
1	Foreign matter	0.001
2	Solubility (water)	2.33
3	Solubility (alcohol)	8.98
4	Moisture content	3.6
5	Total ash	5.8
6	Acid insoluble ash	1.01
7	Water insoluble ash	2.35
8	Water soluble extractive	2.33
9	Alcohol soluble extractive	0.75
10	Ether soluble extractive	0.58

Table 4 represents the physicochemical parameters of *Nannochloropsis oculata*. Based on the solubility it was concluded that alcohol solubility shows 8.98 % and water solubility shows 2.33 with the least presence of foreign matter (0.001%). Moisture content and ash content were also determined which produces above 3.6 % and 5.8 % respectiely. Soluble extractive values such as water, alcohol and ether was founded to be 2.33, 0.75 and 0.58 % for *Nannochloropsis oculata.*

Table 5: Physical characteristics.

Content	Result
Bulk density g/ml	5.21
Trapped density g/ml	8.37
Compressibility index %	37.75
Hausner ratio	1.61
PH	8.6

Based on the physical characteristics the test sample *Nannochloropsis oculata* produced 5.21 g/ml of bulk density and 8.37 g/ml of trapped density. The compressibility index was 37.75 % along with Hausner ratio (1.61) and pH 8.6 respectively which was tabulated in **Table 5.**

Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample,

organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. The pharmacognostic study of *Chlorella vulgaris* showed green in color, produce musty odour which is found to be tasteless. Different Physicochemical parameters such as Total Ash (8.9 g/100g), Acid Insoluble Ash (7.4 g/100g), Water Soluble Ash (2.0 g/100g), Water soluble extractive (3.8 g/100g), Alcohol Soluble extractive (7.9 g/100g) and pH 8.5 was observed for the test sample *Chlorella vulgaris*.[19] Hence, the present result was similar to the result of previous study.

CONCLUSION

The results of the present study indicated the presence of phytochemicals in the microalgae *Nannochloropsis oculata.* The presence of phenolics, pigments and other phytoconstituents were responsible for the potent antioxidant and other bioactivity of the methanolic extract of *Nannochloropsis oculata.* The micro algal biomass can be explored in near future for various medicinal properties to combat various diseases.

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