Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

P-ISSN: 2349-8234
JPP 2017; 6(1): 74-82
Received: 11-11-2016
Accepted: 12-12-2016

Kavya R
Mount Carmel College (Autonomous), Bengaluru, Karnataka, India

Aakanksha Sharma
Mount Carmel College (Autonomous), Bengaluru, Karnataka, India

Dr. Roshni Rao
Department of Botany, Mount Carmel College (Autonomous) Bengaluru, Karnataka, India

Correspondence
Dr. Roshni Rao
Department of Botany, Mount Carmel College (Autonomous) Bengaluru, Karnataka, India

A comparative study of PUFAs in Algae and higher plants

Kavya R, Aakanksha Sharma and Dr. Roshni Rao

Abstract
A right and precise intake of nutrients is the key to a healthy life. Nutrients play a major role in the human diet and are responsible for the various cellular and enzymatic processes and growth of the body. One such category of nutrients is the polyunsaturated fatty acids (PUFAs) that our body cannot synthesize; therefore it is essential that these are taken from external sources to replenish our body. The aim of this study was to find out the effect of nitrogen and salinity on PUFAs in *Ocimum basilicum*, determine the ratio of these PUFAs in some algae and higher plants, and identify the most potent source. It was found that *Chlorella vulgaris* among the selected algae and *Ocimum basilicum* among the higher plants studied, gave the omega-6: omega-3 ratios of 1.93:1 and 0.67:1 respectively. Overall, *Ocimum basilicum* gave the best result among both algae and higher plants.

Keywords: PUFAs, Algae, *Ocimum basilicum*, *Chlorella vulgaris*, Omega-6: omega-3 ratio

1. Introduction
The 21st century is a fast paced world where the dynamic modern lifestyle is leading to stress; with high consumption of fast food one is becoming ignorant towards their body. In such a scenario health and nutrition play a very significant role in one's life. Therefore, it is important that an individual is soundly aware of the diet he or she follows. One such vital component of our diet is polyunsaturated fatty acids (PUFAs) that cannot be synthesized by a human body. Therefore, PUFAs must be provided through the diet in a balanced ratio. PUFAs are lipid based molecules such as triglycerides and phospholipids which are characterised primarily by the number and position of their double bonds, based on which they are classified into two distinct groups omega-3 and omega-6 PUFAs, former being synthesised from alpha-linolenic acid (ALA; 18: 3) and the latter from precursor linoleic acid (LA,18:2) (Catalá, 2013) [1].

These long chain fatty acids provide numerous benefits to the human population, some of them are reduction in cardiac diseases such as arrhythmia, stroke and high blood pressure. In addition, they offer beneficial effects to depression, rheumatoid arthritis and asthma (Romieu *et al*., 2005) [2]; (Schacky *et al*., 2008) [3]. Hence, there is a growing demand for these essential fatty acids. Recent studies in humans show that in addition to the absolute amounts of omega-6 and omega-3 fatty acid intake, the omega-6: omega-3 ratio plays an important role in the general health of human body (Simopoulos, 2016) [4].

The major sources of these PUFAs since a long time has been the marine fish which are very rich in these compounds but due to certain disadvantages such as the presence of contaminants like mercury and other heavy metals, their consumption rate is greatly declining. In addition, fish oil is not suitable for vegetarians and the odour makes it unattractive (Adarme-Vega *et al.,* 2012) [5]. Thus other alternatives are being explored to fill the replacement.

The other potent sources for the Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are the wild oil yielding plants like Walnuts, Figs, Flax seeds, Soya beans, Mustard, Cauliflower but even these face certain drawbacks such as their incapability to metabolise LA and ALA into long chain molecules. Flaxseed oil is the best source of plant omega-3 available and has one of the best omega ratios among all food sources but even this poses many hindrances being the most unstable edible oil, becoming highly toxic on exposure to hot bright sunlight if kept for more than half an hour (Miller *et al.,* 2012) [6]. Other harmful effects of flax in the body is that it increases blood sugar levels which poses a danger to diabetic patients, it is also known to delay blood clotting. This has led to revolutionary interest in scientists to find new resources for obtaining the lipid rich molecules.

Microalgae are photosynthetic micro-organisms that convert the light energy, water and carbon-di-oxide into algal biomass (Munir *et al.,* 2013) [7].

The microalgae require a less harvesting time, need smaller area for cultivation and are easier to maintain making it very suitable to extract oil rich in the lipids. One must be aware that even the fishes derive their omegas from these microalgae. As quoted by Dr. Barbara Levine, associate professor of nutrition in medicine at Weill Medical College of Cornell University, "the purest source of DHA is not the fish itself, but rather what fish consume: the ocean's vegetarian plant algae" (Shukla *et al.,* 2011) [8].

Lipid production by microalgae depends on the species and is affected by culture conditions such as nutrients, salinity, light intensity periods, temperature, pH and even the association with other microorganisms (Richmond, 2004) [9]; (Guschina and Harwood, 2006) [10].

The study focuses on Bligh and Dyer's method and Modified Bligh and Dyer's method for complete lipid extraction. The Bligh and Dyer's method is a rapid method used for the extraction and purification of the total lipids. The Modified Bligh and Dyer's method involves use of 1M NaCl instead of distilled water.

Gas Chromatography- Mass Spectroscopy (GC-MS) was the method which can be used for the estimation and identification of the fatty acid content (Barma *et al.,* 2013) [11]. Fatty acids can be separated and quantified using GC-MS analysis (Abubkar *et al.,* 2012) [12].

Using the above techniques the project aims to compare the lipid content and its constituent fatty acids present in the test algae, *Spirulina platensis*, *Dunaliella salina* and *Chlorella vulgaris* with plant based oils extracted from *Ocimum basilicum* and *Linum usitatissimum*. The project also covers studying these plants under stress conditions like nitrogen starvation and salinity because these factors affect lipid metabolism in algae. A general trend towards accumulation of lipids, particularly TAG, in response to nitrogen deficiency has been observed in numerous species or strains of various microalgae (Yeh *et al.,* 2011) [13]. Based on the literature reviewed, it is clear that amongst the different lipid induction techniques, nitrogen starvation is most widely applied and studied in almost all the microalgae species that can be considered for the commercial production of biodiesel or oil.

In some marine species like *Dunaliella,* it is reported that increase in the salt content elevates the lipids (Takagi *et al.,* 2006) [14]. Hence application of salt is also studied to categorize its potential either as a lipid inducer or reducer in both plants and microalgae.

The goals of the present study can be summarized as follows:

1. To select and identify the best method for oil extraction.
2. To determine which source gives the best ω-6 to ω-3 ratio.
3. To study the effect of nitrogen and salinity on PUFAs in *Ocimum basilicum*.
4. To identify the most suitable and potent microalgae for good sum of lipid production.
5. To determine the factors which lead to higher lipid accumulation.

2. Materials and Methods

2.1 Isolation and Collection

The standard media used for *Spirulina platensis* culture was CFTRI and CHU-10 for *Chlorella vulgaris. Dunaliella salina* was cultured in 3 different media namely Pringsheim's Media, Modified Johnsons Media and Ramaraj Media. Among the three, Ramaraj media gave the best results and showed the maximum growth and was therefore standardized as the culture media for this alga.

They were then made auxenic using microbiological techniques which involves isolation and purification of algae. The algal culture was obtained and was subjected to centrifugation. After centrifuging the algae, the supernatant was discarded and the pellet was retained followed by breaking the cells using sterile glass beads to homogenise the former. To this distilled water was added to make up the final volume to 10 ml. This was then treated with streptomycin. The streptomycin solution was prepared by dissolving 0.002 g of streptomycin in 100 mL of distilled water. 1 mL of this solution was added to 10 mL of algal suspension. The suspension was incubated for 10 minutes at room temperature after which it was subjected to centrifugation at 2000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed 2-3 times with distilled water. To this final pellet 2 mL of distilled water was added. This was the inoculum to be plated. A drop of cell suspension was streaked on an agar plate using a streaking loop in various directions and the plates were incubated in the culture room with 2500-3000 Lux light at 27 ^{0}C.

After 10-15 days of incubation period the individual sterile colonies were picked up and examined under a microscope and also used as inoculum. This method was used for the isolation of algae. By this method *Chlorella vulgaris, Spirulina platensis and Dunaliella salina* algae were isolated and made auxenic.

The other sources chosen for oil extraction were *Linum usitatissimum* and *Ocimum basilicum. Linum usitatissimum* was procured in the form of seeds and for *Ocimum basilicum* leaves were the part used.

2.2 Treatment of *Ocimum basilicum* with different concentrations of NaCl and Urea

The plants of *Ocimum basilicum* were procured from the Medicinal Plants Institute FRLHT, Bengaluru. These plants were then treated with three different concentrations of NaCl solution that were 0.2%, 0.5% and 1% in 2 kg soil. For urea, the concentrations prepared were 40 mg, 60 mg and 80 mg in 2 kg soil. All the above mentioned six concentrations were added to separate pots daily till a period of 15 days to study the effect of these concentrations on the lipid content of *Ocimum basilicum*. After 15 days of this treatment, the leaves were harvested and were used for oil extraction.

2.3 Oil Extraction Method

The extraction of lipids in solution for algae, *Linum usitatissimum* seeds and *Ocimum basilicum* leaves was done by Bligh and Dyer's Method (Bligh and Dyer, 1959) [15] along with Modified Bligh and Dyer's method (Hajra, 1974) [16].

2.4 Sample preparation for oil extraction

In case of algae, different algal solutions were centrifuged separately at a speed of 2000 rpm for 10 minutes until a gram of pellet was retained. To this pellet distilled water was added along with glass beads and shook vigorously for cell disruption and acquires maximum lipids. From this suspension 1 mL was used for oil extraction.

To obtain 1 mL sample from *Linum usitatissimum* and *Ocimum basilicum*, 1gm of seeds for the former and fresh leaves for the latter were first surface sterilized under running tap water to remove any soil and grit. These were then washed with distilled water 3-4 times. Next a drop of Tween 20 was used to cleanse them for about 5 minutes. These were again

washed with distilled water 2-3 times and the plant part was finally sterilized.

1 g of the sterilized sample was added to a mortar and pestle and 10 mL of distilled water was used to homogenise the sample into a fine uniform solution. From this solution 1 mL was utilized for lipid extraction.

For each 1 mL sample, 3.75 mL of both chloroform and methanol were added in the ratio 1:2 and vortexed well. To this 1.25 mL chloroform was added and vortexed well followed by 1.25 mL distilled water. This solution was again vortexed and subjected to centrifugation at 1000 rpm for 5 minutes at room temperature to attain a two phase system (Aqueous top and Organic bottom). The bottom phase was recovered carefully by inserting a micropipette through the upper phase so that the upper phase did not get into the tip of the pipette. To acquire a clean preparation the recovered bottom phase was washed with authentic upper phase (an authentic upper phase utilises 1 mL of distilled water instead of sample and the same procedure mentioned above is followed). To the recovered bottom phase an appropriate amount of authentic upper phase was added (approximately 2.25 mL) and the solution was vortexed well. This formed an upper and a lower layer, out of both the latter was retained and centrifuged at 2000 rpm for 5 minutes. The lower layer in the centrifuge tube had the lipids along with chloroform comprising the total lipid phase.

The Modified Bligh and Dyer's method followed the same protocol as Bligh and Dyer's method but instead used 1 M NaCl solution in place of distilled water.

The lipid phases procured from each sample were added to glass vials. These vials were then kept in a hot water bath at a temperature of 60 ^{0}Celsius to evaporate the chloroform and obtain pure lipids. The lipids attained were identified and analysed quantitatively and qualitatively using GC-MS at Bangalore Test House, Bengaluru and the results were gathered.

2.5 Urea treatment given to *Ocimum basilicum* plants

Plate 1: Before treatment with urea

Plate 2: After 15 days of treatment with different urea concentrations

2.6 NaCl treatment given to *Ocimum basilicum* plants

Plate 3: Before treatment with NaCl

Plate 4: After 15 days of treatment with different NaCl concentrations

Plate 5: *Dunaliella salina*

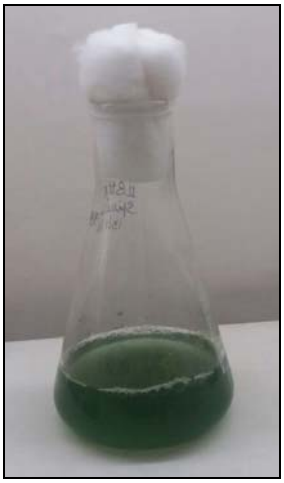

Plate 6: *Spirulina platensis*

Plate 7: *Chlorella vulgaris*

2.7 Modified Bligh and Dyer's method for the total lipid extraction

Plate 8: *Dunaliella salina*

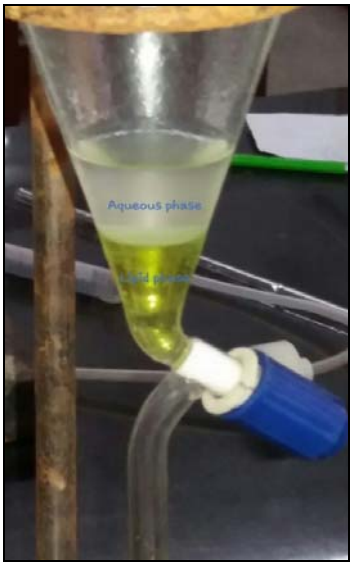

Plate 9: *Chlorella vulgaris*

Plate 10: *Ocimum sanctum*

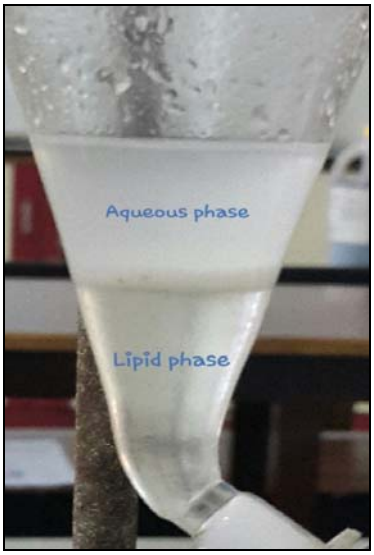

Plate 11: *Linum usitatissimum*

3. Results and Discussion

The lipid phase of algae *Dunaliella salina, Spirulina platensis*, *Chlorella vulgaris* and higher plants *Ocimum basilicum* and *Linum usitatissimum* was extracted using Bligh and Dyer's method and its modified version. The fatty acids of the lipid phase were analysed using Gas Chromatography - Mass Spectrophotometry.

3.1 Comparison of Bligh and Dyer's method and Modified Bligh and Dyer's method

The Modified Bligh and Dyer's method (1 M NaCl) gave higher amount of lipid phase in both algae and higher plants compared to Bligh and Dyer's method. In *Linum usitatissimum*, Modified Bligh and Dyer's showed 3 mL and *Spirulina platensis* showed 3.83 mL of lipid phase whereas the amount of lipid phase in Bligh and Dyer's method in case of *Linum usitatissimum* was 1 mL and in *Spirulina platensis* 3.5 ml. *Ocimum basilicum* did not show any change in the amount of lipid phase (4 mL), when compared to both Bligh and Dyer's method and its modified version (Table1 and Figure1).

In order to improve the above basic method, many modifications have been adopted by researchers. The most

common modification is the addition of 1 M NaCl instead of water as proposed by Hajra, to prevent binding of acidic lipids to denatured lipids (Ranjith Kumar *et al.,* 2015) [17], which in the current study is the modified Bligh and Dyer's method. These results confirm that the Modified Bligh and Dyer's method which uses 1 M NaCl in place of distilled water in the washing procedure is a better process to extract higher amounts of lipids with their minimal loss.

Since the Modified Bligh and Dyer's method gave higher amount of lipid phase, this method was further standardised to obtain the lipid phase. In this case, the estimated lipid phase in both *Ocimum basilicum* and *Dunaliella salina* was 4 mL which was the highest among all other algae and higher plants (Table 2 and Figure 2).

The nitrogen and salinity conditions are known to affect the growth and lipid content of algae, but no such reports are available regarding their effect on higher plants. Hence, in the present study, *Ocimum basilicum* was treated with three different concentrations of urea (source of nitrogen) which were 40 mg, 60 mg and 80 mg in 2 kg of soil respectively (Plate 1 and Plate 2). The plants were also treated with other three different concentrations of NaCl as follows 0.2%, 0.5% and 1% to check the effect of salinity (Plate 3 and Plate 4). The highest lipid phase was 4.2 mL in the plant treated with 40 mg of urea (Table 3 and Figure 3) and 4.6 mL in case of 0.2% NaCl (Table 4 and Figure 4).

This signifies that 40 mg of urea concentration in 2 kg of soil and 0.2% NaCl are the most effective treatments recorded to obtain higher amounts of lipid phase in case of *Ocimum basilicum*. The interpretation according to Table 3 and Table 4 show that with further increase in the concentration of both NaCl and urea, the lipid phase decreases in quantity.

3.2 Fatty Acid Profile for Algae and Higher Plants

Spirulina platensis showed presence of Linoleic acid (omega-6 fatty acid) but linolenic acid, an omega-3 fatty acid was not present.

In *Chlorella vulgaris* both, linolenic acid and linoleic acid were seen. The amount of linoleic acid was 22.07% being higher than linolenic that was 11.4%.

Dunaliella salina showed absence of both linolenic and linoleic acids which are unsaturated in nature.

Linum usitatissimum showed the presence of linolenic acid and linoleic acid. Here linoleic was higher than linolenic acid.

The fatty acids present in *Ocimum basilicum* were linolenic and linoleic. Among the Polyunsaturated fatty acids, the linolenic acid was highest in plant treated with urea concentration 40 mg in 2 kg soil, 23.12%. The percentage of Linolenic acid decreased with increase in urea concentration from 40 mg to 80 mg in 2 kg soil. Thus, higher concentration 80 mg of urea had an inhibitory effect on linolenic acid.

Linoleic acid, which is another PUFA, was recorded highest in plant treated with urea concentration 60 mg in 2 kg soil, 20.13%. More than the concentration of omega-6 and omega-3 individually, the current report say that the ratios of the two is what affects the human health. In the current study ratios obtained were 0.67:1 (ω-6: ω-3) in 40 mg urea treatment in *Ocimum* plants and in 60 mg urea treatment the ratio was 1.24:1. Since a lower omega-6 percentage is better, 40 mg urea treatment had a better ratio than 60 mg urea treatment.

With NaCl treatment, both Linolenic acid and Linoleic were highest in 0.5% NaCl concentration measuring 15.06% and 19.64% respectively. The increase in NaCl concentration from 0.2% to 0.5% caused an increase in the amount of linolenic acid upto a certain extent and after which it decreased. Hence, a very high concentration of NaCl was seen to have an inhibitory effect on omega-3 fatty acids. Regarding linoleic acid, an increase till 0.5% NaCl concentration was seen, after which at 1% NaCl concentration showed no significant change. Although both linolenic and linoleic acid were high in 0.5% NaCl, a better ratio of omega-6: omega-3 (1.2:1) was seen in 0.2% NaCl treatment.

Simopoulos A P, (2002) [18] reported leafy wild plants contain more 18:3ω3 and less 18:2ω6 whereas cultivated plants and seeds are higher in 18:2ω6 with the exception of flax. In our study the highest percentage of Linolenic acid was accounted in *Ocimum basilicum* where all the cultivated plants showed more amount of omega-6 than omega-3 similar to the above quoted reference except the plant treated with 40 mg urea in 2 kg soil which showed more omega-3 (23.12%) than omega-6 (15.64%), which is a better ratio. In case of *Linum usitatissimum* unlike the result recorded by Simopoulos, our study showed higher omega-6 (18.30%) compared to omega-3 (12.39%).

The study by Otles *et al.,* (2001) [19] showed the total amount of polyunsaturated fatty acids was higher in *Chlorella vulgaris*, represented by linoleic acid and alpha-linolenic acid than *Spirulina platensis*. Our study showed similar results. Both *Linum usitatissimum* and *Chlorella vulgaris* gave similar percentage of the linolenic acid (table 5 and graph 5). The linoleic acid content was highest in *Chlorella vulgaris* and least was observed in *Ocimum basilicum* control (table 6 and graph 6). The results were similar indicating that among all the algae, *Chlorella vulgaris* was the only one to show high percentages of both omega-3 and omega-6 fatty acids, thus making it suitable as an alternative source of these PUFAs apart from fish.

We can therefore say that the *Ocimum* plants require a certain amount of urea and NaCl in specific concentrations to yield greater amount of fatty acids and both salinity and urea play an important role in increasing the amount of lipids in these plants. Our readings clearly indicate that control (*Ocimum* plants without urea or NaCl treatment) had least amount of both omega-6 and Omega-3 fatty acids. The treatment with urea and NaCl in *Ocimum basilicum* showed an increase compared to control.

Since *Spirulina platensis* and *Dunaliella salina* are having fewer amounts (*Spirulina platensis*) or no PUFAs (*Dunaliella salina*) makes it more useful for industrial biodiesel purposes because of higher lipid contents than as a source of edible oil.

Among the Higher plants *Ocimum basilicum* showed a better ratio of omega-6 to omega-3 than *Linum usitatissimum*. In Algae, *Chlorella vulgaris* was the only organism that showed both omega-6 and omega-3 fatty acids with a ratio of 1.93:1 (table 7, 8 and figure 7). *Chlorella vulgaris* has the gene n-3 fatty acid desaturase that has shown a decrease in omega-6 and omega-3 ratio from 6:1 to 1:1. This study demonstrates that this gene can dramatically balance the ratio of ω-6/ω -3 PUFAs (Xue *et al.,* 2012).

Simopoulos, (2016) reported a balanced omega-6/omega-3 ratio is important for health and in the prevention and management of obesity. The best ratio of omega-6 to omega-3, 0.67:1 was observed in *Ocimum basilicum* treated with 40 mg of urea in 2 kg soil (table 8, figure 7) compared to the optimum ratio 1:1, when omega-6 fatty acids is less than or equal to omega-3 fatty acids, it maintains a proper balance in the body and can be consumed readily as a source of PUFAs by humans (table 7, 8 and figure 7).

Table 1: Comparison of total lipid phase in Algae and Higher Plants in 1 gram of pellet/seed by Bligh and Dyer's method and Modified Bligh and Dyer's method

Sample	Bligh and Dyer's Method (Volume in mL)	Modified Bligh and Dyer's Method (Volume in mL)
Linum usitatissimum	1	3
Ocimum basilicum	4	4
Spirulina platensis	3.5	3.83

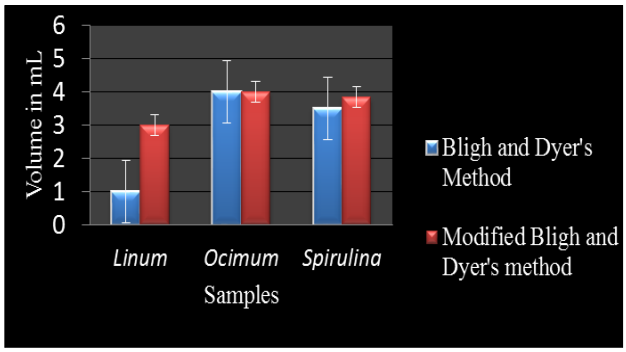

Fig 1: Comparison of total lipid phase in Algae and Higher Plants in 1 gram of pellet/seed by Bligh and Dyer's method and Modified Bligh and Dyer's method

Table 2: Comparison of Total Lipid Phase in Algae and Higher plants in 1 gram pellet/seed by Modified Bligh and Dyer's method

Samples	Modified Bligh and Dyer's Method (Volume in mL)
Linum usitatissimum	3
Ocimum basilicum	4
Spirulina platensis	3.83
Chlorella vulgaris	3.16
Dunaliella salina	4

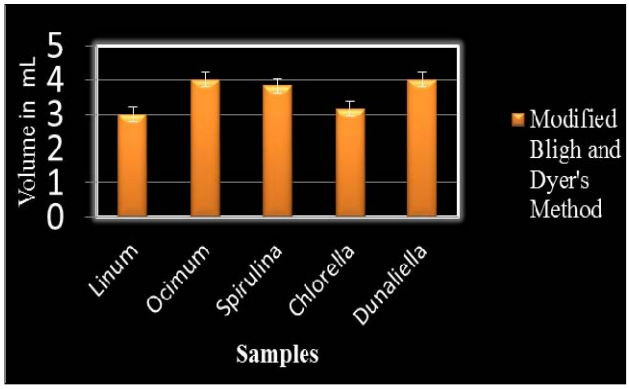

Fig 2: Comparison of Total Lipid Phase in Algae and Higher plants in 1 gram pellet/seed by Modified Bligh and Dyer's method

Table 3: Comparison of total lipid phase in different concentrations of urea added to *Ocimum basilicum*

Samples	Total lipid phase (Volume in mL)
Ocimum basilicum urea 40 mg	4.2
Ocimum basilicum urea 60 mg	4
Ocimum basilicum urea 80 mg	3.9

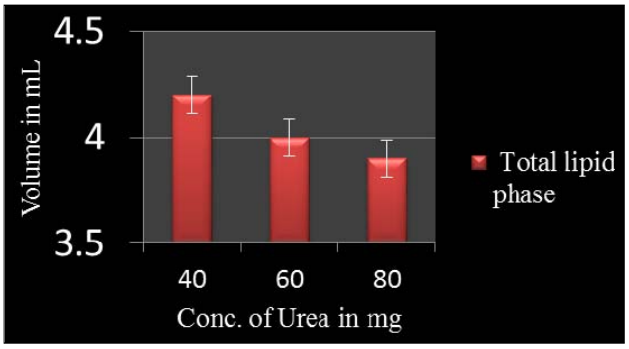

Fig 3: Comparison of total lipid phase in different concentrations of Urea added to *Ocimum basilicum*

Table 4: Comparison of total lipid phase in different concentrations of NaCl added to *Ocimum basilicum*

Samples	Total Lipid phase (Volume in mL)
Ocimum basilicum NaCl 0.2%	4.6
Ocimum basilicum NaCl 0.5%	4.5
Ocimum basilicum NaCl 1%	4.06

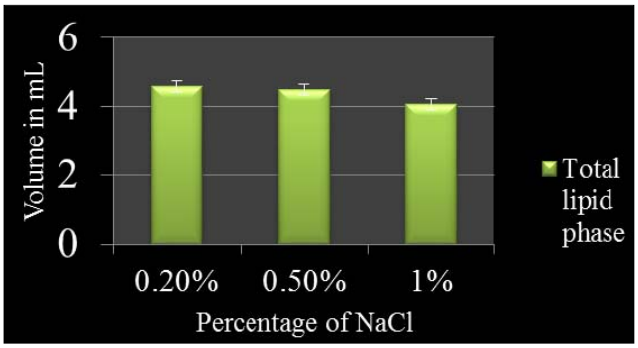

Fig 4: Comparison of total lipid phase in different concentrations of NaCl added to *Ocimum basilicum*

Table 5: Percentage of omega-3 fatty acids (Linolenic acid)

Samples	Percentage of Linolenic acid
Ocimum basilicum Control	2.33
Ocimum basilicum urea 40 mg	23.12
Ocimum basilicum urea 60 mg	16.12
Ocimum basilicum urea 80 mg	12.74
Ocimum basilicum NaCl 0.2%	5.89
Ocimum basilicum NaCl 0.5%	15.06
Ocimum basilicum NaCl 1%	9.8
Linum usitatissimum	12.39
Chlorella vulgaris	11.4

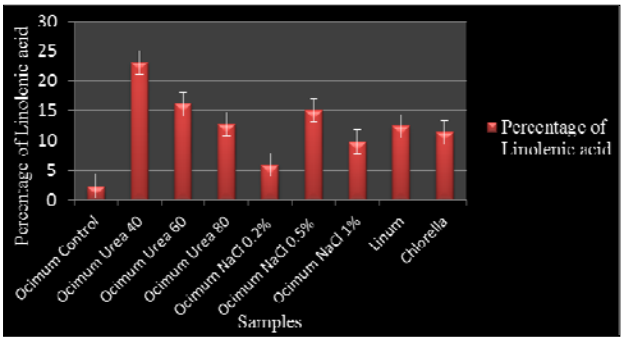

Fig 5: Percentage of omega-3 fatty acids (Linolenic acid)

Table 6: Percentage of omega-6 fatty acids (Linoleic acid)

Samples	Percentage of Linoleic acid
Ocimum basilicum Control	3.48
Ocimum basilicum urea 40 mg	15.64
Ocimum basilicum urea 60 mg	20.13
Ocimum basilicum urea 80 mg	16.04
Ocimum basilicum NaCl 0.2%	7.07
Ocimum basilicum NaCl 0.5%	19.64
Ocimum basilicum NaCl 1%	19.6
Linum usitatissimum	18.3
Chlorella vulgaris	22.07
Spirulina platensis	7.7

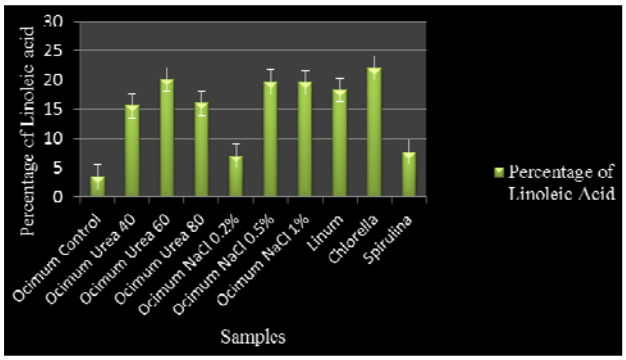

Fig 6: Percentage of omega-6 fatty acids (Linoleic acid)

Table 7: Omega fatty acid composition

Samples	Percentage of Omega-3 fatty acids	Percentage of Omega-6 fatty acids
Ocimum basilicum Control	2.33	3.48
Ocimum basilicum urea 40	23.12	15.64
Ocimum basilicum urea 60	16.12	20.13
Ocimum basilicum urea 80	12.74	16.04
Ocimum basilicum NaCl 0.2%	5.89	7.07
Ocimum basilicum NaCl 0.5%	15.06	19.64
Ocimum basilicum NaCl 1%	9.8	19.6
Linum usitatissimum	12.39	18.3
Chlorella vulgaris	11.4	22.07
Spirulina platensis	0	7.7

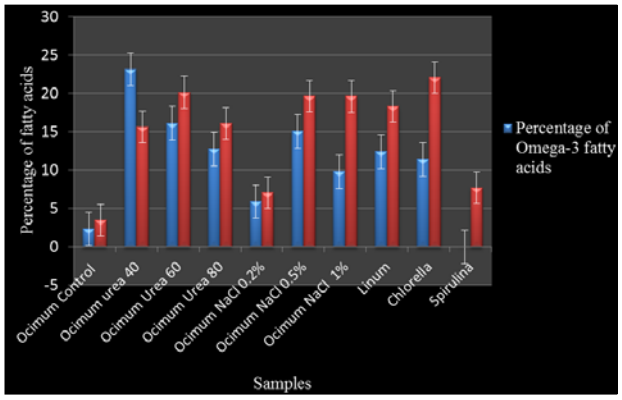

Fig 7: Omega fatty acid composition

Table 8: Omega-6 to Omega-3 ratio

Samples	Omega 6: Omega 3 ratio
Ocimum basilicum Control	1.49:1
Ocimum basilicum urea 40 mg	0.67:1
Ocimum basilicum urea 60 mg	1.24:1
Ocimum basilicum urea 80 mg	1.33:1
Ocimum basilicum 0.2% NaCl	1.2 :1
Ocimum basilicum 0.5% NaCl	1.3:1
Ocimum basilicum 1% NaCl	2:1
Linum usitatissimum	1.47:1
Spirulina platensis	7.7:0
Chlorella vulgaris	1.93:1
Dunaliella salina	NIL

Table 9: Comparison of Total Polyunsaturated Fatty acids in Algae and Higher Plants

Samples	Total Polyunsaturated Fatty Acids
Ocimum basilicum Control	5.81
Ocimum basilicum urea 40 mg	38.76
Ocimum basilicum urea 60 mg	36.25
Ocimum basilicum urea 80 mg	28.78
Ocimum basilicum 0.2% NaCl	12.96
Ocimum basilicum 0.5% NaCl	34.7
Ocimum basilicum 1% NaCl	29.4
Linum usitatissimum	30.69
Spirulina platensis	7.7
Chlorella vulgaris	33.47
Dunaliella salina	NIL

3.3 GC-MS readings

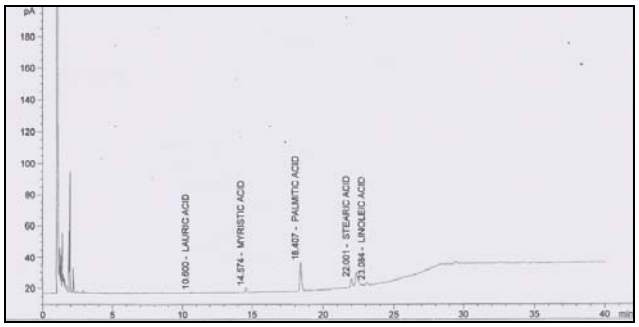

Fig 8: *Ocimum basilicum* control

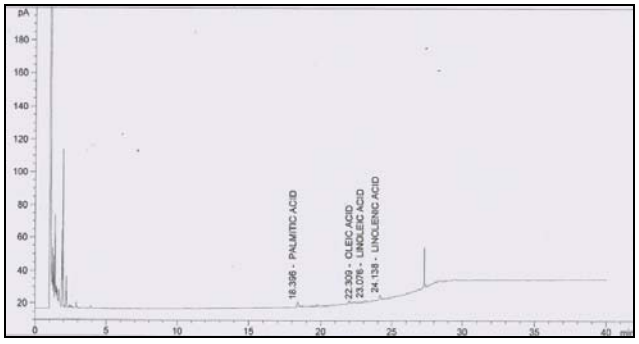

Fig 9: *Ocimum basilicum* treated with 40 mg urea in 2 kg soil

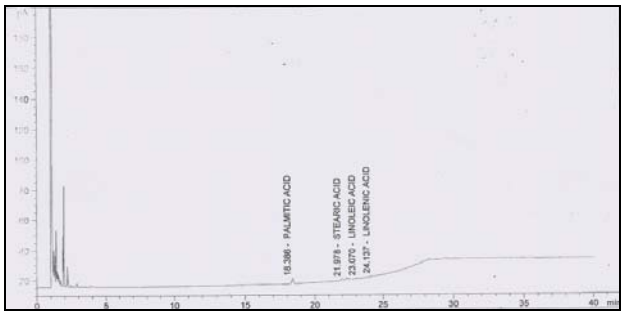

Fig 10: *Ocimum basilicum* treated with 60 mg urea in 2 kg soil

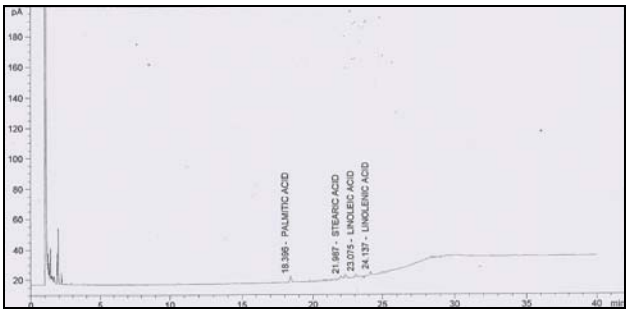

Fig 11: *Ocimum basilicum* treated with 80 mg urea in 2 kg soil

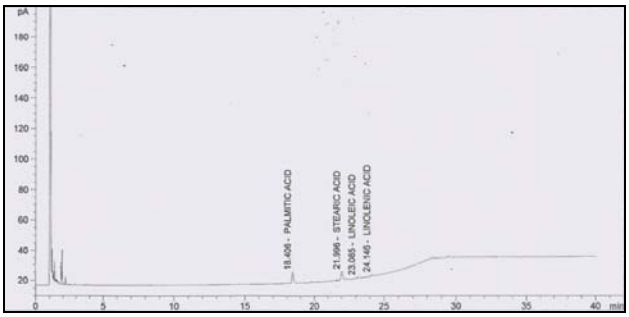

Fig 12: *Ocimum basilicum* treated with 0.2% NaCl in 2 kg soil

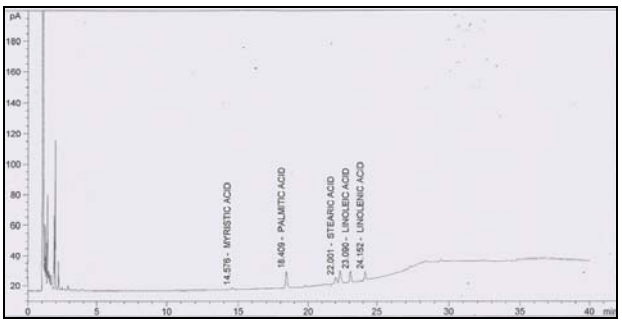

Fig 13: *Ocimum basilicum* treated with 0.5% NaCl in 2 kg soil

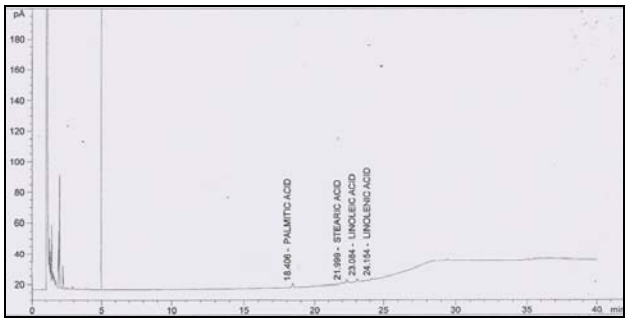

Fig 14: *Ocimum basilicum* treated with 1% NaCl in 2 kg soil

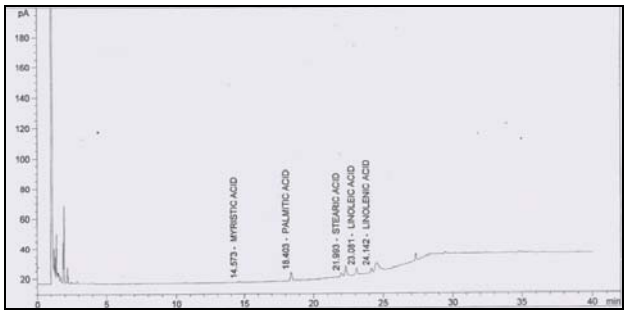

Fig 15: *Linum usitatissimum*

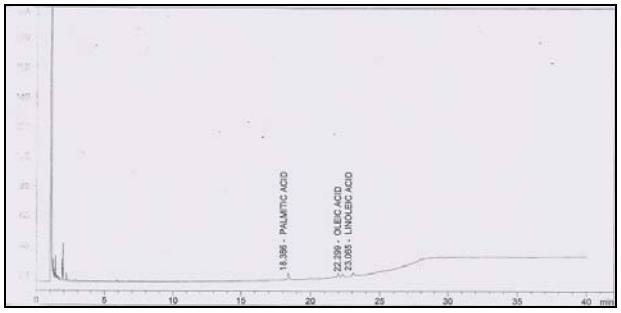

Fig 16: *Spirulina platensis*

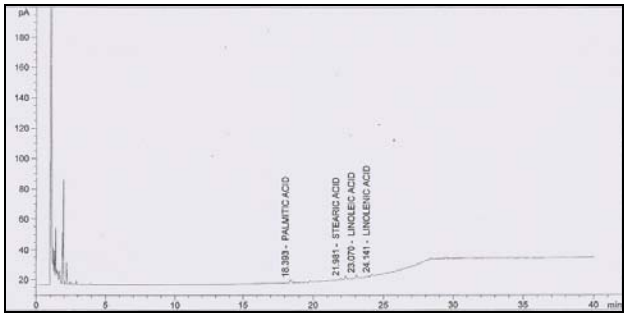

Fig 17: *Chlorella vulgaris*

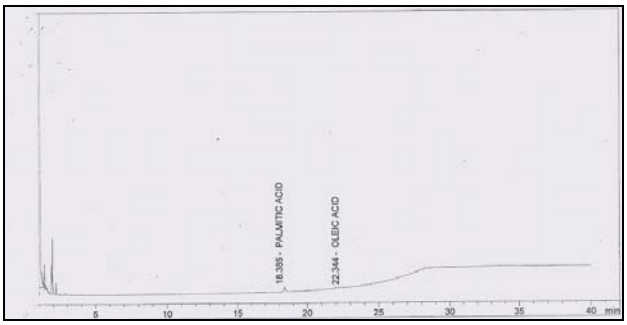

Fig 18: *Dunaliella salina*

4. Conclusion

From the above study, it can be concluded that Modified Bligh and Dyer's method (1 M NaCl) is a better procedure to yield higher amounts of lipids than the usual Bligh and Dyer's method. This method proves to be economical, less hazardous to environment and humans as it does not involve use of harmful explosive solvents. It is also time efficient. Other methods can be further developed which are environment-friendly and cost-effective to extract even higher amounts of lipids for the future use.

PUFAs are an essential component of the daily human diet and have to be taken externally, thus it becomes important to

find alternative sources of these nutrients apart from fish that can be consumed readily not only by non-vegetarians but also by vegetarians. Hence, our study aimed at finding out such sources and involved algae (*Spirulina platensis, Chlorella vulgaris* and *Dunaliella salina*) and higher plants (*Linum usitatissimum* and *Ocimum basilicum*) whose fatty acids profiles were analysed to obtain the best ratio of omega-6 to omega-3 fatty acids, which contributes a significant purpose of proper performance of the human body.

The analysis of the fatty acid profiles of both higher plants and algae done by GC-MS showed *Chlorella vulgaris* to have both Omega-3 and Omega-6 fatty acids among all other algae. Therefore, more research on *Chlorella* can be carried out to make it a potent source of omega-3 and omega-6 fatty acids since still algae is much easier to culture and harvest.

Though *Spirulina platensis* and *Dunaliella salina* have lower contents of PUFAs, they can be exploited for further use in biodiesel production and not for consumption with regard to the increase of PUFAs.

The use of algae should be further investigated as algae are still not well recognised as a potent source of PUFAs and must be promoted as they are ecologically safer and require minimum labour and land for cultivation.

Among the higher plants, *Ocimum basilicum* and *Linum usitatissimum*, *Ocimum basilicum* showed greater omega-3 than omega-6 in urea concentration 40 mg in 2 kg soil. This proportion also proved to have an excellent ratio of these fatty acids.

Overall, *Ocimum basilicum* (40 mg urea in 2 kg soil) which is a higher plant gave the best result for Omega-6 to omega-3 ratio among the three concentrations used and for that reason becomes extremely superior source consumption for PUFAs. The Tulsi leaves are rich in these fatty acids and can be consumed fresh which makes it more acceptable to a common man. Thus, *Ocimum basilicum* can be treated with varying concentrations ranging from 10 mg to 50 mg of urea and NaCl from 0.1% to 0.8% to find out which yields better ratio of omega-6 and omega-3.

Finally from our study we can say that *Ocimum basilicum,* a very popular medicinal plant, also has omega-3 and omega-6 fatty when treated with 40 mg urea. This can provide many benefits to human body like lowering cholesterol, reducing heart disorders and brain development. Therefore *Ocimum basilicum* can become a huge success in the oil and pharmacological industry as a source of PUFAs.

5. Acknowledgement

We would like to thank the UGC for its financial assistance in the form of Minor Research Project and the Principal, Mount Carmel College (Autonomous), Bengaluru for all the facilities provided.

6. References

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