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*Research Article*

# Nutrients and bioactive compounds of the *Lemna gibba* and *Ulva lactuca* as possible ingredients to functional foods

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**ABSTRACT.** *Lemna gibba* freshwater macrophyte and seaweeds *Ulva lactuca* of the middle basin Papaloapan River, southeast of Mexico were chemically characterized in their nutrients and bioactive compounds for possible use in the formulation of functional foods. The proximate chemical analysis showed that ashes contents (g 100 $g^{-1}$ sample) of *L. gibba* and *U. lactuca* were 20.10 and 33.07, crude protein 21.5 and 17.2, lipids 4.45 and 1.7, nitrogen-free extract 32.4 and 38.34, respectively. Significant differences ($P < 0.05$) were found in the chemical composition between the two species of aquatic plants. *L. gibba* resulted in a protein source, and *U. lactuca* resulted in an energy source. They had eight essential amino acids for fish and other aquatic species and were abundant in lysine and methionine. Both aquatic plants had an essential quantity of inulin (functional fiber) this data not been reported. Also, they had xanthophyll and variety of antioxidant (β-carotenes, lutein, lycopene and neoxanthin). *L gibba* had only had one polyunsaturated fatty acid (PUFA; α-linolenic (ALA) 30.31 mg $g^{-1}$). *U. lactuca* had a variety of essential PUFA´s (ALA, LA, AA; 3.93, 6.73 and 0.41 mg $g^{-1}$ of fatty acids, respectively). Based on these results, both of the aquatic plants of the middle basin Papaloapan River studied are susceptible to take advantage in the formulation of functional food, since according to the literature the compounds identified have shown beneficial effects as immunonutrients, immunostimulants, antioxidants or modulators of intestinal flora. In aquaculture production, it is suggested to prove the combined use of these two plants as functional ingredients or some particular component in the diets as prevention strategy of diseases as well as to promote aquaculture sustainable through the use of these plants in the productions.

**Keywords:** *L. gibba*, *U. lactuca*, chemical composition, bioactive compounds, inulin, antioxidant.

## INTRODUCTION

One of the principal challenges in the aquaculture production is ensuring profitable production, preventing the illnesses that can lead to considerable economic losses in the aquaculture sector. The stress factors associated with illness in the cultivation of tilapia include natural phenomena (*e.g.*, hurricanes, cyclones, and torrential rains), inadequate management practices (*e.g.*, population density, cleanliness, use of inadequate substances), deficient water quality, inadequate nutrition, and inadequate diet (Peters *et al.*, 2009; Govind *et al.*, 2012). In the last years, in the field of aquaculture nutrition the functional diets are used because their nutrients and/or bioactive compounds contributes in benefits organic functions, as well as improving health and well-being and reducing the risk of illness (Chasquibol *et al.*, 2003; Zhou *et al.*, 2010; Zheng *et al.*, 2011). For aquaculture production, this represents not the only technology with a positive impact on health and disease prevention, but also an alternative to using of antibiotics for the treatment of

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illness during cultivation (Govind *et al.*, 2012). The use of antibiotics is undesirable because it can lead to resistance in fish and consumers and environmental harm since their disintegration in soil and water can take years (Peters *et al.*, 2009; Zhou *et al.*, 2010; Defoirdt *et al.*, 2011). It is well known that plants produce active compounds according to the environmental conditions and are used for treating some diseases. Exploitation of aquatic plants as a source of edible raw materials for fish has received a little attention, despite the study of some of them and to know some constituents benefits for human and animal health (Mukherjee *et al.*, 2010; Awad & Awaad, 2017). Aquatic plants as *Lemna gibba* (freshwater macrophyte) or *Ulva lactuca* (marine algae) are present most of the year in tropical areas and grow massively in a natural form (Peters *et al.*, 2009; Mukherjee *et al.*, 2010) although the cultivation technique also has been developed (Guy *et al*., 1990; Marinho *et al*., 2013). They are synthesized nutrients and macromolecules complementary to their vital functions in quantities that vary according to the environmental conditions when they are exposed, such as an attack by pathogens, predators, temperature and light changes, and nutritional deficiencies (Buentello *et al.*, 2010; Benjama & Masniyom, 2011). In some regions of the middle basin Papaloapan, *Lemna gibba* is considered a pest and *U. lactuca* is considered a source of pollution of the beaches and an unfortunate aspect for tourism. For the previous, the objective of this work was to identify the macromolecules and bioactive compounds of this aquatic plant for their possible sustainable use in formulating of functional diet for the aquaculture productions.

## MATERIALS AND METHODS

*L. gibba* was collected in its natural habitat in the municipality of Loma Bonita, Oaxaca, and *U. lactuca* from the beaches of the Port of Veracruz, Mexico. Plants were washed with potable water, dried in the sun by turning frequently, and further dried in a convection oven at 35°C to obtain flour from each plant. Proximate chemistry was analyzed for each plant (AOAC, 2006), and included the quantification of total nitrogen and crude protein by the Kjeldahl method (official method 984.13, A-D) (N × 6.25); total lipids by the Soxhlet method (official method 954.02); ash by combustion at 550°C (official method 942.05) and moisture (official method 945.16). Total dietary fiber (TDF), dietary fiber soluble (DFS) and insoluble dietary fiber (IDF) content was determined using enzymatic-gravimetric methods the official AOAC (methods 985.29, 991.43) and the Association of Analyses Chemist, AACC (Methods: 32-07, 32-21, 32-05 32-06, 1985-1987) using a commercial kit Megazyme (Megazyme International Ireland Ltd.) (Peña-Rodríguez *et al.*, 2011). An aminogram was performed using HPLC (official method 982.30 E (a,b,c), chp. 45.3.05), and a fatty acid profile was generated using the fatty acid methyl ester (FAME) concentration technique using gas chromatography (GC) 6850 coupled with a 5975 MSD CVL mass spectrometer (Agilent Technologies) (Mukherjee *et al.*, 2010; Kumar *et al*., 2011). Methanol extracts were obtained for each plant (Domínguez, 1973) to quantify and determine types of sugars following Somogyi-Nelson (González & Peñalosa, 2000), total structural sugars (Wood's technique, 1952) (Din *et al.*, 2009), xanthophyll (official method 970.64), and carotenoids (official method 938.04) (AOAC, 2006) using HPLC. Finally, microbiological analyses were performed which included total and fecal coliform analyses using the NMP technique (NOM-112-SSA1-1994, NOM-113-SSA1-1994), aerobic me-sophylls by the plate accounted technique (NOM-092-SSA-1994), and *Salmonella* (NOM-114-SSA-1994). Determinations were done in triplicate and duplicate, obtaining the arithmetic mean and standard deviation, and analyzed by Student's t-test in SPSS statistical software version 17.0 at 95% ($P < 0.05$) confidence.

## RESULTS

There was a significant difference ($P < 0.05$) between species in the content of all components. The proximate chemical analysis (Table 1) showed that ashes contents (g 100 $g^{-1}$ sample) of *L. gibba* and *U. lactuca* were 20.10 and 33.07, crude protein 21.5 and 17.2, lipids 4.45 and 1.7, nitrogen-free extract 32.4 and 38.34, respectively.

The primary component in both species was the nitrogen-free extract. *L. gibba* had higher crude protein (CP) (21.5%) and lipids (L) (4.45%) content (dry weight) than *U. lactuca* (17.2, 1.7) and the last one had the highest content of ash (33.07). The total dietary fiber (% dry weight) was 21.5 and 9.2, soluble dietary fibers (SDF) were 16.7 and 6.5, and insoluble dietary fiber (IDF) was 4.8 and 2.7, respectively. On the other hand, both aquatic plants presented eight essential amino acids recommended for fish growth by the National Resource Council (NRC, 1993). The amino acid content determined in plants was double of the fish recommendations, with exception of arginine and histidine. *L. gibba* presented the highest content of lysine and methionine (Table 2).

Concerning lipids content, *L. gibba* was significantly ($P < 0.05$) greater than *U. lactuca.* It had higher quantity and variety of saturated, no monosaturated and

**Table 1.** The proximal chemical composition on a dry basis (g 100 $g^{-1}$ sample) of the species of aquatic plants analyzed. Mean of three replicates ± standard deviation. Means in each parameter with different letters (a-b) are significantly different ($P < 0.05$). NR: Non-registered.

| Component | *Lemna gibba* | *Ulva lactuca* |
|---|---|---|
| Ash | [a]20.10 ± 0.33 | [b]33.07 ± 0.45 |
| Crude protein | [a]21.5 ± 0.38 | [b]17.2 ± 0.60 |
| Lipids | [a]4.45 ± 0.65 | [b]1.7 ± 0.37 |
| Total dietary fiber (TDF) | [a]21.5 ± 0.49 | [b]9.2 ± 0.71 |
| Soluble dietary fiber (SDF) | [a]16.7 ± 0.47 | [b]6.5 ± 0.34 |
| Insoluble dietary fiber (IDF) | [a]4.8 ± 0.82 | [b]2.7 ±0.65 |
| Nitrogen free extract | [a]32.4 ± 0.36 | [b]38.34 ± 0.41 |
| Total structural polysaccharides (mg $g^{-1}$) | [a]329 ± 0.031 | [b]274 ± 0.047 |
| Reserve polysaccharides (mg $g^{-1}$) | [a]319 ± 0.026 | [b]422 ± 0.007 |
| Inulin (mg $g^{-1}$) | [a]133.45 ± 19.84 | [b]272.18 ± 9.43 |
| Maltose (mg $g^{-1}$) | NR | 18.326 ± 3.69 |

**Table 2.** Amino acid profile found in *L. gibba* y *U. lactuca* compared with the amino acid requirements for fish by the NRC (1993). Mean of two replicates ± standard deviation. a, b, indicate significant differences ($P < 0.05$).

| Aminoacid | *L. gibba* (%) | *U. lactuca* (%) | NRC (1993) (%) |
|---|---|---|---|
| Arginine | [a]0.003 ± 0.29 | [a]0.004 ± 0.21 | 1.18 |
| Histidine | [a]0.001 ± 0.24 | [a]0.001 ± 0.27 | 0.48 |
| Isoleucine | [a]1.569 ± 0.11 | [b]4.57 ± 0.17 | 0.87 |
| Leucine | [a]0.003 ± 0.12 | [b]9.33 ± 0.14 | 0.95 |
| Lysine | [a]22.33 ± 0.09 | [b]9.24 ± 0.12 | 1.43 |
| Methionine | [a]8.57 ± 0.10 | [b]5.92 ± 0.14 | - |
| Threonine | [a]0.21 ± 0.09 | [b]4.76 ± 0.13 | 1.05 |
| Valine | [a]2.23 ± 0.11 | [b]4.01 ± 0.12 | 0.78 |
| Alanine | [a]27.80 ± 0.13 | [b]9.77 ± 0.15 | - |
| Aspartic acid | [a]8.77 ± 0.12 | [b]10.48 ± 0.14 | - |
| Glutamic acid | [a]23.13 ± 0.12 | [b]15.40 ± 0.15 | - |
| Glycine | [a]1.76 ± 0.11 | [b]6.11 ± 0.12 | - |
| Proline | [a]0.01 ± 0.10 | [a]0.007 ± 0.12 | - |
| Serine | [a]2.03 ± 0.11 | [b]7.64 ± 10.14 | - |
| Tyrosine | [a]1.69 ± 0.11 | [b]3.64 ± 0.11 | - |

only had one polyunsaturated fatty acid (PUFA; α-linolenic (ALA) 30.31 mg $g^{-1}$). *U. lactuca* had a lower contribution of saturated, had two monosaturated (palmitoleic 2.08 mg $g^{-1}$) and variety important PUFA´s (ALA, LA, and AA; 3.93, 6.73 and 0.41 mg $g^{-1}$ of fatty acids, respectively) (Table 3).

The content, total structural polysaccharides quantified were 329 and 274 mg $g^{-1}$, reserve polysaccharides 319 and 422 mg $g^{-1}$, inulin 133.45 and 272 mg $g^{-1}$ and maltose 0 and 18.32 mg $g^{-1}$, *L. gibba* and *U. lactuca* respectively. The fresh and dry samples xanthophyll content in *L. gibba* was significantly greater than green marine alga *U. lactuca* (8.45 and 16.48 g $kg^{-1}$ compared to 2.86 and 1.70 g $kg^{-1}$, respectively) (Table 4). The carotenoids identified in both plants were beta-carotene, lycopene, and lutein; also, *L. gibba* presented α-carotenes, criptoflavin and violaxanthin and *U. lactuca* presented neoxanthin.

Microbiological analyses of *L. gibba* and *U. lactuca* indicated the presence fecal coliforms and totals (0.06 and 1.09 NMP 100 $mL^{-1}$ and 40 and 92 NMP 100 $mL^{-1}$, for each plant respectively) and standard plate count (24 and $98\times10^{-3}$ UFC $g^{-1}$) without exceeded fishery products permissible limits of official Mexicans standards (10 NPM 100 $mL^{-1}$, 100 NPM 100 $mL^{-1}$ and $150\times10^{-3}$ UFC $g^{-1}$). *Salmonella* was absent at 25 g, as set the standard.

## DISCUSSION

Because we want to take advantage of *L. gibba* and *U. lactuca*, from the middle basin of the Papaloapan, in the

**Table 3.** Fatty acids profile of aquatic plants studied.

| Fatty acid | *U. lactuca* (mg $g^{-1}$ of lipids) | *L. gibba* (mg $g^{-1}$ of lipids) |
|---|---|---|
| Saturated | | |
| C12:0, Lauric | 3.4283 | 0.0599 |
| C14:0, Myristic | 21.3033 | 2.5249 |
| C16:0, Palmitic | --- | 32.8270 |
| C18:0, Stearic | 2.5318 | --- |
| C20:0, Arachiric | 0.0856 | --- |
| C21:0, Nonadecanoic | --- | 1.0505 |
| C22:0, Docosanoic | --- | 1.7315 |
| C23:0, Tricosanoic | --- | 0.5386 |
| Monounsaturated | | |
| C16:1, Palmitoleic | 2.0856 | --- |
| C20:1, Gadoleic | 4.7358 | --- |
| Polyunsaturated | | |
| C18:3(9,12,15),α-Linolenic (ALA) | 3.9375 | 30.3135 |
| C18:2ω6, Linoleic (LA) | 6.7319 | --- |
| C20:4ω6, Araquidonic (AA) | 0.4113 | --- |

**Table 4.** Xanthophyll concentration (g xanthophyll $kg^{-1}$) identified in the aquatic plants studied. Mean of two replicates ± standard deviation. a, b, indicate significant difference ($P < 0.05$).

| *L. gibba* | | *U. lactuca* | |
|---|---|---|---|
| Fresh | Dry | Fresh | Dry |
| $^{a}8.45 \pm 0.2347$ | $^{b}16.48 \pm 0.6121$ | $^{a}2.86 \pm 0.5782$ | $^{b}1.70 \pm 0.4591$ |

formulation of functional foods, it was necessary to characterize them chemically and microbiologically. It is known that there exist differences in their chemical composition, due to the physical and chemical factors associated with the habitat of each aquatic plant, such as distribution, temperature, pH, nutrient availability, turbidity, depth, light, and physiological state of the plants (Aguilera-Morales *et al.*, 2005; Mukherjee *et al.*, 2010; Benjama & Masniyom, 2011). The content of chemical compounds in the aquatic plants were within the range reported in other studies; for *L. gibba* has been reported (g 100 $g^{-1}$) a protein interval of 13-33, crude fiber 8-48, ashes 9-25, total lipids 2.3-5, nitrogenous extract free 16-32 (Kalita *et al.*, 2007; Talukdar *et al.*, 2013) while for *U. lactuca* has been reported (g 100 $g^{-1}$) a protein interval of 11-29, total lipids 0.3-2.5, crude fiber 2.8-5.1, ashes 13-46 (Carrillo *et al.*, 2008; Yildirim *et al.*,2009). Based on the content of CP *L. gibba* could be used as a source of protein and *U. lactuca* a source of energy (NRC, 1993; Cozzolino, 2000) or to find the best combination. For example, the protein requirement of some aquatic species such as tilapia fish in the fattening stage is 35 to 25% (Furuya, 2010), *L. gibba* could be used as a protein complement and source of lysine and methionine essential for the growth of this species (Frikha *et al.*, 2011; Shuuluka *et al.*, 2013). Several determined amino acids satisfy the nutritional needs of critical aquatic species like tilapia, trout and shrimp between others (Halver & Hardy, 2011; Kumar *et al.*, 2011; Hanne *et al.*, 2014). Notwithstanding the above, the protein amounts in aquatic plants studied were similar to other protein sources that are traditionally used for animal nutrition, such as soy paste and amaranth (Hanne *et al.*, 2014).

*U. lactuca* presented greater mineral content. The variations ash content of the aquatic plants is related to each one's ability to store minerals according to their aquatic environment (Frikha *et al.*, 2011), though this may also be due to the association between cations and polysaccharides (Chasquibol *et al.*, 2003; Frikha *et al.*, 2011). The difference in total lipids contents (which includes fatty acids, phospholipids, sterols, vitamins, hydrocarbons, and pigments) between the studied species of aquatic plants was due to the season of the year during which they were harvested and the influence of the environment on them (Dewanji, 1993; Aguilera-Morales *et al.*, 2005; Hanne *et al.*, 2014). Respect to polyunsaturated fatty acids (PUFAs), it should be considered that dried plants contain a higher quantity due to oxidation, which, at the same time,

depends on several factors (species, texture, time of exposure to air, sun and high temperatures, and storage time and conditions) (Sánchez-Machado *et al.*, 2004; Mukherjee *et al.*, 2010; Kumar *et al.*, 2011). The amount of α-linolenic quantified in *L. gibba* was higher than those reported by Mukherjee *et al.* (2010) and near to reported by Yan *et al.* (2013) (47.14 mg $g^{-1}$ fatty acid). To *Ulva*, Yaich *et al.* (2011) reported that the saturated fatty acids 16:0 and 22:0 and monoun-saturated fatty acid 18:1 were the primary fatty acids. Yu-Qing *et al.* (2016) also reported fatty acid saturated dominance. In this study there was also the dominance of saturated fatty acids; however, the quantities and variety of PUFA´s are to consider their use (3.93 and 6.73 mg $g^{-1}$, ALA and LA respectively). These are essential polyunsaturated fatty acid that takes part in the inhibition of prostaglandin synthesis; it has anti-inflammatory properties and is associated with disease prevention. They play a critical role as phospholipid components and are essential for the growth and productive output in fish and are also natural ligands for a class of anti-inflammatory transcription factors known as a peroxisome-proliferating receptor (Mukherjee *et al.*, 2010; Kumar *et al.*, 2011). Govind *et al.* (2012) state that these fatty acids can improve nerve transmission, participate in molecular mechanisms of cellular membranes, reduce the formation of LDL cholesterol and triglycerides in the liver, and improve immunity by increasing defenses and participating in hormone synthesis. According to the U.S. National Library of Medicine (2017), the fatty acids C21:0 and C22:0 have antioxidant properties and antimicrobial.

On the other hand, it has been published on the chemical characterization of polysaccharides mainly in *Ulva* sp., this parameter in this study was agreement with literature in green algae (Lahaye & Jegou, 1993; Ray, 2006). Differences polysaccharides content between the species is explained by the nitrogen content of the aquatic environment that affect the biosynthesis of the algal cell polysaccharides as well as the protein and the pigments (Lahaye & Ray, 1996). Ortiz *et al.* (2006) reported for *U. lactuca* TDF (total dietary fiber) 55.4-60.5% (dry weight) values, which was superior to this study. Benjama & Masniyom (2011) reported to *Ulva* sp. 8.7-39.6% (dry base) of IDF and 51.3-62.2 SDF. For *L. gibba* we found no literature reporting TDF, SDF, and IDF. The chemical characterization and analyses of polysaccharides of *Lemna* sp. are oriented to the potential biofuel because they are rich in cellulose and starch. The amounts *L. gibba* and *U. lactuca* soluble fiber as inulin were appreciable (272.18-133.45 mg $g^{-1}$), and represent 67% of the reserve sugars which had not been reported in this species and are of great importance, since they are highly fermentable for the production of short-chain fatty acids (SCFA), which are an energy substrate or food for beneficial bacteria (prebiotic effect) as *Enterococcus* that decreases colon pH by secreting lactic acid and promote vitamin synthesis, principally B-complex, which strengthens the immune system. Notwithstanding, SCFA's (butyrate, propionate, and acetate) can be metabolized in muscle to obtain energy (acetate), synthesize lipids and glucose (propionate), produce mucous and some proinflammatory cytokines (butyrate) (Leary & Lovell, 1975; Lahaye, 1991; Chasquibol *et al.*, 2003). In addition to all of these benefits, the solubility is directly proportional to the property of functional capacity to retain water, a characteristic that is highly technologically exploitable to increase the resulting feed's stability in water (Quitral *et al.*, 2012).

The significant difference in the content of xanthophyll and carotenoids fresh and dry samples between species of the aquatic plants studied due to physiological state and environment condition aquatic (Furuya, 2010; Zhou *et al.*, 2010), although drying process its components are susceptible to light and high temperatures because have long-chain liposoluble structures with conjugated double-bonds (Ortiz *et al.*, 2006; Abdel-Aal *et al.*, 2013). Xanthophyll and carotenoids determined in *L. gibba* and *U. lactuca* are potent antioxidants, implicated in the detriment of some illnesses since they participate in the immune response, in the neutralization of reactive oxygen and nitrogen species produced during cellular metabolism (Martino *et al.*, 2002; Madrigal & Sangronis, 2007; Frikha *et al.*, 2011). Regulate the expression of some genes, such as those that produce γ-interferon, which is responsible for regulating inflammatory and immune responses (Benjama & Masniyom, 2011; Quitral *et al.*, 2012; Abdel-Aal *et al.*, 2013).

From a microbiological point of view, *L. gibba* and *U. lactuca* collected in the basin of the Papaloapan do not represent a risk for use in the feeding of aquatic organisms as not exceeded the permissible limits by the Mexican Official Standards. Thus both the species of aquatic plants meet the safety limits regarding bacteriological criteria. Regarding other researches and *U. lactuca*, the contents microorganism were low in *L. gibba* probably for its ability reduce populations of bacteria due to the components of its wall cellular (Dewedar & Bahgat, 1995; Abou El-kheir *et al.*, 2007). The coliform total and fecal and standard plate count values of *U. lactuca* were high respect to referred by Abirami & Kowsalya (2011) (<1 to 3 $g^{-1}$ coliform and 40 cfu $g^{-1}$ standard plate count). It is important to consider that the content of microorganisms is in function in the environment where these aquatic plants are collected.

## CONCLUSIONS

The freshwater macrophyte *L. gibba* and marine alga *U. lactuca* of the middle basin Papaloapan River, Mexico could be used in the formulation of functional foods for aquatic organism since to that these aquatic plants contain exploitable quantities of nutrients and bioactive compounds, which according to the literature could provide substantial benefits for health and disease prevention. *L. gibba* turned out to be a protein source rich in xanthophyll; they presented α-carotenes, criptoflavin and violaxanthin and high quantity of ALA fatty acid. *U. lactuca* turned out to be an energy source with an important amount of variety PUFAs and was also a source of xanthophyll and carotenoid. Both aquatic plants contained eight essential amino acids recommended to aquatic species, and they were an important source of lysine and methionine. They had soluble polysaccharides, inulin, β-carotenes, lutein, and lycopene. Microbiologically *L. gibba* and *U. lactuca* can be used in fish food formulation.

This study is a contribution to sustainable aquaculture promotes the use of undesirable aquatic plants with attractive organoleptic characteristics for several species aquaculture interest commercial. According to the feeding aim, aquatic plants studied can be used in fresh or flour or maybe to extract any metabolites of interest. In aquaculture production, it is suggested to prove the combined use of these two plants as functional ingredients or some particular component in the diets as prevention strategy of diseases as well as to promote aquaculture sustainable through the use of these plants in the productions.

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