Original Article

Biochemical Profile of *Enteromorpha linza* (L.) J.Ag. in hare Island, Thoothukudi, Tamilnadu, India

John Peter Paul J*. and Muthu Sheeba M.

Department of Botany, St. Xavier's College (Autonomous), Palayamkottai – 627 002

ABSTRACT

The aim of the study was to investigate the biochemical profile of *Enteromorpha linza* (L.) J. Ag. in Hare island, Thoothukudi, Tamil Nadu, India. The biochemical composition such as total carbohydrates, total proteins, total lipids, total phenols, total chlorophylls and total carotenoids were estimated using the standard procedure in fresh plant material. The preliminary phytochemical analysis was performed by Harborne method. The phytochemical screening of methanol extract was estimated using the standard procedure for UV-Vis spectroscopic, HPLC and FTIR. In the present study it was found that *Enteromorpha linza* (L.) J. Ag. contained high level of carbohydrates (33.8% FW), followed by proteins (20.8% FW), lipids (1.74% FW), phenols (1.65% FW), chlorophylls (0.038%) and low level of carotenoids (0.021%). The UV-Vis phytochemical profile of methanol extract of *Enteromorpha linza* (L.) J. Ag. was analyzed at 240 to 800nm. The results of FTIR analysis showed different peaks at 495.67, 538.10, 609.46, 651.69, 727.11, 732.18, 827.41, 939.27, 993.27, 1116.71, 1191.93, 1388.65, 1463.87, 1622.02, 2264.27, 2503.43, 2640.37, 2678.94, 2742.59, 2850.59, 2920.03, 2954.74, 3014.53, 3321.19, 3379.05 and 3402.20. It was confirmed the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds. The qualitative HPLC fingerprint profile of methanol extract of *Enteromorpha linza* (L.) J. Ag. was selected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. The profile displayed two prominent peaks at the retention time of 1.417min and 2.190min followed by four moderate peaks were observed at the retention time of 0.215min, 0.298min 0.395min and 1.417min respectively. The biochemical analysis of *Enteromorpha linza* (L.) J. Ag. showed the presence of various phytochemicals. The results of the present study supplement the usage of the studied plant which possesses several bioactive compounds and used as food and

Address for Correspondence

Department of Botany, St. Xavier's College(Autonomous), Palayamkottai-627002 Tamil Nadu, India.
Tel:+ 91-9442955038.
E-mail: johnarock2008 @yahoo.com

medicine.

Keywords: Seaweed, *Enteromorpha linza*, Biochemical profile, HPLC, UV-Vis, FTIR.

INTRODUCTION

The marine environment representing approximately half of the global biodiversity, is an enormous resource for new compounds. Seaweeds which are also known as marine macro algae are a habitat of both marine and brackish water environment. Seaweeds are found in the intertidal and subtidal region up to where photosynthetic light of 0.01% prevails and also in the coastal region between high tide and low tide. As the first organism in marine food chain, seaweeds provide nutrients and energy for all other living organisms[1].

Seaweeds provide shelter and habitat for many coastal animals. Seaweeds are also traditionally consumed in different part of the world. Recently human consumption of green algae (Chlorophyceae), brown algae (Phaeophyceae) and red algae (Rhodophyceae) is high in Asia, mainly in Japan, China and Korea. In Asian countries, seaweeds are often consumed as marine vegetables. Japanese people are the main consumers with an average of 1.6 kg (dry weight) per year per capita[2]. Seaweed can be eaten by humans as food and are sources of useful industrial products such as phycocolloids, carrageenan, alginates and agar. Algal phycocolloids find use in the food industry as thickening and emulsifying agents. Some of the algae are used to prepare soil conditioner for horticulture. Other uses include medicine, animal feed, cosmetics and fish bait[3].

A number of research studies have been conducted to investigate the phytochemicals present in seaweeds especially carbohydrates, proteins, lipids, phenols[4-6]. The chemical composition of tropical seaweeds was estimated seasonally and reported that the protein content of green seaweeds was greater than the brown and red seaweeds. Furthermore protein content of seaweeds also found higher concentration in green seaweed compared with some higher plants. Lipid extracts of some edible seaweed showed antioxidant activity and synergistic effect with the tocopherol[7] also. Seaweeds are also used as manure for agricultural and horticultural crops due to the presence of minerals, trace elements and plant growth regulators which occur in water soluble form and enhances the disease resistance in field crops[8,9]. Hence, the present work was aimed to screen and evaluate the biochemical profile of *Enteromorpha linza* (L.) J. Ag. in Hare Island, Thoothukudi, Tamil Nadu, India.

MATERIALS AND METHODS

Collection of Sample

Enteromorpha linza(L.) J. Ag. (Figure 1) is a green seaweed shows much attention in the recent years as it has potential to supplement native vegetation. *Enteromorpha linza* (L.) J. Ag. were collected from Hare island, Thoothukudi in the south east coast of Tamil Nadu, India during the month of January 2014. Samples were rinsed with sea water to remove debris and epiphytes. The entire epiphytes were removed using soft brush. In the laboratory, the seaweeds are once again washed in freshwater and stored in refrigerator for further analysis.

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Estimation of Biochemicals

The total carbohydrates[10], Proteins[11], lipids[12], phenols[13], chlorophylls[14] and carotenoids[15] were estimated by standard method in fresh plant sample.

Preliminary Phytochemical analysis

The different extracts were tested for alkaloids, anthraquinones, catechin, flavonoids, glycosides, phenolic groups, reducing sugars, saponins, steroids, tannins and terpenoids. Phytochemical screening of the extracts was carried out according to the standard methods[16].

Preparation of extracts

For the preparation of different extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 3g powdered samples were packed in Soxhlet apparatus and extracted with ethanol, acetone, benzene, choloroform and petroleum ether water for 8h separately.

Test for Alkaloids

1ml of 1% HCl was added to the 2ml of extract in a test tube and was treated with few drops of Mayer's reagent. A creamy white precipitate indicated the presence of alkaloids.

Test for Anthraquinones

2ml extract was mixed with benzene and 1ml 10% ammonia solution was added. The presence of a pink, red or violet color indicates the anthraquinones.

Test for catechin

2ml extract was mixed with Enrlich reagent and few drops of Conc. HCl. Formation of pink colour indicate the presence of catechin.

Test for flavonoids

A few drops of 1% NH_3 solution was added to 2 ml of extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

Test for Glycosides

2ml of 50% H_2SO_4 was added to the 2ml of extract in a boiling tube. The mixture was heated in boiling water bath for 5 min. 10ml of Fehling's solution was added and boiled. A brick red precipitate indicated the presence of glycosides.

Test for phenolic groups

To 1ml extract, add 2ml distilled water followed by few drops of 10% Ferric chloride. The formation of blue or black colour indicates the presence phenolic groups.

Test for reducing sugars

5-8 drops Fehling's solution was added to 2ml extract. The mixture was heated in boiling water bath for 5 min. A red-brick precipitate shows the presence of reducing sugars.

Test for Saponins

2ml of extract was shaken vigorously with 5ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

Test for Steroids

0.5 ml of hot acetic anhydride was added with 2ml of ethanolic extract. The mixture was treated with Libermann reagent. The appearance of a ring of blue green showed the presence of sterol and steroids.

Test for tannins

To 2ml extract, 1ml of distilled water and 1-2 drops of ferric chloride solution was added and observed for brownish green or a blue black coloration.

Test for terpenoids

2ml extract was mixed with 2ml of $CHCl_3$ in a test tube. 3ml Conc. H_2SO_4 was carefully added along the wall of the test tube to form a layer. An interface with a reddish brown coloration was confirmed the presence of terpenoids.

UV-Vis spectral analysis

The crude extracts containing the bioactive compound was analyzed spectroscopically for further confirmation. The crude extracts of *Enteromorpha linza* (L.) J. Ag. were scanned in a wavelength ranging from 310-900nm using a Shimazdu spectrophotometer and characteristic peaks were detected.

HPLC Analysis

The HPLC method was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, a Rheodyne injector fitted with a 20µl loop and an auto injector SIL-10AT. A Hypersil® BDS C-18 column (4.6 × 250mm, 5µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1ml/min at ambient temperature (25-28°C). The mobile phase consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45µm and sonicated before use. Total running time was 15min. The sample injection volume was 20µl while the wavelength of the UV-Vis detector was set at 254nm.

Instrumentation

An isocratic HPLC (Shimadzu HPLC Class VP series) with two LC- 0 AT VP pumps (Shimadzu), a variable wave length programmable photo diode array detector SPD-M10A VP (Shimadzu), a CTO- 10AS VP column oven (Shimadzu), a SCL-10A VP system controller (Shimadzu), a reverse phase Luna 5□ C18 (2) and Phenomenex column (250 mm X 4.6mm) were used. The mobile phase components Methanol:water (45:55) were filtered through a 0.2µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270kgf/cm^2. The column temperature was maintained at 27°C. 20µl of the respective sample and was injected by using a Rheodyne syringe (Model 7202, Hamilton).

FTIR analysis

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum.

RESULT AND DISCUSSION

Biochemical Profile of *Enteromorpha linza* (L.) J. Ag.

The amount of total carbohydrate, total protein, total lipid, total phenols, total chlorophylls and total carotenoids of *Enteromorpha linza* (L.) J. Ag. are presented in the in the Table 1. The concentration of carbohydrates was found to be 338mg/g (33.8%) in fresh weight. In the present study, protein content showed remarkable amount of 208mg/g (20.8%). The total lipids and phenols contents in *Enteromorpha linza* (L.) J. Ag. were found relatively low compared to total carbohydrates and total proteins. The seaweed contains 174mg/g of lipids (1.74%) and 165mg/g (1.65%) of phenols (Figure 2).

The total chlorophylls and total carotenoids were observed 3.88mg/g (0.038%) and 2.14mg/g (0.021%) respectively (Figure 3).

Carbohydrate is one of the important components for metabolism and it supplies the energy needed for respiration and other most important processes. Proteins have crucial functions in all the biological processes. Their activities can be described by enzymatic catalysis, transport and storage, mechanical sustentation, growth and cellular differentiation control. Lipids are rich in -C = O- bonds, providing much more energy in oxidation processes than other biological compounds. They constitute a convenient storage material for living organisms. In general, seaweeds exhibit low lipid contents[17]. In seaweeds the lipids are widely distributed, especially in several resistance stages. Total chlorophyll content was the summative value of the chlorophyll 'a' and chlorophyll 'b'. Therefore it showed that similar trend and concentration gradient like the constituting two above mentioned parameters. Total chlorophyll content was also recorded highest in green algae when compared to red algae. Thus carotenoid concentrations were found to be varied in different algal groups and collaborate with the earlier report. The phycobilin content was observed to be more in red algae than in green algae. Most of the red seaweeds contain higher amount of phycoerythrin in addition to chlorophyll[18].

Preliminary Phytochemical analysis

In the phytochemical analysis of *Enteromorpha linza* (L.) J. Ag., eleven different types of secondary metabolites (alkaloids, anthroquinones, catechins, flavonoids, glycosides, phenolic groups, reducing sugars, saponins, steroids, tannins and terpenoids) were tested in seven different extracts of *Enteromorpha linza* (L.) J. Ag. Thus, out of (1x7x11) 77 tests for the presence or absence of the above compounds, 50 tests gave positive results and the remaining gave negative results. The 50 positive results show the presence of alkaloids, anthraquinones, catechin, flavonoids, glycosides, phenolic groups, reducing sugars, saponins, steroids, tannins and terpenoids. Phenolic groups show the maximum presence, being found in seven different extracts and saponin in six extracts followed by alkaloids, catechin, glycosides and tannins found in only five extracts. Among the seven different extracts, the benzene, methanol and ethanol extracts showed the presence of the maximum number (9) of compounds. Next to benzene, methanol and ethanol extracts showed the presence of eight compounds and the aqueous and chloroform extracts showed the presence of seven compounds and the acetone extract showed five compounds (Table 2).

UV-VIS Spectrum Analysis

The UV-VIS fingerprint profile of the methanol extract of *Enteromorpha linza* (L.) J. Ag. was selected at the wavelength of 240nm to 800nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 269, 329, 410 and 425 with the absorption 1.677, 0.732, 0.511 and 0.322 respectively (Table 3 and Figure 4).

HPLC Analysis

The qualitative HPLC fingerprint profile of the methanol extract of *Enteromorpha linza* (L.) J. Ag. was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. The methanol extract prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in the *Enteromorpha linza* (L.) J. Ag. Six compounds were separated at different retention times of 0.215min, 0.298min, 0.395min, 1.075min, 1.417min and 2.190min respectively. The profile displayed two prominent peaks at the retention times of

1.417min and 2.190min and some moderate peaks were also observed at the retention times of 0.215min, 0.298min 0.395min and 1.417 (Figure 5).

FTIR Analysis

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The crude powder of *Enteromorpha linza* (L.) J. Ag. was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of FTIR analysis showed different peaks at 495.67, 538.10, 609.46, 651.69, 727.11, 732.18, 827.41, 939.27, 993.27, 1116.71, 1191.93, 1388.65, 1463.87, 1622.02, 2264.27, 2503.43, 2640.37, 2678.94, 2742.59, 2850.59, 2920.03, 2954.74, 3014.53, 3321.19, 3379.05 and 3402.20. It was confirmed the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds etc (Figure 6).

Seaweeds are known for the richness in polysaccharides, minerals and certain vitamins[19], but also contain bioactive substances like proteins, lipids and polyphenols with antibacterial, antiviral and antifungal properties as well as many others[20]. Therefore seaweeds give great potential as a supplement in functional food or for the extraction of compounds. Physiologically active substances in seaweeds affect the maintenance of human homeostasis directly[21]. Seaweeds are low in calories from a nutritional perspective. The lipid content was low and even though the carbohydrate content was high, most of this is dietary fibres and not taken up by the human body. However, dietary fibres are good for human health as they make an excellent intestinal environment[22].

Halogenated compounds are produced naturally, mainly by seaweeds, dispelling the widespread notion that these chemicals are only of man-made origin[23]. Halogenated compounds are dispersed in several different classes of primary and secondary metabolites, including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons[25]. In many cases, they possess biological activities of pharmacological interest, including antibacterial[25] and anti-tumoural[26]. The most notable producers of halogenated compounds are seaweeds in the marine environment[27],[28]. The compounds are predominantly derivates of esquiterpenes diterpenes, triterpenes, acetogenins, fatty acids and brominated indoles. In addition to antimicrobial and cytotoxic properties also play multifunctional ecological roles such as acting as a feeding defendant[29-31].

Seaweed extracts with different solvents such as water, ethanol, methanol, ethyl acetate, petroleum ether and chloroform have shown antibacterial, anti-inflammatory and anti-pathogenic effects. The antibacterial effect of seaweed extracts was seen in several gram-negative bacteria strains and the extracts were non toxic against non targeted larvae[32]. Anti-inflammatory effect was seen in oedema and erythema of mice, but there was also decreased motor activity in rodents when seaweed extract was added to their diet[33]. Another study on rodents improved the survival of stroke-prone hypertensive rats when given 5% seaweed powder in the diet. Similarly, seaweed powder supplement in rat diet (5% wet weight) attenuated the development of hypertension and its related diseases and improved survival rates[34]. Methanol extract of seaweeds were furthermore preventive against DNA damage[35,36]. The latter effect was also seen by enzymatic extracts of other brown seaweed[37]. Seaweed extract stimuli in plant science and agriculture are described in recent publications[38,39].

CONCLUSION

The biochemical profile of the present study suggests that *Enteromorpha linza* (L.) J. Ag. have considerable carbohydrates, proteins, lipids, phenols, chlorophylls and carotenoids for the use of food and pharmaceutical industry as a source in preparation of nutrient supplements, medicine and fine chemical synthesis. The carbohydrates content was higher however, phenol values were lower. It was found that *Enteromorpha linza* (L.) J. Ag. was appeared to be interesting potential sources of plant food proteins owing to their high carbohydrate level. In addition, eleven different types of secondary metabolites (alkaloids, anthroquinones, catechins, flavonoids, glycosides, phenolic groups, reducing sugars, saponins, steroids, tannins and terpenoids) were also observed. The methanol profile of *Enteromorpha linza* (L.) J. Ag. showed the presence of four compounds at the nm of 267, 329, 410 and 425 with the absorption 1.677, 0.732, 0.511 and 0.322 respectively using UV-Vis Spectrophotometer. The qualitative HPLC fingerprint profile of the methanol extract of *Enteromorpha linza* (L.) J. Ag. showed the presence of six compounds at different retention times of 0.215min, 0.298min, 0.395min, 1.075min, 1.417min and 2.190min. The profile displayed two prominent peaks at the retention times of 1.417min and 2.190min. It was confirmed the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds with the help of FT-IR.

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Table 1. Biochemical profile of *Enteromorpha linza* (L.) J. Ag. in Thoothukudi

Name of the Biochemicals	Concentration of Biochemicals (mg/g)	Amount of Biochemicals (%)
Total Carbohydrates	338*	33.8*
Total Proteins	208*	20.8*
Total Lipids	174*	1.74*
Total Phenols	165*	1.65*
Total Chlorophylls	3.88*	0.038*
Total Carotenoids	2.14*	0.021*

*An average of Triplicates

Table 2. Preliminary Phytochemical analysis of crude extracts of *Enteromorpha linza* (L.) J.Ag.

Phytochemicals	Solvents						
	Aqueous	Methanol	Ethanol	Acetone	Benzene	Chloroform	Petroleum Ether
Alkaloids	-	+	+	-	+	+	+
Anthra-quinones	-	+	+	-	+	+	-
Catechin	+	-	+	+	+	-	+
Flavonoids	-	+	+	-	+	+	-
Glycosides	+	+	+	-	+	+	-
Phenolic groups	+	+	+	+	+	+	+
Reducing sugars	+	+	-	-	+	-	-
Saponins	+	+	+	+	+	-	+
Sterols & Steroids	-	+	-	+	-	+	-
Tannins	+	-	+	-	+	+	+
Terpenoids	+	-	-	+	-	-	+

Table 3. UV-Vis Spectroscopic peak value of methanol extract of *Enteromorpha linza*(L.) J. Ag.

Nm	269	329	410	425	520	570	610	670	710	780
Abs	1.677	0.732	0.511	0.322	0.239	0.128	0.123	0.101	0.036	0.011

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Figure 1. Natural habit of *Enteromorpha linza* (L.) J. Ag.

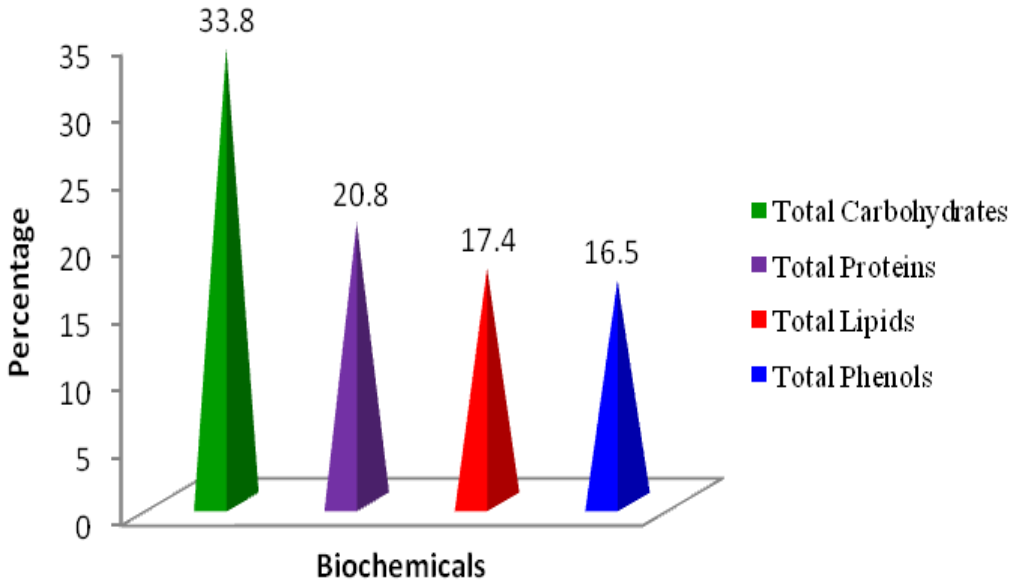

Figure 2. Concentration of various Biochemicals of *Enteromorpha linza*(L.) J.Ag.(%)

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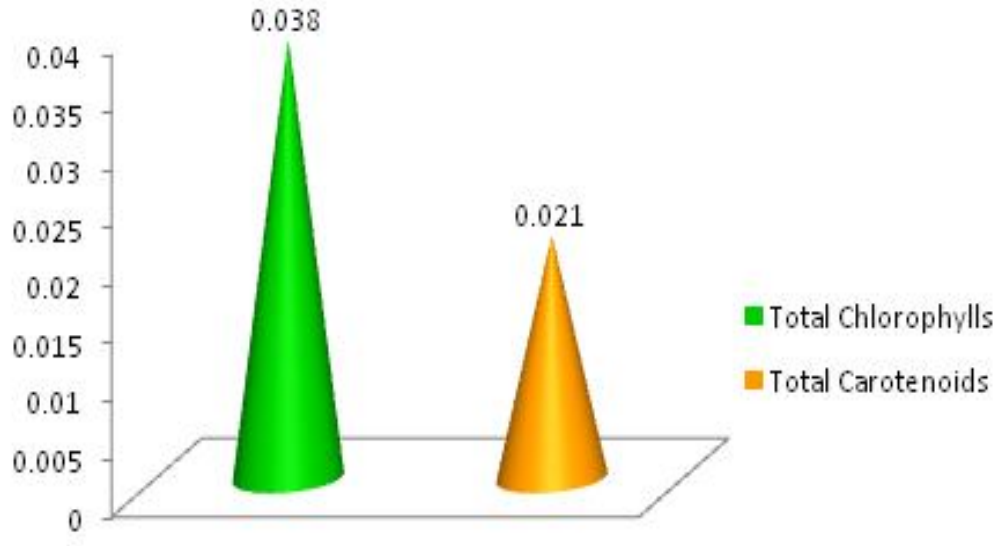

Figure 3. Pigment Concentration of *Enteromorpha linza* (L.) J. Ag. (%)

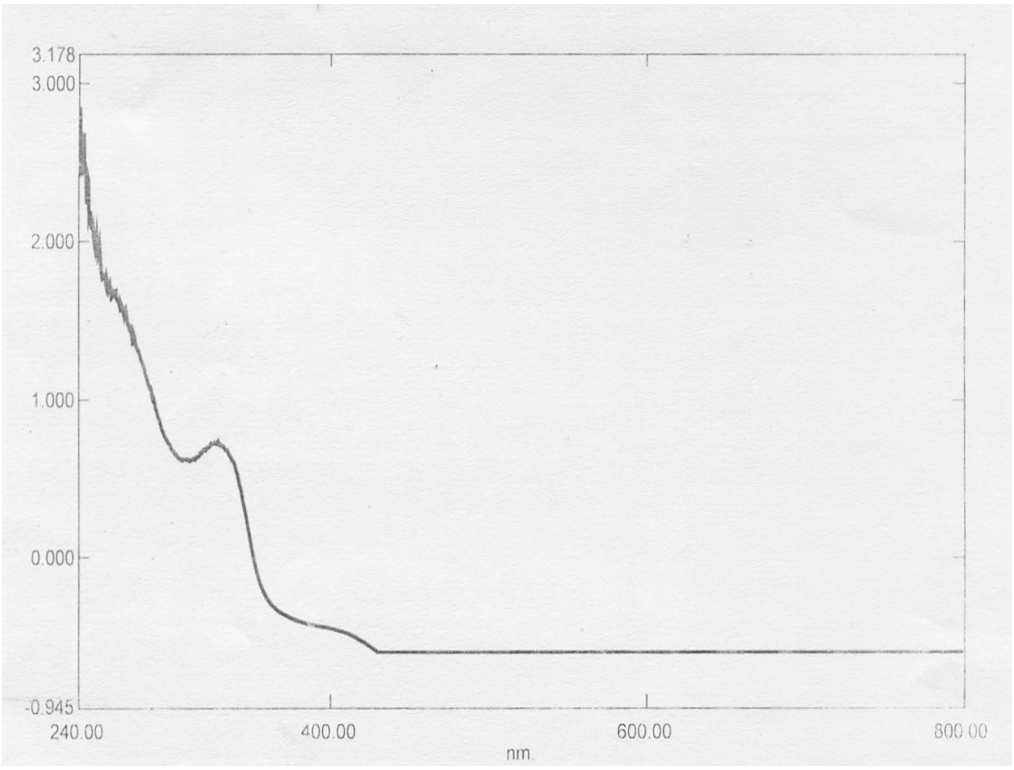

Figure 4. UV-Vis absorption spectra of methanol extract of *Enteromorpha linza* (L.) J. Ag.

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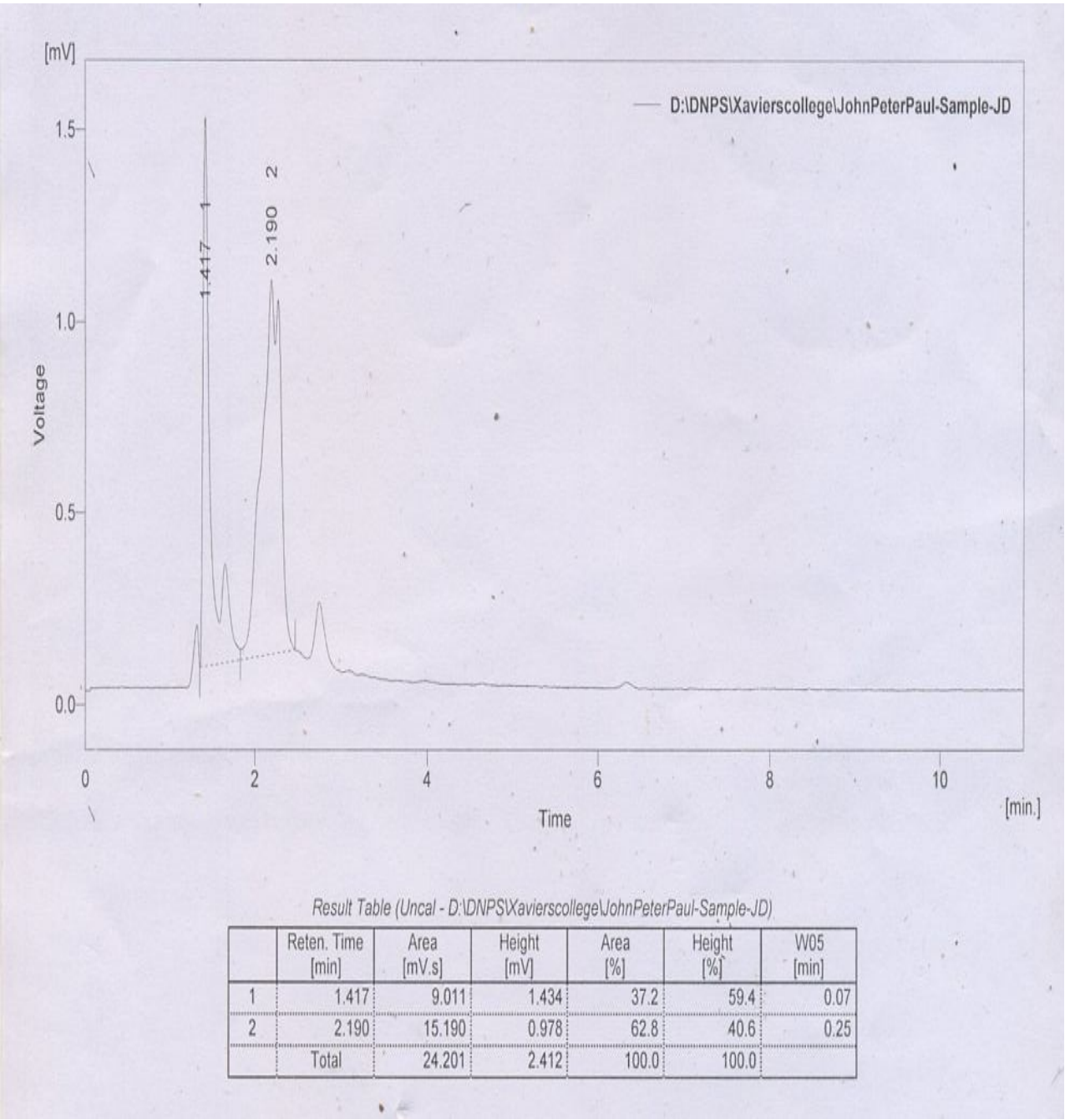

Result Table (Uncal - D:\DNPS\Xavierscollege\JohnPeterPaul-Sample-JD)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.417	9.011	1.434	37.2	59.4	0.07
2	2.190	15.190	0.978	62.8	40.6	0.25
	Total	24.201	2.412	100.0	100.0	

Figure 5. HPLC analysis of methanol extract of *Enteromorpha linza* (L.) J. Ag.

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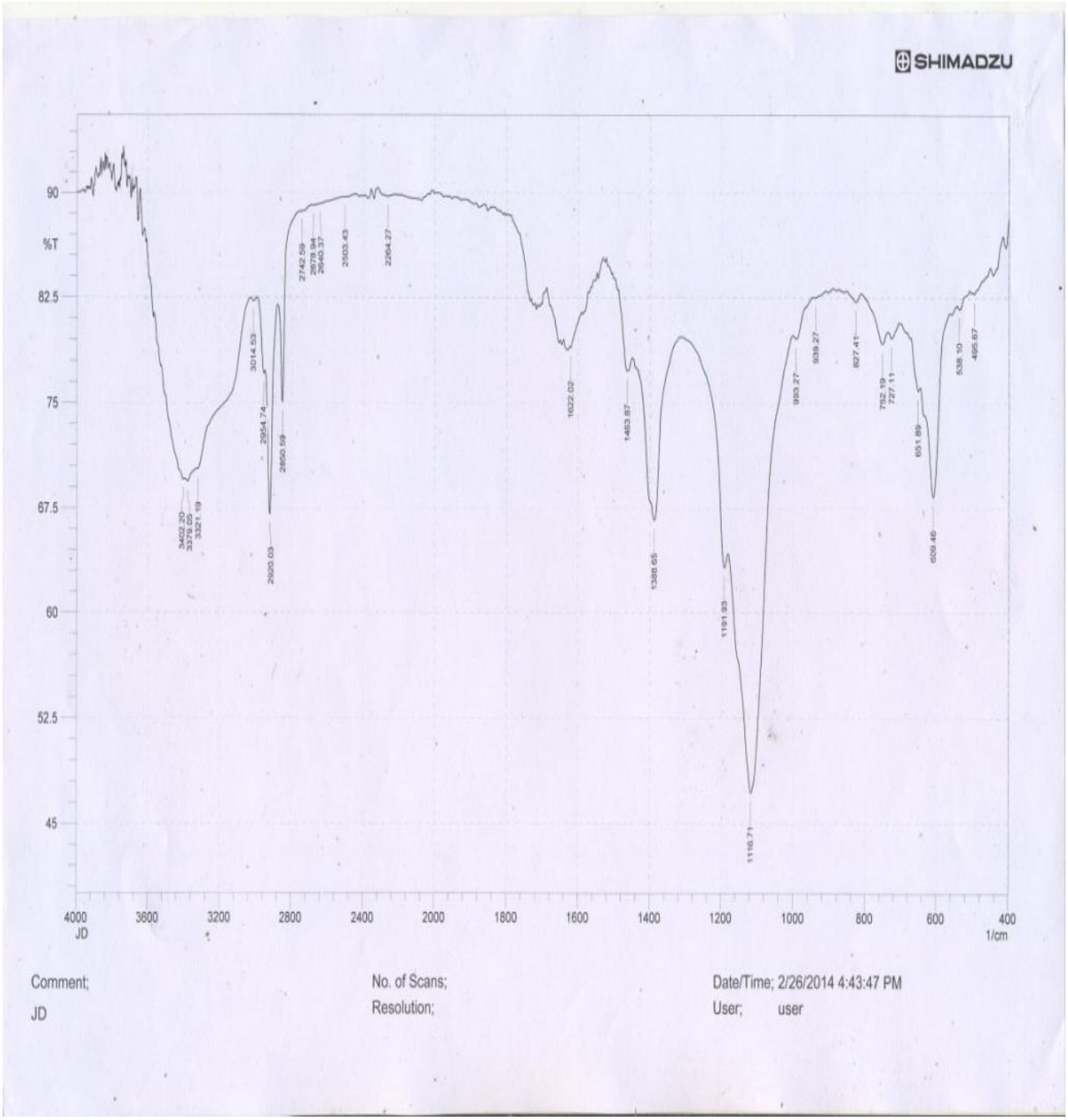

Figure 6. FTIR analysis of methanol extract of *Enteromorpha linza* (L.) J. Ag.