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Phytochemicals of algae, *Arthospira platensis* (spirulina) *Chlorella vulgaris* (chlorella) and *Azolla pinnata* (azolla)

Manjula Thangaraj [1], Bhavan Periyakali Saravana [1,*], Jayakumar Thanasekaran [2], Sheu Joen-Rong [3], Manjunath Manubolu [4] and Kavitha Pathakoti [5]

[1] *Department of Zoology, Bharathiar University, Coimbatore 641046, Tamil Nadu, India.*
[2] *Department of Ecology and Environmental Sciences, Pondicerry University, Puducherry 605014, India.*
[3] *Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei 11031, Taiwan.*
[4] *Department of Environmental Health Sciences and Organismal Biology, Ohio State University, Ohio 43212, USA.*
[5] *Department of Biology, Jackson State University, Jackson, MS 39217, USA.*

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Abstract

The present investigation was carried out to understand the proximate composition, and the secondary phytochemical compounds and their metabolites present in algae, spirulina, chlorella and azolla. Spirulina contains a rich source of crude protein (58.94%), followed by chlorella (47.08%) and azolla (21.82%). The content of crude fat was higher in chlorella (5.68%), followed by azolla (4.00%) and spirulina (1.54%). The crude fibre level was found to be higher in azolla (26.21%), followed by chlorella (2.87%) and spirulina (1.00%). The gross energy was higher in chlorella (4,377 kcal/kg), followed by spirulina (4,183 kcal/kg) and azolla (3,295 kcal/kg). The secondary phytochemical analysis revealed presence of alkaloids, terpenoids, flavonoids, tannis, polyphenols, saponins, cardiac glycoside and quinones in these algae at different levels. The GC-MS analysis showed presence of 11 metabolic compounds, of which 5 from petroleum etheric extract and 6 from methanolic extract. The chlorella showed presence of 14 secondary metabolites, of which 5 from petroleum etheric extract and 9 from methanolic extract. In azolla, there were 13 secondary metabolic compounds, of which 6 from petroleum etheric extract and 7 from methanolic extract. Therefore, a total of 38 secondary metabolites, which includes 12 different bioactive principle compounds, were identified in these algae. Docosane was found to be present both in spirulina and azolla. Similarly, Tetradecanoic acid was found to be present both in spirulina and chlorella. The remaining ten bioactive principle compounds are species specific, such as 9-octadecenal; and Hexadecanoic acid are for spirulina, Dodecanoic acid; 6,7-Dimethoxy-2-tetralone; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Phenol, 2-methoxy-5-(1-propenyl)-,(E); Cycloheptane,1,3,5-tris(methylene); 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol; and Loliolide are for chlorella, and cis-2-[2-(hydroxymethyl)cyclopentyl]ethanol is for azolla. Thus, these algae are rich in natural organic compounds. Their pharmaceutical and nutraceutical potencies need to be studied.

Keywords: Spirulina; Chlorella; Azolla; Phytochemicals; Bioactive Compounds

1. Introduction

Microalgae belong to Protista, are a collection of unicellular or basic multicellular photosynthetic organisms. A variety of microalgae (red, green, brown and blue green in colors) makes a possibly rich source for different bioactive compounds with applications in nutraceutical, cosmetic, pharmaceutical, and medicinal industries [1]. Moreover, they have a high reproductive potential and grow very fast, hence they serve as model organisms in biotechnological studies

* Corresponding author: Bhavan Periyakali Saravana
Department of Zoology, Bharathiar University, Coimbatore 641046, Tamil Nadu, India.

[2]. Microalgae are one of the richest sources of protein in addition to polysaccharides, carotenoids, phycobiliproteins, polysaccharides, vitamins and sterols [3]. Like dark leafy green veggies, for example spinach, spirulina is the best source of magnesium. Feeding microalgae to elderly people or animals has been demonstrated to protect from age-dependent diseases, particularly cardiac hypertension or hyper-lipidemia [4].

The blue-green micro alga, *Arthospira platensis* (spirulina) consists of different phytochemicals of medicinal applications including cancer, tuberculosis and inflammation [5, 6, 7]. It is used in weight loss therapy and also used to reduce fatigue, boost body energy, lower bad cholesterol (LDL) and triglyceride levels, and stimulate the immune system for fighting viral infections. Spirulina is rich in protein, vitamins (vitamin A, vitamin B-12, and vitamin E), carotenoids, iron, amino acids and fatty acids to boost the overall health and provide anti-aging, anti-inflammatory and anti-cancer effects. It reduces blood pressure. With a powerful chelating agent, spirulina reaches deep into bodily tissues and takes out toxins and helps them up for excretion. It tones the skin and encourages cell turnover to promote a more youthful-looking complexion [8] and radiant appearance, as well as preventing acne build up, inflammation and swelling. Spirulina fights free radicals and, therefore, can prevent skin damage that can lead to wrinkles and signs of aging. Spirulina contains a high concentration of zeaxanthin, an important nutrient linked to ocular health. As such, it may help to reduce the risk of cataracts and age-related macular degeneration. Besides consumption, this alga is used as an ingredient in shampoos and conditioning treatments for helping hair growth and slows down the appearance of gray/white hairs. Spirulina contains thyroid-supporting minerals like iodine, and the amino acid tyrosine, which act together and make up one form of thyroid hormone. Spirulina protects the liver, facilitates healthy digestion, and can help to control diabetes also. *Arthrospira* is also used in aquaculture as it provides food for zooplankton, fish, crustacean, shellfish and bivalves. Due to lack of cellulose in its cell wall, 85–95% is assimilated by the organism. Its cells have mucopolymermurein that is easily digested by the digestive enzymes secreted by fish [9]. *A. platensis* can enhance the natural mucous layer of the skin resulting in a shiny appearance of the fins and skin [10]. It enhances the colour of red tilapia [11].

Chlorella vulgaris (chlorella) is one of the most cultivated eukaryotic green microalga. It is extensively used in the pharmaceutical and beauty care products industry and as a health food and feed supplement [12, 13]. *C. vulgaris* has been reported to have immune-modulating and anticancer properties [14, 15]. Chlorella is highly nutritious it has 60% protein, all essential amino acids, rich in n-3 long chain polyunsaturated fatty acids, vitamins (pro vitamin A, vitamins C, B_1, B_2,B_5, B_6, B_{12}, Biotin, E, D and K) and minerals includes iron, potassium, magnesium, phosphorus and calcium, and carotenoids like beta-carotene and lutein [16]. These nutrients fight cell damage in our bodies and help reduce the risk of diabetes, cognitive disease, heart problems, and cancer. Chlorella has a very good antioxidant property and also inhibits lipid peroxidation [17]. It is believed that chlorella has many health benefits, it helps to get rid of toxins, keeps our immune system strong (by production of powerful immune chemical, interferon), boost our brain power (due to B12), makes us feel young and energetic with glowing, beautiful skin, promotes healthy liver, eases digestion, relieves constipation, and stabilizes blood sugar and blood pressure (15 Health Benefits of Chlorella: Matthew DiLorenzo, DC: Chiropractor. https://www.dilorenzochiropractic.com/blog/15-health-benefits-of-chlorella). The tryptophan found in chlorella is a sleep-enhancing amino acid used by the brain to produce neurotransmitters, serotonin and melatonin that help us relax and go to sleep [18, 19]. Chlorella bio transforms estradiol into estrone, helps in clearing estrone and leaving estriol (the antioxidant form of estrogen), alone [20]. *Chlorella vulgaris* has a significant ability to bioaccumulate testosterone [21].

The aquatic macro alga, *Azolla pinnata* (azolla) is called floating fern, mostly grown in ponds and backwaters of rivers. It proliferates without inorganic nitrogen fertilization. It is a symbiotic nitrogen fixing plant [22, 23]. Actually it grows in symbiosis with a photosynthetic bacterium that takes nitrogen from the atmosphere and shares it with the fern. Azolla has wide applications such as fertilizer (a green manure), animal fodder, antioxidant, antiviral, antimicrobial, anticarcinogenic, anti-inflammatory, anti-diabetic, hepato- and gastro-protective, neuro-protective, cardio-protective and anti-hypertensive effects due to its high content in proteins, flavonoids, hormones, alkaloids, phenols, triterpenoid derivatives essential amino and fatty acids, vitamins (vitamin A, vitamin B12, Beta carotene), growth promoter intermediaries and minerals [24-26]. Azolla has been suggested as a foodstuff for human consumption, however, no long-term studies of the safety of eating Azolla have been made. *A. pinnata* in combination with other natural feed ingredients can be beneficial to fish quail, rabbit, duck, pig etc [27]. It is used to control mosquitoes, by covering the water surface as a green mat over it. It may also be used for the production of hydrogen fuel, the production of biogas and the reduction of ammonia volatilization which accompanies the application of chemical nitrogen fertilizer. Azolla can fertilize itself. *A. pinnata* can clean up the environmental pollutants in the phytoremediation of domestic and industrial wastewater [28]. In the view of the above mentioned facts, this study was aimed to analyze the primary and secondary phytochemicals of spirulina, chlorella and azolla, in order to assess their nutritional and medicinal potentials.

2. Material and methods

2.1. Collection and identification of spirulina, chlorella, and azolla

The pure cultures of *A. platensis* and *C. vulgaris* were collected from M/S Ecolive Exports, Ayalur, Gobichettipalayam, Tamil Nadu, and India. Similarly, the pure culture of *A. pinnata* was collected from the azolla cultivation and Research Centre, Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu, India. The specimen/ herbarium of these species were morphologically identified and authenticated with the Botanical Survey of India, Coimbatore. These algae were mass cultured under laboratory conditions.

2.2. Preparation of algal powders

The spirulina, chlorella, and azolla were sun dried for three days until they became crispy while retaining their greenish coloration. The dried algae were then milled using a mixie to produce algae meals. The powdered sample was stored in sterilized containers for further use.

2.3. Analysis of proximate compositions of the primary phytochemicals of algae

Powders of spirulina, chlorella, and azolla were subjected to proximate composition analysis (crude protein, etheric extract, total ash, moisture and total carbohydrate) following the methodology of Castell and Tiews [29] as given in AOAC [30].

2.4. Preparation of solvent extracts of algae

The powdered samples of spirulina, chlorella, and azolla (75g each) were taken and packed in Whatmann No. 1 filter paper and put into soxhlet apparatus. The extracts were successively soaked with 300 ml (1:4 w/v) of a polar solvent, methanol (99.9% purity, Changshu Yangyuan Chemicals, China), and a non-polar solvent, petroleum ether (99.98% purity, SRL Pvt. Ltd. India) individually and separately for 6-9 h each (30 to 36 cycle) until a clear colorless solution was obtained. The extracts were filtered by using double layered muslin cloth and concentrated at 40-50 °C using rotary vacuum evaporator (ROTAVAP). The extracts obtained were vacuum-dried under 40°C and used for further investigation. The extracts obtained appeared as dark green, gummy solid.

2.5. Qualitative screening and quantitative analysis of secondary phytochemicals

The qualitative tests were used to identify the presence of specific constituents, and quantitative tests were used to determine the amount of active constituents present. The extracts were subjected to analysis to detect the presence of following biomolecules using the standard qualitative procedures as described by Trease and Evans [31]. Each secondary phytochemical deducted was quantitatively estimated, particularly total flavonoids, total tannins and total phenols by following the methods of Brighente *et al*., [32], Julkunen-Titto [33] and Siddhuraju *et al*., [34], respectively.

2.6. Analysis of secondary phytochemical metabolites

The petroleum etheric and methanolic extracts of spirulina, chlorella, and azolla were subjected to Gas Chromatography-Mass Spectrum **(**GC-MS) analysis (Thermo GC-trace ultra ver-5.0; Thermo MS-DSQ-II; ZB 5-MS capillary standard non-polar column (30 mts, 0.25 mm id, 0.25 µm film) for identification of different secondary phytochemical metabolic compounds, at South India Textile Research Association (SITRA), Coimbatore, Tamil Nadu, and India. The relative percentage constituent was expressed as percentage with peak area normalization. Peaks resolved with relative abundance of 0-100 were considered as major compounds. To show the minor peaks, the chromatogram was magnified. Identification of various components present in these extracts were assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. National Institute of Standard and Technology (NIST4) and WILEY9 [**35**] on-line library sources were used for matching the identified components.

3. Results and discussion

3.1. Proximate composition of primary phytochemicals

The proximate compositions of spirulina powder contains, crude protein (58.94%), crude fat (1.54%), crude fibre (1.0%), ash (12.22%), moisture (9.62%), total carbohydrate (16.68%), gross energy (4183 kcal/kg), sand and silica (0.31%), and mineral salts of calcium (0.30%), phosphorous (1.08%) and total salt (1.17 %) [Table 1]. The proximate compositions of chlorella powder contains, crude protein (47.08%), crude fat (5.68%), crude fibre (2.87%), ash

(7.82%), moisture (9.91%), total carbohydrate (26.64%), gross energy (4377 kcal/kg), sand and silica (0.80%), and mineral salts of calcium (0.80%), phosphorous (0.96%) and total salt (1.59 %) [Table 1]. According to Belasco [36], *C. vulgaris* contains 10% minerals and vitamins, 5% fibre, 20% carbohydrates, 20% fat, and 45% protein (w/w, dry basis). The proximate compositions of azolla powder contains, crude protein (21.82%), crude fat (4.00%), crude fibre (26.21%), ash (22.81%), moisture (9.27%), total carbohydrate (15.79%), gross energy (3295 kcal/kg), sand and silica (3.74%), and mineral salts of calcium (2.30%), phosphorous (1.19%) and total salt (4.08%) [Table 1].

When compared all the three alage, spirulina contains a rich source of crude protein (58.94%), followed by chlorella (47.08%) and azolla (21.82%). The content of crude fat was higher in chlorella (5.68%), followed by azolla (4.00%) and spirulina (1.54%). The crude fibre level was found to be higher in azolla (26.21%), followed by chlorella (2.87%) and spirulina (1.00%). The content of ash was higher in azolla (22.81%), followed by spirulina (12.22%) and chlorella (7.82%). The level of moisture was almost equal in all the three algae (9.27-9.91%). The total carbohydrate level was found to be higher in chlorella (26.64%), followed by spirulina (16.68%) and azolla (15.79%). The gross energy was higher in chlorella (4,377 kcal/kg), followed by spirulina (4,183 kcal/kg) and azolla (3,295 kcal/kg) [Table 1].

Table 1 Proximate composition of primary phytochemicals and mineral contents of algae, *A. platensis*, *C. vulgaris* and *A. pinnata*

Proximate composition (% on dry wt. basis)	***A. platensis***	***C. vulgaris***	***A. pinnata***
Crude protein	58.94	47.08	21.82
Ether extract (crude fat)	1.54	5.68	4.00
Crude fibre	1.00	2.87	26.21
Ash	12.22	7.82	22.81
Moisture	9.62	9.91	9.27
Total nitrogen free extract (carbohydrate)	16.68	26.64	15.79
Gross energy (kcal/kg)	4183	4377	3295
Sand and silica (acid insoluble ash)	0.31	0.80	0.56
Calcium	0.30	0.80	2.30
Phosphorous	1.08	0.96	1.19
Total salt	1.17	1.59	4.08

3.2. Secondary phytochemicals

The preliminary phytochemical screenings of spirulina, chlorella, and azolla extracted with different solvents are presented in Table 2. The petroleum etheric extract of spirulina showed the luxurious presence of alkaloids, moderate presence of terpenoids and flavonoids, and poor presence of tannins and polyphenol. The methanolic extract of spirulina showed the luxurious presence of tannins and polyphenols, and moderate presence of saponins, cardiac glycosides and quinones. The petroleum etheric extract of chlorella, showed the luxurious presence of alkaloids, moderate presence of terpenoids and flavonoids, and poor presence of tannins and polyphenol. The methanolic extract of chlorella showed the luxurious presence of polyphenols and saponins, and moderate presence of tannins, cardiac glycosides and quinones.

It has been reported that the crude extract of azolla contains phyto-constituents such as tannins, phenolic contents and flavonoids which are responsible for antioxidant activity [37]. *A. pinnata* contains the flavonoids 3-Deoxyanthocyanins [38]. It possesses antimicrobial [39], hepatoprotective [40] and antiulcer effects [25]. In the present study, the petroleum etheric extract of azolla showed the luxurious presence of terpenoids, moderate presence of alkaloids, tannins and polyphenol, and poor presence of flavonoids. The methanolic extract of azolla showed the luxurious presence of polyphenols and saponins, and moderate presence of tannins and cardiac glycosides. In total, the presence of alkaloids, terpenoids, flavonoids, tannins, polyphenols, saponins, cardiac glycosides and quinons have been confirmed in spirulina, chlorella, and azolla.

In spirulina, the quantity of flavonoids was found to be maximum in petroleum etheric extract, whereas the quantity of tannins and phenols were found to be maximum in methanolic extract. The similar trends for these three primary

compounds were seen in chlorella, and azolla as well [Table 3]. Among these three primary compounds, quantity wise, tannins was the maximum followed by phenols and flavonoids in sprulina, whereas in chlorella, and azolla, total phenols were found to be maximum, followed by tannins and flavounoids [Table 3].

Table 2 Qualitative detection of secondary phytochemicals in petroleum etheric and methanolic extracts of *A. platensis*, *C. vulgaris*, and *A. pinnata*

Secondary Phytochemicals	**Algae**					
	A. platensis		***C. vulgaris***		***A. pinnata***	
	Petroleum ether	**Methanol**	**Petroleum ether**	**Methanol**	**Petroleum ether**	**Methanol**
Alkaloids	+++	ND	+++	ND	++	ND
Terpenoids	++	ND	++	ND	+++	ND
Flavonoids	++	ND	++	ND	+	ND
Tannins	+	+++	+	++	++	++
Polyphenols	+	+++	+	+++	++	+++
Saponins	ND	++	ND	+++	ND	+++
Cardiac glycosides	ND	++	ND	++	ND	++
Quinones	ND	++	ND	++	ND	+

+, poorly present; ++, moderately present; +++, luxuriantly present; -, absent

Table 3 Quantity of secondary phytochemicals present in petroleum etheric and methanolic extracts of *A. platensis*, *C. vulgaris* and *A. pinnata*

Secondary Phytochemicals (Mg/g dry wt.)	**Algae**					
	A. platensis		***C. vulgaris***		***A. pinnata***	
	Petroleum ether	**Methanol**	**Petroleum ether**	**Methanol**	**Petroleum ether**	**Methanol**
Total flavonoids	4.83 ±0.05	1.42±0.05	4.48 ±0.05	1.23 ±0.13	3.56 ±0.25	1.13 ±0.35
Total tannins	2.98 ±0.16	6.86 ±0.35	2.66 ±0.28	4.97 ±0.27	2.87 ±0.04	4.64 ±0.26
Total phenols	2.74 ±0.45	5.93 ±0.25	2.14 ±0.45	5.84 ±0.28	2.18 ±0.12	5.54 ±0.26

Each value is mean ± standard deviation of three individual observations (n=3)

Algae are worthy natural sources against infectious agents [40, 41]. The secondary phytochemicals serve as health tonic in aquaculture nutrition [42]. Akaloids (morphine, atropine, quinine etc.,) are biologically and therapeutically active compounds that have numerous medical applications [43]. In the present study, spirulina, chlorella, and azolla showed presence of alkaloids. However, methanolic extracts showed their absence.

Terpenoids are anti-parasitic, anti-fungal, anti-bacterial, anti-viral, anti-inflammatory, anti-malarial, anti-allergenic, anti-spasmodic, anti-hyperglycemic, anti-cancer, and immune modulatory properties [44-46]. Terpenoids have insecticidal properties as well [47]. In the present study, spirulina, chlorella, and azolla showed presence of terpenoids. However, methanolic extracts showed their absence.

Flavonoids, the major group of phenolic compounds are famous for containing a broad spectrum of chemical and biological activities [48]. Flavonoids act as antipyretic, and antioxidants. They scavenging free radicals, protect against inflammatory disorders, allergies, diarrhea, platelet aggregation, ulcers, hepatotoxins, cancer and heart disease, possess anti-ageing, anti-bacterial, anti-viral and vasodilator, analgesic, spasmolytic properties due to the presence of epicatechin, quercetin and luteolin [49-52]. In the present study, spirulina, chlorella, and azolla showed presence of

flavonoids. However, methanolic extracts showed their absence. The presence of flavonoids in *Tetraselmis* and *Oscillatoria* have been reported by Rajendran *et al*., [53] by using four solvents extracts namely, acetone, ethanol, methanol and chloroform.

Tannins possess strong antioxidant, antiviral, antibacterial, anti-inflammatory and antioxidant properties for possible therapeutic applications [54, 55]. They are astringent substances, having the capacity to combine with tissue proteins and precipitate them. Therefore they are used as mild antiseptics in treatment of diarrhea, and to check small haemorrhages [56]. In the present study, spirulina, chlorella, and azolla showed presence of tannins. Many tannins containing drugs are used in the treatment of piles, inflammation, burns and as astringent [57].

Phenols are associated with diverse functions, including photosynthesis, protein synthesis, allelopathy and nutrient uptake [58]. Phenolics have antimicrobial, antiviral, cytotoxic, anti-mutagenic and anti-carcinogenic activities [59-61]. Phenolic compounds are responsible for blockage of specific enzymes that cause inflammatory disorders. They also protect platelets from clumping through modification of the prostaglandin pathways [62]. In the present study, spirulina, chlorella, and azolla showed presence of phenols. Phenolic compounds act as antioxidants by chelating metal ions, banning radical formation and enhancing the antioxidant endogenous system.

Saponins are bioactive compounds with a wide range of medicinal properties, including haemolytic, hypo-cholesterolemic, anti-carcinogenic, anti-inflammatory, and antibacterial and antioxidant activities [63, 64]. It has been reported that saponins can kill tumor cells by triggering tumor cell death via different signaling pathways, by activating death receptors [65], targeting mitochondria [66], and inducing oxidative stress [67]. The present study showed presence of saponins in spirulina, chlorella, and azolla. However, petroleum etheric extracts showed their absence.

The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal [68]. Cardiac glycosides and catecholamine are agents of choice in treatment of congestive cardiac failure [69]. The present study showed presence of cardiac glycosides in spirulina, chlorella, and azolla. However, petroleum etheric extracts showed their absence.

Quinones are compounds very much used in pharmacopoeia in the treatment of malaria [70] and more recently of tumours [71]. They are a good source of anti-inflammatory, antibacterial and immune modulating potentials [72]. The present study showed presence of quinones in spirulina, chlorella, and azolla. However, petroleum etheric extracts showed their absence.

It has been reported that phenols, tannins and flavonoids found in *Spirulina platensis* considered as potential therapeutic agents that counteract some diseases depending on their free radical scavenging activity [73-75]. *A. platensis* contains antioxidants, carbohydrates, pigments (zeaxanthin, myxoxanthophyll), proteins, polyunsaturated fatty acids, minerals, and it serves as a feedstock for biofuels [76-80]. It has been reported that *Spirulina* is well known for its high content of proteins (60–70%), vitamins such as vitamin B12 (8 ppm) and provitamin A (0.2%), minerals such as iron (0.1%), and polyunsaturated fatty acids especially the ω-6-fatty acids (up to 29.4–31.5% of the total fatty acids) [81-83].

According to Jong-Yuh and Mei-Fen [84] *C. vulgaris* is a good food source and contains protein (60%), lipids (20%), chlorophylls and β carotenes, soluble vitamins, choline, dietary fiber and essential minerals like iron, calcium, potassium, magnesium and phosphorous [85, 86]. It is considered as a good antioxidant source and bioactive secondary metabolites [87]. Chlorella and spirulina are generally regarded as safe for animal and human consumption and can be used as a source of nutraceutical compounds for its intervention in animal or human health problems [88, 89]. Many researchers have focussed them as alternative sources of protein, vitamins and minerals, and it acknowledged the nutraceutical abilities of these microalgae. Bioactive compounds derived from microalgae can be sourced directly from primary metabolism, such as proteins, fatty acids, vitamins, and pigments, or can be synthesized from secondary metabolism (polyphenols and other antioxidants) [90].

A. pinnata grows in association with the blue-green algae, *Anabaena azollae*, and is considered to be a promising feed because of its good nutritive value, the ease of cultivation, and high productivity [91, 92]. *A. pinnata* appears as a good source of protein (25% to 30%) and contains almost all essential amino acids that are superior to wheat bran, maize, offals etc. [93, 94]. This plant is naturally rich in minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese, zinc etc., apart from appreciable quantities of vitamin A, precursor beta-carotene, and vitamin B12 [95]. It has also been reported that Azolla contains carotenoids, some probiotics and biopolymers [96]. Due to the nutritional properties, it is used for feeding in animals [97-100].

3.3. Secondary phytochemical metabolites

GC-MS analysis of spirulina showed presence of 11 secondary metabolic compounds, of which 5 from petroleum etheric extract and 6 from methanolic extract. The chlorella showed presence of 14 compounds, of which 5 from petroleum etheric extract and 9 from methanolic extract. In azolla, there were 13 compounds, of which 6 from petroleum etheric extract and 7 from methanolic extract [Table 4; Figures 1-6]. The details of bioactive compounds detected in these algal extracts are presented in Table 5.

The petroleum etheric extract of spirulina showed two bioactive compounds, Docosane, and 9-Octadecenal [Table 5; Figure 1], its methanolic extract also showed two compound Tetradecanoic acid, and Hexadecanoic acid [Table 5; Figure 2]. The petroleum etheric extract of chlorella revealed the presence of three bioactive compounds, Dodecanoic acid, 6, 7-Dimethoxy-2-tetralone and 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol [Table 5; Fig. 3], whereas the methanolic extract showed five bioactive compounds, Phenol, 2-methoxy-5-(1-propenyl)-, (E), Cycloheptane, 1, 3, 5-tris (methylene), 4-(6, 6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol, Tetradecanoic acid and Loliolide [Table 5; Figure 4]. The petroleum etheric and methanolic extracts of azolla showed presence one bioactive compound in each, Docosane [Table 5; Figure 5] and cis-2-[2-(hydroxymethyl) cyclopentyl] [Table 5; Figure 6], respectively. Therefore, collectively twelve bioactive principle compounds (Docosane; Tetradecanoic acid; 9-octadecenal; Hexadecanoic acid; Dodecanoic acid; 6,7-Dimethoxy-2-tetralone; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Phenol, 2-methoxy-5-(1-propenyl)-, (E); Cycloheptane, 1,3,5-tris(methylene); 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol; Loliolide; and cis-2-[2-(hydroxymethyl)cyclopentyl] ethanol) were detected from *A. platensis*, *C. vulgaris* and *A. pinnata*. Among the twelve bioactive principle compounds, Docosane was found to be present both in *A. platensis* and *A. pinnata*. Similarly, Tetradecanoic acid was found to be present both in *A. platensis* and *C. vulgaris*. Among the remaining ten bioactive principle compounds, 9-octadecenal; and Hexadecanoic acid represent *A. platensis*, Dodecanoic acid; 6,7-Dimethoxy-2-tetralone; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Phenol, 2-methoxy-5-(1-propenyl)-, (E); Cycloheptane, 1,3,5-tris(methylene); 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol; and Loliolide represent *C. vulgaris*, and cis-2-[2-(hydroxymethyl)cyclopentyl] ethanol represents *A. pinnata*.

3.3.1. Docosane (C22H46)

Its other names are: n-Docosane; Heneicosane; and Methyl-Dokosan. It is an alkane having antibacterial properties [101, 102]. It is used to synthesize structural composites with thermal energy storage/release capability [103, 104]. Docosane is a hydrocarbon lipid molecule that is hydrophobic, practically insoluble in water, and relatively neutral. It has a waxy taste. It is found in highest concentrations in lemon balms detected in all spices, lindens, papaya, and sunflowers. Docosane is found in the essential oils from plants, such as dill (PMID: 25154406), *Periploca laevigata* Aiton subsp. *angustifolia* (Apocynaceae) (PMID: 22439883).

3.3.2. Tetradecanoic acid (C14H28O2)

Its other names are: Myristic acid; n-Tetradecanoic acid; and Crodacid. It is also a common saturated fatty acid. Its salts and esters are commonly referred to as myristates. It was first isolated from nutmeg (*Myristica fragrans*) by Playfair Lyon [105]. Both lauric acid and myristic acid have positive effects on high density lipid (good cholesterol) level [106, 107]. The lauric and myristic acids have high hydrophobicity and act as lipid anchors in bio membranes of eukaryotic cells. Tetradecanoic acid used as an antioxidant, lubricant, hypercholesterolemic, cancer-preventive, and cosmetic [108-110].

3.3.3. 9-octadecenal (C18H34O)

Its synonymous names are: (E)-Octadec-9-enal; Octadecenyl aldehyde; and (9E)-9-Octadecenal. It belongs to the class of organic compounds known as long chain fatty aldehydes. It is an unsaturated fatty acid (Oleic acid (omega-9)) found in animal and vegetable oils, such as olive oil, avocados, almonds, peanuts, sesame oil, pecans, pistachio nuts, cashews, hazelnuts, macadamia nuts, etc. It occurs naturally in greater quantities than any other fatty acid. It lowers heart attack risk and atherosclerosis, and aids in cancer prevention. It is essential but technically not an EFA (essential fatty acid), because the human body can manufacture a limited amount [111]. It is used to flavour baked goods, candy, ice cream and sodas [112].

Table 4 Secondary phytochemical metabolic compounds detected in petroleum etheric and methanolic extracts of *A. platensis*, *C. vulgaris* and *A. pinnata*, through GC-MS.

Algae	Petroleum ether			Methanol		
	Peak RT	**Compounds**	**Molecular formula**	**Peak RT**	**Compounds**	**Molecular formula**
A. platensis	4.02	Docosane (Alkane)	$C_{22}H_{46}$	6.86	Formic acid, butyl ester	$C_5H_{10}O_2$
	10.00	5-Iodo-5-(1'-naphthyl)-1-phenoxypent-4-en-2-ol	$C_{21}H_{19}IO_2$	14.92	2-Bromolauric acid	$C_{12}H_{23}BrO_2$
	14.82	2-Propanone	C_3H_6O	24.20	Tetradecanoic acid (myristic acid)	$C_{14}H_{28}O_2$
	17.03	9-Octadecenal (Oleic acid)	$C_{18}H_{34}O$	26.92	Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$
	28.04	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	29.84	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	$C_{27}H_{54}$
	--	--	--	30.96	N-Ethyl-N-methyl-4-nitrosobenzenamine	$C_9H_{12}N_2O$
C. vulgaris	10.62	2-Methyl-2-[2-dimethyl (phenyl)silylprop-2-en-1-yl] tetrahydrofuran	$C_{16}H_{24}OSi$	3.91	Phosphonic acid, phenyl-, methyl phenyl ester	$C_{13}H_{13}O_3P$
	15.02	Z-Phenyl(4-pyrimidinyl) methanoneoxime	$C_{11}H_9N_3O$	7.14	5-hydroxy-1-deutero-1,2-pentadiene	C_5H_7DO
	17.85	Dodecanoic acid*	$C_{12}H_{24}O_2$	10.02	Phenol, 2-methoxy-5-(1-propenyl)-, (E)	$C_{10}H_{12}O_2$
	21.72	6,7-Dimethoxy-2-tetralone	$C_{12}H_{14}O_3$	15.00	2-Bromolauric acid	$C_{12}H_{23}BrO$
	28.80	3,7,11,15-Tetramethyl-2-hexadecen-1-ol*	$C_{20}H_{40}O$	18.13	Cycloheptane, 1,3,5-tris(methylene)	$C_{10}H_{14}$
	--	--	--	22.24	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol	$C_{14}H_{22}O$
	--	--	--	26.91	5,5,5-Trifluoro-2-methyl-4-trifluoromethyl-1,3-pentadiene	$C_7H_6F_6$
	--	--	--	30.05	Tetradecanoic acid	$C_{14}H_{28}O_2$
	--	--	--	32.90	Loliolide	$C_{11}H_{16}O_3$
A. pinnata	13.02	5,5-Dideuteriomethoxycyclohexane	$C_7H_{12}D_2O$	13.00	1,2-Dihydro-1,4-diphenylphthalazine	$C_{20}H_{16}N_2$
	17.58	Piperidine, 1,4-dimethyl	$C_7H_{15}N$	18.52	Methyl hydrogen 2,2'-dimethoxy-1,1'-binaphthalene-3,3'-dicarboxylate	$C_{25}H_{20}O_6$
	22.15	Docosane*	$C_{22}H_{46}$	29.08	4-Benzyl-2,4,6-triphenyl-4H-thiopyran	$C_{30}H_{24}S$
	31.09	(S)-(4S,5S)-4-Methoxymethyl-5-phenyl-2-{(Z)-[2-(N-1-	$C_{27}H_{28}N_2O_2$	32.18	2',5'-Bis(bromomethyl)-1,1':4',1"-terphenyl	$C_{20}H_{16}Br_2$

		phenylethylamino)-2-phenyl] ethenyl}-2-oxazoline				
	33.78	5-Iodo-5-(1'-naphthyl)-1-phenoxypent-4-en-2-ol	$C_{21}H_{19}IO_2$	35.04	1,3,4-Thiadiazol-2-amine, 5-(pentylthio)	$C_7H_{13}N_3S_2$
	36.12	8,9:14,15-dibenzo-2,4,6,16,18,20-docosahexaene-10,12-diynedial	$C_{30}H_{22}O_2$	36.10	cis-2-[2-(hydroxymethyl)cyclopentyl] ethanol*	$C_8H_{16}O_2$
	--	--	--	42.43	Dethiobiotin	$C_{10}H_{18}N_2O$

RT, Retention time; P, Probability; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

Table 5 Bioactive secondary phytochemical metabolic compounds detected in petroleum etheric and methanolic extracts of A. platensis, C. vulgaris and A. pinnata, through GC-MS

Algae	**RT**	**Bioactive compounds**		**Biological properties (by literature only)**
		Petroleum ether	**Methanol**	
A. platensis	4.02	Docosane	--	Antibacterial activity (Waage and Hedin, 1985).
	17.03	9-Octadecenal	--	Antimicrobial and anti-inflammatory activities (Chinonye *et al*., 2019).
	24.20	--	Tetradecanoic acid	Antioxidant, anticancer, hypercholesterolemic, larvicidal, repellant and nematicide activities (Diana and Parthipan, 2015; Priya and Subhashini, 2016).
	26.92	--	Hexadecanoic acid	Anti-inflammatory, antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, haemolytic, 5-alpha reductase inhibitor, mosquito larvicide activities (Kumar *et al*., 2010; Awa *et al.,* 2012).
C. vulgaris	10.02	--	Phenol, 2-methoxy-5-(1-propenyl)-, (E)	Anesthetic, allergenic, antibacterial, anti-inflammatory, antioxidant, anti-pyretic, anti-bacterial, anti-septic and anti-cancer activities (Hadi *et al.,* 2016).
	17.85	Dodecanoic acid	--	Anti-microbial, nematicide and pesticide activities (Markkas and Govindharajalu, 2015).
	18.13	--	Cycloheptane, 1,3,5-tris (methylene)	Aroma chemical and aroma precursor (deJong and Heijmen, 1980).
	21.72	6,7-Dimethoxy-2-tetralone		Antiseptic and anesthetic activities (Sulochana *et al.,* 2016).
	22.24	--	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol	Melamine, and dyes effects (Kumar *et al.,* 2014).
	28.80	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	--	Antimicrobial, anti-inflammatory, anti-cancer, diuretic, anti-tuberculosis, insecticidal, anti-oxidant activities (Das and Himaja, 2014; Parthipan *et al.,* 2015).

	30.05	--	Tetradecanoic acid	Antioxidant, anticancer, hypercholestrolemic, larvicidal, repellent, nematicide activities (Diana and Parthipan, 2015; Priya and Subhashini, 2016).
	32.90	--	Loliolide	Antioxidant and cell protective (Yang *et al.,* 2011).
A. pinnata	22.15	Docosane	--	Antibacterial activity (Waage and Hedin 1985).
	36.10	--	Cis-2-[2-(hydroxymethyl) cyclopentyl] ethanol	Antimicrobial activity (Ramya *et al.,* 2015).

RT, Retention time; P, Probability; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

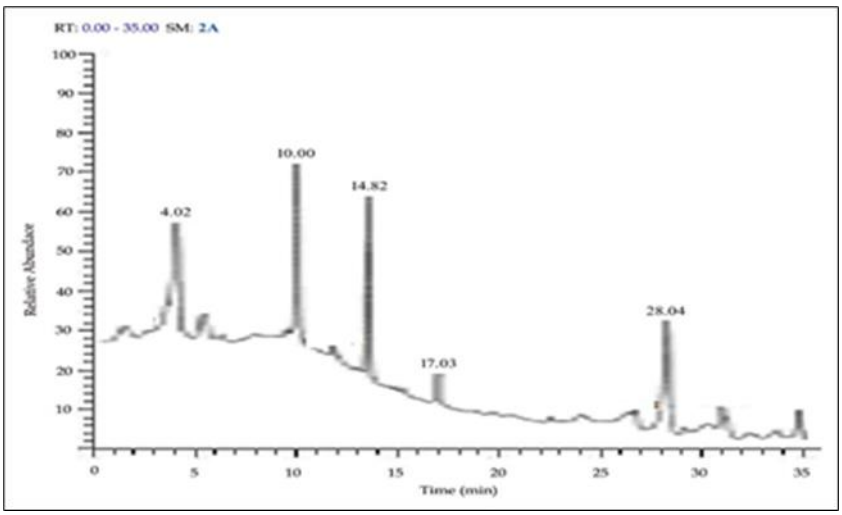

Figure 1 GC-MS chromatogram of *A. platensis* extracted with petroleum ether (Relative abundance, 0-100)

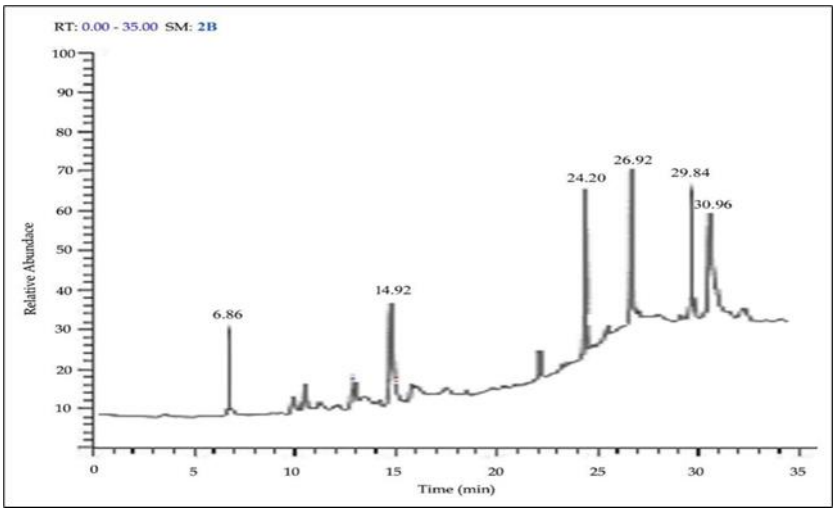

Figure 2 GC-MS chromatogram of A. platensis extracted with methanol (Relative abundance, 0-100)

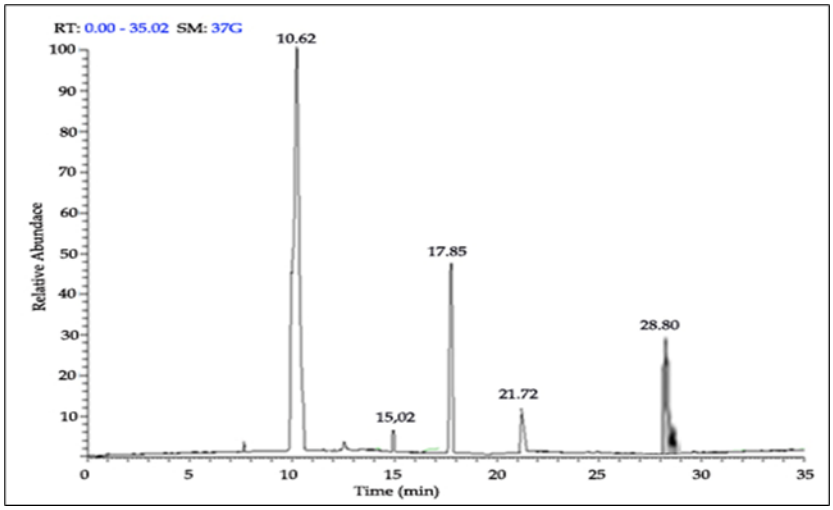

Figure 3 GC-MS chromatogram of *C. vulgaris* extracted with petroleum ether (Relative abundance, 0-100)

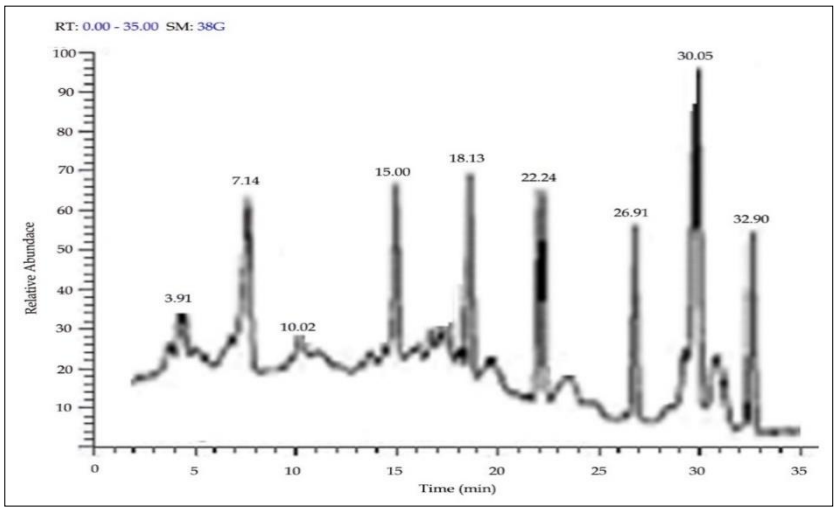

Figure 4 GC-MS chromatogram of *C. vulgaris* extracted with methanol (Relative abundance, 0-100)

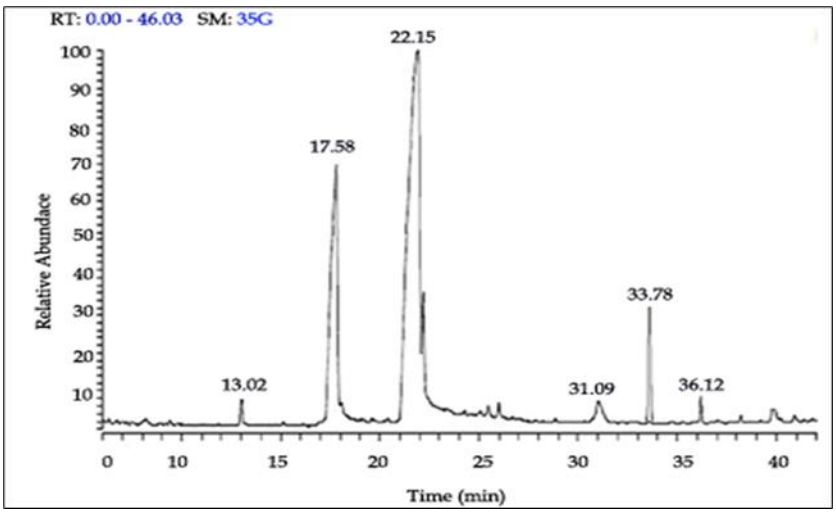

Figure 5 GC-MS chromatogram of *A. pinnata* extracted with petroleum ether (Relative abundance, 0-100)

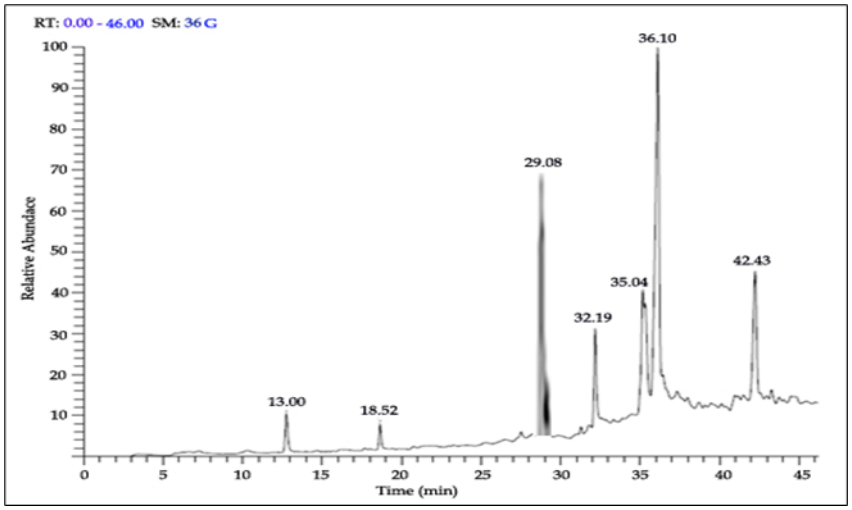

Figure 6 GC-MS chromatogram of *A. pinnata* extracted with methanol (Relative abundance, 0-100)

3.3.4. Hexadecanoic acid (C16H32O2)

Its other names are: Palmitic acid; Cetylic acid; and Palmitate. It is the most common saturated fatty acid found in animals, plants and microorganisms [113, 114]. Palmitates are the salts at physiologic pH (7.4), and esters of palmitic acid has the ability as antioxidant, flavor, antifibrinolytic, hypocholesterolemic, anti-androgenic, lubricant, hemolytic, 5-alpha reductase inhibitor, nematicide, and anti-alopecic [109]. The excess carbohydrates in the body of humans and animals are converted to palmitic acid. It is the first fatty acid produced during fatty acid synthesis and is the precursor to longer fatty acids. Palmitate negatively feeds back on acetyl-CoA carboxylase, which is responsible for converting acetyl- CoA to malonyl-CoA, which in turn is used to add to the growing acyl chain, thus preventing further palmitate generation. Excess consumption of palmitic acid increases the risk of developing cardiovascular disease due to increase in low density lipid level in the blood [115]. Among all fatty acids, palmitic acid has the strongest effect in boosting the metastatic potential of CD36+ metastasis-initiating cells in mouse models [116].

3.3.5. Dodecanoic acid (C12H24O2)

Its other names are: Lauric acid; N-Dodecanoic acid; and Dodecylic acid. It is a common saturated fatty acid, a component of triglycerides found in coconut and palm kernel oils, and leaves extract of *Avicennia A. marina*. It is also found in human breast milk, cow's milk and goat's milk [117, 118]. It increases total serum cholesterol when compared to many other fatty acids in terms of an increase in high density lipids, the "good'' blood cholesterol [106]. In contrast, lower high density lipids correlates with a decrease in atherosclerotic risk [107]. It is used in treatment for acne [119, 120], viral infections including influenza, swine flu, avian flu, the common cold, fever blisters, cold sores, genital herpes caused by herpes simplex virus, genital warts caused by human papilloma virus and HIV/AIDS. It is also used for preventing the transmission of HIV from mothers to children [121]. Antibacterial and antifungal activities of the algal extracts were reportedly because of the presence of Lauric, palmitic (hexadecanoic acid), linolenic, linoleic, oleic, stearic (Octadecanoic acid) and myristic acids (Tetradecanoic acid) [122]. Further, it is also used in treatment of bronchitis, gonorrhoea, yeast infections, chlamydia, intestinal infections caused by a parasite called *Giardia lamblia*, and ringworm. Lauric acid is considered to be a safer fat than trans-fats in food preparations. It is safe for pregnant and breast-feeding women in food amounts [123].

3.3.6. 6, 7-Dimethoxy-2-tetralone (C12H14O3)

Its synonymous names are: 6,7-Dimethoxy-3,4-dihydronaphthalen-2(1H)-one; 6,7-Dimethoxy-3,4-dihydro-1H-naphthalen-2-one; and 6,7-Dimethoxyl-2-tetralone. It is a starting material for many dopaminergic compounds. It is used in the synthesis of 2-bromotetralones by undergoing bromination. It was also used as a precursor to quinolines with dopaminergic activity, naphthols with anti-inflammatory activity and benzophenanthridine alkaloids with antitumor activity [124]. It has been used to synthesize natural alkaloids, cyclic amino acids and as novel antagonists of human TRPVI [125-128]. It was also used as a precursor to quinolines with dopaminergic activity, naphthols with anti-inflammatory activity and benzophenanthridine alkaloids with antitumor activity.

3.3.7. 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (C20H40O)

Its other names are: Phytol; 3, 7, 11, 15-Tetramethylhexadec-2-en-1-ol; and 2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-. Phytol is a constituent of chlorophyll, vitamin K, vitamin E, and other tocopherols produced in all photosynthesizing plants. Phytol is a very hydrophobic molecule, practically insoluble in water, but soluble in most organic solvents. It is a colourless clear liquid with a floral, balsamic or waxy odour and a green, berry, tropical or waxy taste. It is a diterpenoid and a long-chain primary fatty alcohol commonly used as an aromatic ingredient in many fragrance compounds. Some of its lipophilic analogs show potent antitubercular activity. It has many biological activities such as anxiolytic, metabolism modulating, cytotoxic, antioxidant, apoptosis-inducing, anti-nociceptive, anti-inflammatory, anti-cancer, diuretic, anti-tuberculosis, insecticidal, anti-oxidant, immune-modulating, and antimicrobial effects [129- 131].

3.3.8. Phenol, 2-methoxy-5-(1-propenyl)-,(E) ($C_{10}H_{12}O_2$)

Its synonymous names are: Isoeugenol; Isochavibetol; 2-Methoxy-5-[(E)-1-propenyl] phenol; and 2-Methoxy-5-[(E)-prop-1-enyl] phenol. It is an isomer of eugenol. It also known as propenylgualacol, belongs to the class of organic compounds known as methoxyphenols (containing a methoxy group attached to the benzene ring of a phenol moiety). Isoeugenol is also classified as a phenylpropene, a propenyl-substituted guaiacol, it may occur as either the cis (Z), a pale yellow liquid or Trans (E), crystalline isomers. Isoeugenol is very slightly soluble in water and soluble in organic solvents. It can be prepared from eugenol by heating. In addition to its industrial production via eugenol, it can also be extracted from certain essential oils especially from clove oil and cinnamon. Therefore, it has a spicy, sweet, carnation-like odour and tastes of sweet spice and clove. It is found naturally in a wide number of foods, spices and plants including basil, blueberries, cinnamon, cloves, coffee, dill, ginger, nutmeg, thyme and turmeric. It is also a component of wood smoke and liquid smoke. It is one of several phenolic compounds responsible for the mould-inhibiting effect of smoke on meats and cheeses. Isoeugenol (specifically the acetate ester) has also been used in the production of vanillin. Isoeugenol is one of several non-cannabinoid phenols found in cannabis plants [132]. It is a widely used food flavouring agent and a perfuming agent. As a food flavouring agent, it is responsible for the flavour of nutmeg, as a fragrance, it is extensively used as a scent agent in consumer products such as soaps, shampoos, perfumes, detergents and bath tissues. However, isoeugenol is a strong contact allergen [133].

3.3.9. Cycloheptane, 1, 3, 5-tris (methylene) (C10H14)

Its other names are: 1,3,6-Trimethylenecyclo-heptane; 1,3,5-Trimethylenecycloheptane#; and 1,3,5-Tris (methylene) cycloheptane. Cycloheptane is a cycloalkane. It is a non-polar solvent for the chemical industry and as an intermediate in the manufacture of chemicals and pharmaceutical drugs. It is used in the manufacture of synthetic resins and rubber adhesives and also as a blowing agent in the manufacture of polyurethane insulating foam, and is found in many domestic appliances such as refrigerators and freezers as a refrigerant, replacing alternatives such as CFC-11 and HCFC-141b. Cycloheptane vapour is irritating to the eyes and may cause respiratory depression if inhaled in large quantities.

3.3.10. 4-(6, 6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol (C14H22O)

Its other name is (4Z)-4-(6, 6-Dimethyl-2-methylene-3-cyclohexen-1-ylidene)-2-pentanol. It is a secondary amyl alcohol and a pentanol. It derives from a hydride of a pentane. It is used as a solvent and an intermediate in the manufacturing of other chemicals. It is a colorless liquid with a fuel-like odor at room temperature. Pentanol is used to help manufacture pharmaceuticals, herbicides, antioxidants, extractants, cosmetics etc.

3.3.11. Loliolide (C11H16O3)

Its synonymous names are: Loliolid; and (3S, 5R)-Loliolide. It is a natural bioactive ubiquitous monoterpenoid lactone present in a variety of plants including marine algae and medicinal plants such as psychoactive plant *Salvia divinorum*, *Acalypha*, *Brachystemma calycinum*, *Calendula officinalis* L. flowers and many more [134]. Loliolide exerted inhibitory activity against cellular senescence in human dermal fibroblasts diminishing senescence-associated β-galactosidase activity (SA-β-gal), the level of p21 protein, and the level of reactive oxygen species in senescent cells induced by adriamycin treatment. As a selective estrogen receptor modulator, it can activate ERβ and ERα and simultaneously express estrogenic activity. Loliolide can be applied as medicinal or food compositions to improve estrogen deficiency-related symptoms and can be used as selective estrogen receptor modulator (SERM) in menopause women. Recently loliolide was characterized as a potent hepatitis C virus (HCV) entry inhibitor, which may act as a candidate of antiviral agent against hepatitis C [135]. Loliolide was suggested to be a potential anti-adipogenic agent, which may be utilized as a lead compound in anti-obesity treatment. It is also used as an anti-bacterial, anti-inflammatory, anti-depressive, and anti-cancer agent [134].

3.3.12. Cis-2-[2-(hydroxymethyl) cyclopentyl] ethanol (C8H16O2)

Its other names are: cis-1, 2-Cyclohexanedimethanol; cis-Cyclohexane-1, 2-diyldimethanol; 1, 2-Cyclohexanedimethanol, (1R,2S)-rel-; and [(1S,2R)-2-(Hydroxymethyl) cyclohexyl] methanol. It is one of the components in fungicide, insect repellent etc., it is used as a reactant in the acylation of alcohols with an acid anhydride or acid chloride. It is a substrate in the synthesis of high-density polycyclic aviation fuel by the Guerbet reaction. It is an alkylating agent in the preparation of alkylated aromatic compounds using Fe^{3+}-montmorillonite catalyst via Friedel–Crafts alkylation reaction. Cyclohexanedimethanol is a mixture of isomeric organic compounds. It is a colorless low-melting solid used in the production of polyester resins.

At the outset, algae are rich in carbohydrate (up to 60%) with medium or high amounts of proteins (10–47%), low in lipids (1–3%) and variable contents of minerals (7–38%), vitamins (A and E), alkaloids, carotenoids, flavonoids, terpenoids, saponins, glycosides, phenolics, sterols and polyunsaturated fatty acids [136-139]. The secondary metabolites are natural chemical substances which are produced from the metabolisms for defense purposes from the predators [140]. Due to the high content of major bioactive compounds (polyunsaturated fatty acids, sulfated polysaccharides, phycosterols, heat-induced proteins, phenolic compounds and pigments like carotenoids) in the algae [141], they could possess therapeutic benefits such as anti-diabetic, antioxidant, anti-inflammatory, antibacterial, antiviral, and antitumoral [142-151] on human and animal health.

4. Conclusion

In general, the nutrient and phytochemical composition of algae could vary depending on the season, the place, the soil and the morphology of the plants. The phytochemical analysis revealed presence of alkaloids, terpenoids, flavonoids, tannis, polyphenols, saponins, cardiac glycoside and quinones in spirulina, chlorella and azolla. The presence of bioactive principle compounds reflects the potential of particular alga for specific activity. They can be isolated, purified and characterized to use as medicine for various ailments. Thus, algae like spirulina, chlorella and azolla can be taken as a source of important bioactive principle compounds which can be used in formulation of drugs by the pharmaceutical industries. Further work under progress is checking out some of the bioactive principle organic compounds for their *in-vitro* anticancer activities.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest.

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