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Research Article
Proximate and Total Fatty Acid Composition of Some Aquatic Macrophytes in the Nile River Rayahs, Egypt

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Abstract

Background and Objective: *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Eichhornia crassipes* are economically important and widely distributed macrophytes species. The objective of the current study was to evaluate and compare the variations in proximate and fatty acid composition of these aquatic macrophytes with respect to macrophyte species and water characteristics of the sampling sites. **Materials and Methods:** Macrophytes were collected from five sites of River Nile Rayahs. A proximate analysis and high performance gas chromatography (HPGC) were used for evaluation of the nutritional components. **Results:** The results demonstrate a significance differences ($p>0.05$) between the three studied species regarding their nutritional components, in addition to their differential response to the different water variables. All the studied plants had a high organic matter content with the highest nitrogen free extract value (NFE) 77.55% in sample of *E. crassipes* from site 5. The highest protein, digestible crude protein, protein/lipid, protein/nitrogen free extracts, protein/energy contents, nitrogen and potassium contents were found in *C. demersum* from site 5. Moreover the highest metabolized energy, energy value of organic matter and Zn contents were recorded for *M. spicatum* from site 3. HPGC analysis of fatty acids indicate the presence of 10 saturated fatty acids (SFA), 6 monounsaturated (MUSFA) and 5 polyunsaturated (PUSFA), varied significantly in distribution with plant species and sampling site. **Conclusion:** This study reveals the economic potential of these macrophytes as a natural food resources for fish and animals especially *C. demersum* and *M. spicatum* from site 5.

Key words: Macrophytes, nutritional value, HPGC analysis, metabolized energy, Nile River Rayahs

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aquatic macrophytes, besides serving as a base of aquatic food chain and important components of food web dynamics[1,2], they are considered as one of the potential sources of food and fodder for humans, animals and fishes[3-6]. In addition to their used in medicine[7,8] and as a source of some antimicrobial substances[9-11]. But not all aquatic macrophytes are suitable to be used as a potential food source as they are known to differ widely in their nutritional composition depending up on species, part of plant used, location and season[5,12,13]. So the knowledge of factors affecting their nutritional composition is very importance in order to evaluate their food potential[14]. Since fish is the most important source of essential fatty acids that originate from artificial food or phytoplankton and seaweeds in the food chain[15] and required in humans diet[16,17]. Studies on plants lipids have been focused on due to its high essential fatty acids that cannot be synthesised by humans, fish and animals.

Nothing was found in literature concerning the fatty acids profiles of the Egyptian macrophytes species *M. spicatum*, *C. demersum* and *E. crassipes*, so in view of this and due to the known variation in nutritional composition of aquatic macrophytes in different habitats. The aim of this study was to evaluate and determine variations of nutritional components in *M. spicatum*, *C. demersum* and *E. crassipes* from River Nile Rayahs in terms of estimating their proximate and fatty acid composition. Regarding the negative impact of heavy metals on the food value[18,19] measurement of some heavy metals was also involved.

MATERIALS AND METHODS

Study site: The Nile River bifurcates at El Kanater City to two main branches (Damietta and Rosetta branches) and four Rayahs (Rayah are the main irrigation Canals in Egypt). The Rayahs include El Rayah El-Tawfiki (T), El Rayah El-Menoufy (M), El Rayah El-Behery (B) and El Rayah El-Nasery (N). From the flora of these water courses three macrophytes species (*Myriophyllum spicatum* L., *Eichhornia crassipes* (Mart.) Solms and *Ceratophyllum demersum* L.) related to three families (Haloragaceae, Pontederiaceae and Ceratophyllaceae respectively) and representing different plants life forms were selected.

Collection and preparation of samples: The three selected macrophytes species were collected in August, 2018 (the time of their maximum growth period) from El Rayah El-Menoufy (M) and El Rayah El-Behery (B), however samples of *M. spicatum* were collected from the four Rayahs, in addition to the Nile River bifurcate point (RN). At each Rayah one site was only selected for samples collection (Fig. 1 and Table 1). Three samples were prepared for each macrophyte species. The collected macrophytes were kept with water in polyethylene bags and transferred to the laboratory. Where they were identefied[20], cleaned, washed and additional moisture was removed before weighted. A part of each plant sample was dried at 100°C to constant weight for estimation of dry matter. The other part was dried separately at 50°C to constant weight, ground into fine powder and kept for chemical analysis.

Proximate analysis: Water content (WC) was considered as the loss in mass from wet sample at room temperature and drying at 100°C. Ash percentage was determined after Egyptian Pharmacopoeia[21]. Plant elements were extracted using sulphuric acid and perchloric acid following the method of Burgski[22]. Nitrogen (N) content was assessed by Kjeldahl method[23]. Total Protein (P) content was calculated by multiplying the nitrogen content by a factor[24] of 6.25. Potassium (K) content was detected by flame photometry and total phosphorus (P) was determined following the method of Umoren *et al.*[25]. Heavy metals in all plants extracts were measured by atomic absorption spectrophotometer (Perkin Elmer 2100).

Calculated parameters: Nitrogen free extractives (NFE) was determined following the equation applied by Pádua *et al.*[26]:

$$\text{NFE (DW \%)} = 100-(\text{EE}+\text{CP}+\text{Ash})$$

where, EE is the ether extract (total lipids) and CP is the crude protein.

Digestible crude protein (DCP) was estimated following the equation of Demarquilly and Weiss[27]:

$$\text{DCP (DW \%)} = 0.929\ \text{CP}-3.52$$

Metabolized energy was determined according to Pantha[28], using the values of 3.4 for carbohydrate, 8.1 for lipid and 4.2 for protein:

$$\text{ME (Kcal g}^{-1}\text{ DW)} = \frac{3.4\ \text{NFE}+8.1\ \text{EE}+4.2\ \text{CP}}{100}$$

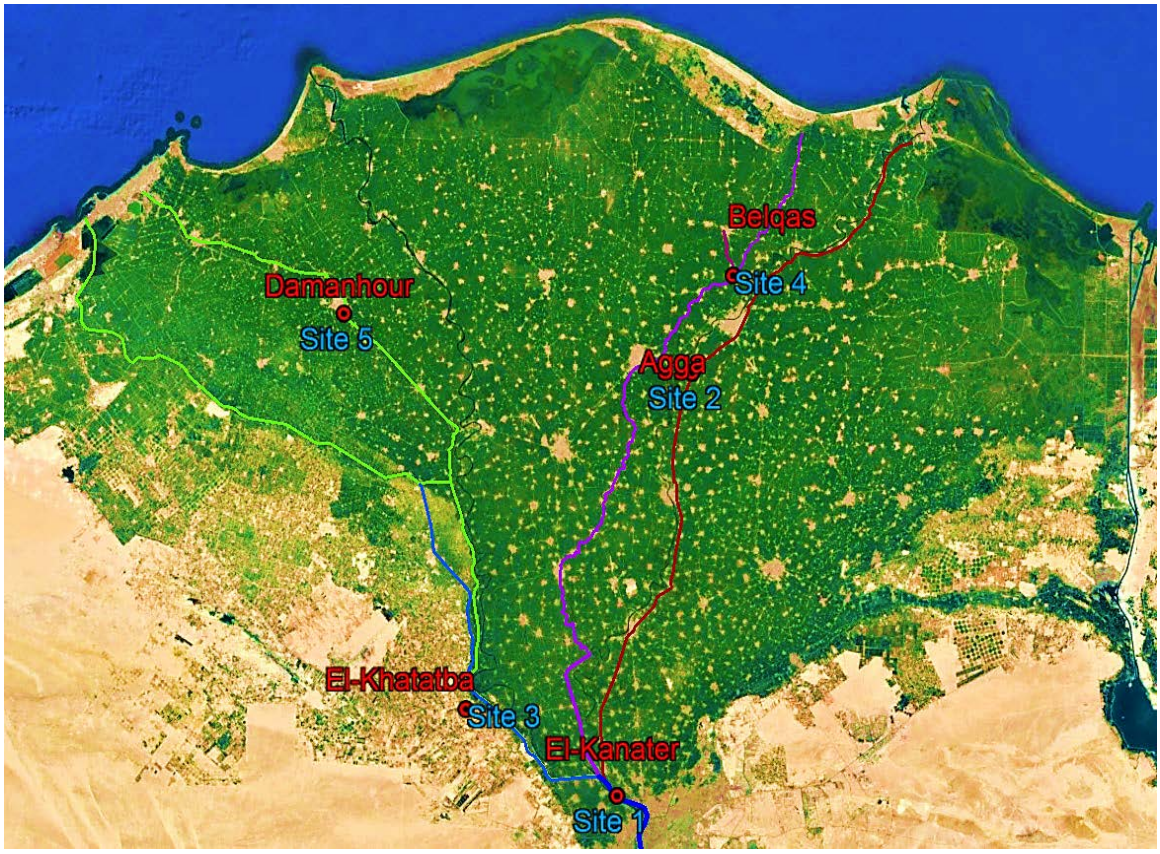

Fig. 1: Map of River Nile Rayahs with the selected sampling sites

Table 1: Details, latitude and longitude of the sampling locations

Rayahs	Site code	Site name	Latitude	Longitude
RN	1	River Nile bifurcates point	30°9'6.79"	31°9'27.89"
T	2	Aga	30°54'22.5"	31°16'51.29"
N	3	El-Khatatba	30°19'57.76"	30°48'57.5"
M	4	Belqas	31°07'59.9"	31°22'50.1"
B	5	Damanhour	31°00'46.5"	30°28'52.8"

The energy content (EV) was determined on the basis of biochemical composition (g g^{-1} DW) using the standard conversion factors[29] for lipids 9.45, carbohydrates 4.10 and protein 5.65 and expressed as Kcal g^{-1} DW where, cal is the calorie and DW is the dry weight.

Fatty acid analysis: Lipid contents in all plants species were extracted and estimated following Bligh and Dyer[30]. Fatty acid methyl esters (FAMEs) were prepared and analyzed following[31] (BS EN ISO 5508. In a clean tube take 0.1-0.2 g of plant lipids, add 10 mL of 0.2 mol L^{-1} methanolic sulphuric acid and 2 mL of benzene, close the tube well and put it in a water bath at 90°C for 90 min. Cool, add 10 mL petroleum ether shake strongly and separate out the ethereal layer in a dry tube. Evaporate to dryness and the analysis was performed in a gas chromatograph HP (Hewlett Packard) 6890 GC under the following conditions.

GC condition:

- Detector: FID (Flame Ionization Detector)
- Detector temperature: 250°C
- Injection temperature: 220°C, injection volume 3 µL, split ratio 50 1
- Column: HP-INNOWax (polyethylene glycol), 60 and 0.25 mm ID, 0.2 µm film thickness
- Carrier gas: Nitrogen, gas flow 2 mL min^{-1}
- Oven program: Initial temp. 140°C for min

Table 2: List of standard fatty acids used in the present investigation

Carbon No.	Common name	Carbon No.	Common name
C4:0	Butyric acid	C18:3αω3	Linolenic acid
C6:0	Caproic acid	C18:3ϒω6	ϒ Linoleic acid
C8:0	Caprylic acid	C20:0	Arachidic acid
C12:0	Lauric acid	C20:1	Cis-11-eicosenoic acid
C13:0	Tridecanoic acid	C20:2	Cis-11,14-eicosadienoic acid
C14:0	Myristic acid	C20:3ω3	Cis-11,14,17-eicosatrienoic acid
C14:1	Myristoleic acid	C20:3ω6	Cis-8,11,14-eicosatrienoic acid
C15:0	Pentadecanoic acid	C20:4ω6	Arachidonic acid
C15:1	Cis-10-pentadecenoic acid	C20:5ω3	Cis-8,11,14,17-eicosapentaenoic acid (EPA)
C16:0	Palmitic acid	C21:0	Heneicosanoic acid
C16:1	Palmitoleic acid	C22:0	Behenic
C17:0	Heptadecanoic acid	C22:1ω9	Erucic
C17:1	Cis-10-heptadecanoic acid	C22:2	Cis-13,16-docosadienoic
C8:0	Stearic acid	C22:6ω3	Cis-4,7,10,13,16,19-docosahexaenoic acid (DHA)
C18:1 cω9	Oleic acid	C23:0	Tricosanoic acid
C18:1 tω9	Elaidic acid	C24:0	Lignoceric acid
C18:2cω6	Linoleic acid	C24:1	Nervonic acid
C18:2tω6	Linolelaidic acid		

Fatty acids were identified by comparing the retention times of experimental samples to those of known standards (Table 2).

Statistical analysis: Different statistical techniques were used to assess the significance of difference of different macrophytes components in relation to plant species and water characteristics of sampling sites. Including, ANOVA test, Duncan's multiple range test at $p<0.05$ (XL Stat version 2014) and the canonical correspondence analysis[32] (CCA using CANOCO V. 4.0).

RESULTS

Main nutritional components: As shown in results (Table 3 and 4), the studied macrophytes species were characterized by their high water contents which ranged between 89.14-94.71% DW with the highest for *E. crassipes*. Among the studied samples *M. spicatum* from site 1 was shown to have the highest ash content 34.25%, whereas the highest organic matter content (91.55%) was recorded in the same species from site 3. All samples contained a moderate protein content with the highest value of protein, digestible crude protein, P/L, P/NFE, P/E, N, K (13.13, 8.67, 2.92, 0.20, 3.38, 2.1 and 4.8% DW, respectively) for *C. demersum* from site 5. Lipid contents ranged from 3.10-6.30% DW in *M. spicatum* from site 1 and from site 4 respectively, while NFE showed higher value (77.55% DW) in sample of *E. crassipes* from site 5. In addition, the highest value of ME (3.43 Kcal g^{-1} of DW), EV (4.18 Kcal g^{-1} of DW) were recorded for *M. spicatum* from site 3.

From the results (Table 5), it is obvious that both macro and micro-elements showed a significant difference between different plant samples. During this study both nitrogen and potassium were found within high level (2.10 and 4.85% DW respectively) in samples of *C. demersum* from site 5, meanwhile samples of *M. spicatum* from sites 4 showed the highest phosphorus contents (3.31×10^{-2} DW %). A wide pattern of variation could be observed with regard to the microelements, both iron and manganese occurred in relatively higher concentrations compared to zinc and copper. The highest iron and manganese level was detected in samples of *M. spicatum* from site 5 (8.11 and 1.99 mg g^{-1} DW respectively), however the highest Zn content 49.75 µg g^{-1} DW was found in *M. spicatum* from site 3 and the highest copper contents (21.25 µg g^{-1} DW) was recorded for *E. crassipes* from site 4.

Fatty acids profile: Results in Table 6-8 revealed the presence of different fatty acids, varied significantly in their numbers and concentrations with reference to the plant species and sampling site. As compared with the other studied species *M. spicatum* from site 5 was recorded to have the highest number and concentration of TFA (19 fatty acid, 9.51 g/100 g of lipid) in addition to the highest TUSFA (4.21 g/100 g of lipid) content, while the lowest numbers and the lowest fatty acids concentrations were recorded for samples of same species from site 1 and 2 (9 fatty acids and 1.53 g/100 g of lipid respectively). However, the highest concentrations of TSFA were found in *C. demersum* followed by *M. spicatum* from site 5 (5.41 and 5.30%, respectively).

Saturated FAs: Ten of saturated fatty acids were detected in the studied plants (Table 6). *Myriophyllum spicatum* from

Table 3: Mean values of the nutritional composition (% of DW) of the different studied plant species from different sites

Plants	Sites	WC	Ash	OM	NFE	Protein	Lipid
M. spicatum	1	89.14^{e}	34.25^{a}	65.75^{i}	56.53^{i}	6.13^{h}	3.10^{i}
	2	89.60^{e}	14.38^{g}	85.62^{c}	73.52^{d}	7.00^{g}	5.10^{e}
	3	90.23^{d}	8.45^{i}	91.55^{a}	77.05^{b}	9.25^{e}	5.25^{d}
	4	90.24^{d}	15.47^{f}	84.53^{d}	68.17^{e}	10.06^{d}	6.30^{a}
	5	92.32^{c}	21.96^{b}	78.04^{h}	60.73^{h}	11.81^{b}	5.50^{b}
E. crassipes	4	94.05^{a}	18.92^{c}	81.08^{g}	65.85^{g}	10.63^{c}	5.38^{c}
	5	93.55^{b}	10.34^{h}	89.66^{b}	77.55^{a}	8.31^{f}	3.80^{g}
C. demersum	4	94.34^{a}	17.18^{d}	82.82^{f}	73.71^{c}	5.69^{i}	3.42^{h}
	5	94.71^{a}	16.05^{e}	83.95^{e}	66.34^{f}	13.13^{a}	4.50^{f}

WC: Water contents, OM: Organic matter, NFE: Nitrogen free extracts, $^{a\text{-}i}$Significance differences ($p<0.05$) between different samples

Table 4: Mean values of digestible crude protein (DCP), metabolized energy (ME), energy value of organic matter (EV), P/L, P/E and P/NFE of the studied plant species expressed on dry weight basis

Plants	Sites	DCP (%)	ME (Kcal g^{-1})	EV (Kcal g^{-1})	P/L (%)	P/NFE (%)	P/E
M. spicatum	1	2.17^{h}	2.43^{h}	2.96^{e}	1.98^{d}	0.11^{e}	2.07^{e}
	2	2.98^{g}	3.21^{d}	3.89^{c}	1.37^{h}	0.10^{f}	1.80^{ef}
	3	5.07^{e}	3.43^{a}	4.18^{a}	1.76^{e}	0.12^{d}	2.21^{d}
	4	5.83^{d}	3.25^{c}	3.96^{b}	1.60^{g}	0.15^{c}	2.54^{c}
	5	7.45^{b}	3.01^{g}	3.68^{d}	2.15^{c}	0.19^{a}	3.21^{b}
E. crassipes	4	6.35^{c}	3.12^{f}	3.80^{c}	1.97^{d}	0.16^{b}	1.19^{h}
	5	4.20^{f}	3.29^{b}	4.01^{b}	2.19^{b}	0.11^{e}	2.07^{e}
C. demersum	4	1.76^{i}	3.02^{g}	3.67^{d}	1.66^{f}	0.08^{g}	1.55^{g}
	5	8.67^{a}	3.17^{e}	3.89^{c}	2.92^{a}	0.20^{a}	3.38^{a}

$^{a\text{-}h}$Significance differences ($p<0.05$) between different samples

Table 5: Mean values of macro and microelements of the studied plant species expressed on dry weight basis

Plants	Sites	N (%)	P×10^{-2} (%)	K (%)	Zn (µg g^{-1})	Cu (µg g^{-1})	Fe (mg g^{-1})	M (mg g^{-1})
M. spicatum	1	0.98^{g}	2.43^{f}	1.90^{i}	13.50^{i}	10.75^{f}	4.57^{c}	0.72^{c}
	2	1.12^{f}	2.18^{h}	3.71^{f}	29.75^{g}	13.75^{d}	3.14^{d}	0.57^{d}
	3	1.48^{d}	1.50^{i}	3.91^{d}	49.75^{a}	9.50^{g}	1.06^{f}	0.42^{e}
	4	1.61^{c}	3.31^{a}	4.42^{b}	39.75^{c}	14.00^{c}	4.76^{c}	0.59^{d}
	5	1.89^{b}	2.75^{d}	4.10^{c}	41.50^{b}	18.75^{b}	8.11^{a}	1.99^{a}
E. crassipes	4	1.70^{i}	2.93^{b}	3.11^{g}	35.25^{d}	21.25^{a}	3.05^{e}	0.26^{f}
	5	1.33^{e}	2.31^{g}	3.80^{e}	34.75^{e}	13.50^{d}	0.86^{g}	0.23^{g}
C. demersum	4	0.91^{h}	2.81^{c}	2.33^{h}	32.75^{f}	12.00^{e}	5.11^{b}	0.91^{b}
	5	2.10^{a}	2.68^{e}	4.85^{a}	15.50^{h}	7.75^{h}	3.36^{d}	0.52^{d}

$^{a\text{-}i}$Significance differences ($p<0.05$) between different samples

Table 6: List of saturated fatty acids detected in the studied macrophytes species

	M. spicatum										*C. demersum*				*E. crassipes*			
	Site 1		Site 2		Site 3		Site 4		Site 5		Site 4		Site 5		Site 4		Site 5	
Fatty acids (FA)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)
Saturated fatty acids (SFA)																		
C6:0	nd	nd	1.575	0.024	nd	nd	nd	nd	0.18	0.017	nd	nd	nd	nd	1.863	0.044	nd	nd
C8:0	nd	nd	0.919	0.014	1.36	0.060	0.63	0.025	nd	nd	1.097	0.041	0.521	0.042	2.887	0.068	0.861	0.066
C12:0	nd	nd	2.185	0.034	2.15	0.096	3.22	0.128	1.53	0.146	3.751	0.141	1.971	0.160	4.736	0.111	3.185	0.245
C13:0	4.57	0.166	5.992	0.092	5.87	0.260	7.74	0.308	4.35	0.414	9.659	0.363	5.034	0.407	13.439	0.316	8.461	0.651
C14:0	5.79	0.210	6.273	0.962	6.02	0.267	8.31	0.331	6.68	0.636	10.349	0.389	5.386	0.436	13.831	0.325	9.17	0.705
C15:0	3.07	0.111	3.112	0.048	3.35	0.148	4.75	0.189	2.24	0.213	2.012	0.076	3.377	0.273	7.699	0.181	4.561	0.351
C16:0	32.64	1.184	38.01	0.582	34.55	1.532	37.32	1.485	35.18	3.346	34.878	1.311	42.171	3.413	20.634	0.485	30.369	2.337
C17:0	nd	nd	nd	nd	0.77	0.034	1.15	0.046	0.72	0.069	0.824	0.031	0.669	0.054	nd	nd	0.692	0.053
C18:0	7.29	0.264	3.375	0.517	4.32	0.191	5.59	0.222	4.69	0.447	7.919	0.297	5.307	0.429	3.978	0.094	5.399	0.415
C20:0	nd	nd	nd	nd	nd	nd	nd	nd	0.14	0.014	1.005	0.038	2.443	0.197	nd	nd	1.726	0.133
Sum	53.37	1.935	61.4443	0.942	58.429	2.588	68.751	2.734	55.739	5.302	71.49	2.687	66.87	5.411	69.06	1.624	64.424	4.956

nd: Not detected

Table 7: List of unsaturated fatty acids detected in the studied macrophytes species

	M. spicatum										*C. demersum*				*E. crassipes*			
	Site 1		Site 2		Site 3		Site 4		Site 5		Site 4		Site 5		Site 4		Site 5	
Fatty acids (FA)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)	FA (%)	Lipid (g %)
Monounsaturated fatty acid (MUSFA)																		
C14:1	4.929	0.179	5.467	0.084	5.433	0.241	7.185	0.286	3.769	0.358	8.925	0.336	4.717	0.382	12.589	0.296	8.096	0.623
C15:1	nd	nd	1.115	0.017	0.632	0.028	1.792	0.071	1.848	0.176	0.904	0.034	1.621	0.131	nd	nd	0.34	0.026
C16:1	6.319	0.229	6.629	0.102	4.744	0.210	5.721	0.228	6.61	0.629	3.269	0.123	4.306	0.349	3.07	0.072	2.747	0.211
C17:1	nd	nd	nd	nd	1.199	0.053	1.504	0.060	0.491	0.047	0.75	0.028	1.126	0.091	nd	nd	0.501	0.039
C18:1c	15.999	0.580	8.563	0.131	6.698	0.297	5.028	0.200	3.844	0.366	6.725	0.253	11.476	0.929	7.14	0.168	16.403	1.262
C20:1	nd	nd	nd	nd	2.803	0.124	nd	nd	2.717	0.258	nd	nd	nd	nd	nd	nd	nd	nd
Sum	27.247	0.988	21.774	0.334	21.509	0.953	21.23	0.845	19.279	1.834	20.573	0.774	23.246	1.882	22.799	0.536	28.087	2.161
Polyunsaturated fatty acid (PUSFA)																		
C18:2cω6	19.374	0.703	16.78	0.257	19.695	0.873	8.186	0.326	21.361	2.031	0.939	0.035	1.795	0.145	4.23	0.104	5.181	0.399
C18:3 α(w3)	nd	nd	nd	nd	0.362	0.016	1.834	0.073	1.015	0.097	2.482	0.093	1.535	0.124	nd	nd	nd	nd
C20:3w3	nd	nd	nd	nd	nd	nd	nd	nd	1.265	0.120	1.605	0.060	2.235	0.181	nd	nd	nd	nd
C20:4w6	nd	nd	nd	nd	nd	nd	nd	nd	1.338	0.127	2.906	0.109	1.492	0.121	3.704	0.087	2.31	0.178
C22:6w3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.82	0.228	nd	nd	nd	nd
Sum	19.374	0.703	16.78	0.257	20.057	0.889	10.02	0.399	24.919	2.375	7.932	0.297	9.877	0.799	7.934	0.191	7.491	0.577

nd: Not detected

site 1 was shown to have the lowest number of SFA (5 fatty acids), however samples from site 5 were characterized by the highest numbers and concentrations of SFA. The most abundant FA in this group were palmitic acid and myristic acid. However, the long chain fatty acid arachidic acid was present only in all plant samples from site 5 in addition to samples of *C. demersum* from site 4.

Monounsaturated FAs: Six of monounsaturated fatty acids (MUFA) were detected (Table 7). In the studied plants the levels of oleic acid and myristoleic acid were found as the highest two fatty acids in this group within the range of 3.844-16.403 and 3.769-12.589%, respectively. In addition, *M. spicatum* from sites 3 and 5 was characterized by the presence of the MUSFA cis-11-Ecosenoic acid (C20:1).

Polyunsaturated FAs: The total polyunsaturated fatty acids ranged from 7.491% of FA in samples of *E. crassipes* from site 5-24.919% of FA in *M. spicatum* from site 5. All plant species from different sites contained linoleic acid (C18:2c an omega-6 fatty acid), however polyunsaturated fatty acids (C 18:3 αw3 an omega-3 fatty acid) linolenic acid and C20:3w3 were detected in *M. spicatum* from sites 4 and 5 and in samples of *C. demersum*, while absent in samples of *E. crassipes* (Table 7).

DISCUSSION

Results in Table 3-8 shows a-significant difference in all nutritional components with plant species and sampling site ($p>0.05$). Similar observations were recorded by Haroon *et al.*[5] and Shaltout *et al.*[33]. All plant species were characterized by their high nutritional composition which represented by their high NFE contents ranged from 56.53-77.55%, that exceed the dietary requirements of shellfish and fish and the range of some rough fodder material for carbohydrates (from 10-30% and from 27.8-51.9%, respectively)[34-35]. In comparison with other aquatic plants and with the same plant species from different sites the values exceed the values recorded for *Echinochloa stagnina* 45.7 and 53.7%[3,33], *Nymphaea lotus* and *Pistia stratiotes* 30.4-35.1%[4] and that reported for *Eichhornia crassipes* 38.9, 54.1 and 40.89-49.24%[3,33,13].

Protein have important function in all biological processes[36] and the organism requirements of proteins were varied depending on age and species of the cultured organisms[37] in addition to the rearing environment. The range of protein content of (5.69 and 13.13%) as recorded in this study is lower than that require for aquatic animals diet (32-52%)[34,35], but lie in the proper level reported for terrestrial animal diet (6-12%) depending on the animal species[38]. The results of the present study are comparable to those reported by Haroon[12] for *Saccharum spontanum*, *Ceratophyllum demersum*, *Potamogeton pectinatus* and *Potamogeton crispus* (7.8, 9.81, 10.13 and 14.50% DW respectively), *Echinochloa stagnina*, *Eichhornia crassipes* and *Ceratophyllum demersum*[33] and *Echinochloa stagnina*, *Eichhornia crassipes* and *Potamogeton tomentosum*[3], lower than that reported by Olele[39], for *Pistia stratiotes*, *Eichhornia crassipes* and *Ceratophyllum demersum* (15.80±0.80, 21.65±0.65 and 19.65±0.65%, respectively) and Abdel Shafy *et al.*[13] for *Eichhornia crassipes*

Table 8: Comparative total fatty acids, total saturated, total unsaturated and saturated to unsaturated fatty acids of the studied plant species from different sites

Plants	Sites	TFA	TSFA	TUSFA	TSFA/TUSFA
M. spicatum	1	3.63±0.10^{g}	1.94±0.10^{g}	1.69±0.06^{e}	1.15±0.06^{j}
	2	1.53±0.12^{i}	0.94±0.11^{i}	0.59±0.07^{i}	1.59±0.05^{f}
	3	4.43±0.10^{d}	2.59±0.02^{f}	1.84±0.04^{d}	1.41±0.06^{g}
	4	3.98±0.02^{e}	2.74±0.05^{d}	1.24±0.06^{f}	2.21±0.04^{b}
	5	9.51±0.01^{a}	5.30±0.03^{b}	4.21±0.01^{a}	1.26±0.02^{h}
E. crassipes	4	2.36±0.04^{h}	1.63±0.05^{h}	0.73±0.06^{h}	2.23±0.04^{c}
	5	7.70±0.11^{c}	4.96±0.06^{c}	2.74±0.05^{b}	1.81±0.06^{e}
C. demersum	4	3.76±0.03^{f}	2.69±0.02^{e}	1.07±0.03^{g}	2.51±0.03^{a}
	5	8.09±0.06^{b}	5.41±0.05^{a}	2.68±0.05^{c}	2.02±0.06^{d}

TFA: Total fatty acids, TSFA: Total saturated fatty acids, TUSFA: Total unsaturated fatty acids, TSFA/TUSFA: Ratio of total saturated to total unsaturated fatty acids, results are expressed as g/100 g of lipid, $^{a-i}$Significance differences ($p<0.05$) between different samples

12.9-17.59% DW this may be related to the part of plant used, time and place of samples collection.

However, lipid was found relatively within low concentration (from 3.10-6.30% DW), these values are being higher than that reported for some rough fodder materials[35] (0.5-3.1%). It was also higher than that reported for other hydrophytes from different water courses like: *Potamogeton crispus*, *Potamogeton pectinatus*, *Polygonum tomentosum*, *C. demersum* and *Saccharum spontanum*[12] (2.40, 2.02, 2.0, 1.99 and 1.2% DW, respectively). *Nymphea lotus* and *Pistia stratiotes*[8] (2.3-3.5%) and *Eichhornia crassipes*[13] (2.27-4.79%).

Energy is essential for the maintenance of many life processes, so the ability of a food to supply energy is of a great importance in detecting its nutritional value to animals[30]. Metabolized energy results (2.43-3.43 Kcal g^{-1}) in this study were found to be higher than that reported for *P. stratiotus* and *N. lotus* (2.04, 2.05 Kcal g^{-1}, respectively)[4].

As recorded in the literature the higher of protein to energy ratio (P/E), the better is the diet[40]. So, on the basis of high P/E values for *C. demersum* and *M. spicatum* from site 5 (3.38 and 3.21, respectively) it may be inferred that these two species are suitable for incorporation in fish and animal diets.

As recorded by Polisini and Boyed[41] and Jobling[42] adequate levels of essential mineral nutrients are one of the important aspects of plant nutritive quality but excessive concentration of these minerals in plant tissue lowers its nutritional value, moreover the highest concentrations of microelements are considered to be toxic[43]. From the result obtained, it is obvious that with the exception of *M. spicatum* from site 1, the ash content of the studied plant species (8.45-21.96%) lies in the range of some green roughages (from 8.6% in maize to 14.2% in cowpea) commonly used as livestock feed[44]. Depending on the results of Snow and Ghaly[40] all investigated plants contain sufficient amount of P, Cu and Zn to meet the dietary requirements of aquatic animals and exceed that of K, Fe and Mn.

High number of different fatty acids groups (19 fatty acids) were detected in 2 species, *M. spicatum* and *C. demersum* in site 5 while 16 FA types were detected in *E. crassipes*, which was higher than that reported for other macrophytes from different water courses by Haroon *et al.*[45], Haroon[4] and Haroon and Abdel-Aal[46] (6, 12 and 6 fatty acids respectively). Five of saturated fatty acid included tridecanoic, myristic, pentadecanoic, palmitic and stearic acid were detected in all plant species, in which palmitic and myristic acid were found in higher concentrations especially in *C. demersum* from site 5 and *Eichhornia crassipes* from site 4 respectively.

Concerning the USFA, Table 7 showed that three MUSFA were presence in all studied plants. Myristoleic and oleic acid were the highest concentration in *E. crassipes* from site 4, 5 respectively. *Myriophyllum spicatium* was characterized by the highest concentrations of TUSFA (4.21±0.01 g/100 g of lipid). Other previously studies of Haroon *et al.*[45] and Haroon[4] recorded arachidic acid as the major constituent of *Inula erithmoides* and *Potamogeton pectinatus* extracts, however palmitic acid represent the highest constituent of *Halimione portulacoides* fatty acids. In addition, myristic acid represents the highest amount of fatty acids separated from *P. stratiotes* shoots (28.3%) and *N. lotus* leaves (24.0%) extracts, while linolenic acid represents the major constituent of *N. lotus* stem (34.0%) fatty acids.

The polyunsaturated fatty acids, linoleic acid (C18:2c ω-6) and arachidonic (C20:4 α ω-6) acids were detected in all plant species but varied with the sampling sites, however α-linolenic acid (C18:3 α (ω-3) and Cis-11,14,17-Eicosatrienoic acid (C20:3 ω-3) were absence from *E. crassipes* and docosahexaenoic acid (DHA, 22:6 ω-3) was only detected in *C. demersum* from site 5. Although, *C. demersum* from site 5 was recorded to have the highest number (5) of PUSFA, *M. spicatum* was found to have the highest concentration (2.375% g of lipids).

Results in Table 9 and Fig. 2a shows the differential responses of different plant components to the different water variables. The highest protein values were found in all plant species from site 5 which was characterized by the highest water COD, NO_3 and PO_4 values compared with other sites. At

Table 9: Simple linear correlation coefficient between the measured plants components and water physicochemical parameters

Variables	WC	Ash	OM	NFE	Pro	Lipid	ME	EV	N	P ×10^{-2}	K	Temperature (°C)	EC (µg S cm^{-1})	pH	DO (mg L^{-1})	BOD (mg L^{-1})	COD (mg L^{-1})	NH_3 (µg L^{-1})	NO_2 (µg L^{-1})	NO_3 (µg L^{-1})	PO_4 (µg L^{-1})
WC (%)	1																				
Ash (%)	-0.240	1																			
OM (%)	0.240	-1.000	1																		
NFE (%)	0.154	-0.903	0.903	1																	
Protein (%)	0.379	-0.204	0.204	-0.220	1																
Lipid (%)	-0.185	-0.381	0.381	0.047	0.592	1															
ME	0.220	-0.975	0.975	0.797	0.365	0.567	1														
EV	0.234	-0.970	0.970	0.779	0.397	0.575	0.999	1													
N (%)	0.088	-0.231	0.231	-0.083	0.717	0.350	0.305	0.333	1												
P ×10^{-2} (%)	0.344	0.319	-0.317	-0.440	0.250	0.215	-0.221	-0.210	0.020	1											
K (%)	0.138	-0.599	0.599	0.234	0.786	0.672	0.706	0.727	0.817	0.034	1										
Temperature (°C)	0.513	0.079	-0.076	-0.196	0.372	-0.073	-0.054	-0.032	0.211	0.357	0.019	1									
EC (µg S cm^{-1})	-0.022	-0.324	0.324	0.363	-0.217	0.393	0.352	0.333	-0.385	0.266	0.026	-0.555	1								
pH	0.498	0.120	-0.126	-0.217	0.3174	-0.074	-0.092	-0.073	0.126	0.259	-0.046	0.992	-0.483	1							
DO (mg L^{-1})	-0.473	0.037	-0.035	0.025	-0.164	-0.143	-0.083	-0.084	0.153	-0.592	0.107	-0.784	-0.047	-0.843	1						
BOD (mg L^{-1})	0.387	0.201	-0.204	-0.312	0.375	-0.245	-0.203	-0.181	0.497	0.228	0.294	0.137	-0.417	0.071	0.287	1					
COD (mg L^{-1})	0.730	-0.184	0.188	-0.025	0.598	0.060	0.221	0.244	0.539	0.440	0.513	0.422	-0.184	0.374	-0.181	0.800	1				
NH_3 (µg L^{-1})	0.359	-0.059	0.055	0.050	-0.031	0.242	0.105	0.097	-0.382	0.714	-0.174	0.368	0.5329	0.463	-0.850	-0.407	0.051	1			
NO_2 (µg L^{-1})	0.137	-0.330	0.333	0.200	0.343	0.066	0.323	0.337	0.556	-0.298	0.584	-0.392	-0.077	-0.486	0.695	0.679	0.527	-0.665	1		
NO_3 (µg L^{-1})	0.511	-0.385	0.386	0.151	0.650	0.071	0.398	0.423	0.739	-0.125	0.648	0.383	-0.498	0.277	0.076	0.678	0.798	-0.390	0.685	1	
PO_4 (µg L^{-1})	0.279	-0.407	0.407	0.207	0.555	0.046	0.403	0.425	0.716	-0.439	0.589	0.308	-0.622	0.190	0.208	0.479	0.520	-0.584	0.630	0.929	1

Fig. 2(a-b): CCA-biplot ordination diagram of the macrophytes components in relation to the different water variables, (a) Axes F1 and F2: 58.03% and (b) Axes F1 and F2: 74.83%

DCP: Digestible crude protein, NFE: Nitrogen free extracts, ME: Metabolized energy, EV: Energy value, N: Nitrogen, P: Phosphorus, K: Potassium, OM: Organic matter, WC: Water contents, P/L: Protein/lipid, P/E: Protein/energy value, P/NFE: Protein/nitrogen free extracts, Temp: Water temperature, TN: Total nitrogen, TP: Total phosphorus, EC: Electrical conductivity, TRANS: Transparency, DO: Dissolved oxygen, BOD: Biochemical oxygen demand, COD: Chemical oxygen demand, TFA: Total fatty acids, TSFA: Total saturated fatty acids, TUSFA: Total unsaturated fatty acids, TSFA/TUSFA: Ratio of total saturated to total unsaturated fatty acids

Table 10: Simple linear correlation coefficient between the different fatty acids components and the water physicochemical parameters

Variables	TFA	TSFA	TUSFA	TSFA/TUSFA	Temperature (°C)	EC (µg S cm^{-1})	pH	DO (mg L^{-1})	BOD (mg L^{-1})	COD (mg L^{-1})	NH_3 (µg L^{-1})	NO_2 (µg L^{-1})	NO_3 (µg L^{-1})	PO_4 (µg L^{-1})
TFA	1													
TSFA	0.9810	1												
TUSFA	0.9602	0.8878	1											
TSFA/TUSFA	-0.2025	-0.0319	-0.4354	1										
N (%)	0.7676	0.7670	0.7183	-0.1771										
P×10^{-2} (%)	0.0537	0.1186	-0.0448	0.5703										
K (%)	0.4955	0.5367	0.4030	0.0589										
Temperature (°C)	0.5089	0.5440	0.4223	0.2656	1									
EC (µg S cm^{-1})	-0.6466	-0.5809	-0.6979	0.5419	-0.5556	1								
pH	0.4155	0.4564	0.3270	0.3283	0.9925	-0.4830	1							
DO (mg L^{-1})	0.0060	-0.0730	0.1214	-0.6694	-0.7847	-0.0476	-0.8431	1						
BOD (mg L^{-1})	0.7695	0.7653	0.7238	-0.1851	0.1371	-0.4173	0.0718	0.2877	1					
COD (mg L^{-1})	0.8006	0.8576	0.6634	0.2745	0.4262	-0.1842	0.3743	-0.1861	0.8009	1				
NH_3 (µg L^{-1})	-0.3631	-0.2667	-0.4793	0.8234	0.3685	0.5329	0.4638	-0.8550	-0.4037	0.0510	1			
NO_2 (µg L^{-1})	0.5511	0.5329	0.5413	-0.3191	-0.3922	-0.0773	-0.4863	0.6956	0.6797	0.5271	-0.6655	1		
NO_3 (µg L^{-1})	0.9252	0.9331	0.8514	-0.1201	0.3835	-0.4988	0.2779	0.0761	0.6788	0.7982	-0.3905	0.6850	1	
PO_4 (µg L^{-1})	0.8318	0.8068	0.8122	-0.3457	0.3085	-0.6229	0.1909	0.2082	0.4793	0.5202	-0.5847	0.6308	0.9292	1

the same time both lipid and NFE showed no clear relation with water physicochemical parameters. This is not in agreement with Tucker and Debusk[47] they mentioned a seasonal variation in carbohydrates, protein and crude fibres contents of *E. crassipes* cultured outdoors with constant nutrient availability. In addition a significant positive correlation was observed between nitrogen (N) content in all plants tested and water NO_3 and PO_4 contents. However, Nichols and Keeney[48] recorded lowest nitrogen content in summer samples of *Myriophyllum spicatum*, even though available nitrogen in the sediment was highest value at that time.

The correlation coefficient of different fatty acids and water physicochemical characteristics is presented in Table 10 that indicates the positive relationship between different fatty acid groups and water BOD, COD, NO_3 and PO_4. On the other hand the lowest TFA, TSFA and TUSFA were recorded for plant samples from site 2 which characterized by the highest water EC values. It was found that the three plant species were riche in their nutritional components, especially samples from site 5. Depending on the present results together with the previously recorded information the potential of these macrophytes as a natural food resources for fish and animals could be recommended, but some work concerning safety and toxicity is still needed.

CONCLUSION

The present work confirmed the nutritional importance of the three studied macrophytes species. All the studied species were found to be important sources of essential fatty acids (EFAs), in addition to their high organic matter, nitrogen free extract, potassium, ferrous and manganese contents that lay within the range of aquatic animal diet. The variation in their nutritional components with respect to species and location was observed. *Ceratophyllum demersum* and *Myriophyllum spicatum* from site 5 being the most suitable feeding items for animals and fish, as it contains high protein, lipid, P/E and fatty acids contents.

SIGNIFICANCE STATEMENT

This study discover the nutritional potential of *M. spicatum*, *C. demersum* and *E. crassipes* that can be beneficial for consumers to find a new natural food resources suitable to reduce the cost of commercial feeds. To support a recommendation for consumption of these aquatic plants as a natural food a full nutritional profile and screening for the presence of anti-nutritive factors, the presence of which could limit utilization prospects must be involved.

This study may help the researchers to determine the most suitable conditions that needed for cultivation of a good quality plants.

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