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PHYTOCHEMICAL PROFILE OF LEMON BASIL GROWN IN AQUAPONIC CULTURE

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Abstract

A traditional aromatic culinary plant, basil (Ocimum spp.) also offers a wide range of potential health benefits, including antioxidant, anti-inflammatory and anticarcinogenic effects. Multiple varieties cultivated around the world, ranging from the traditional Ocimum basilicum - sweet basil to Genovese basil, cinnamon basil, purple basil, holy basil, etc, were investigated for their therapeutic properties due to their high content of antioxidant compounds. The aim of this study was the phytochemical analysis of a lemon-flavored basil, with seeds procured from Kiepenkerl. To promote a circular economy, the lemon basil was grown together with yeast biomass fed fish in an aquaponic system. The samples consisted of basil grown with: different percentages of yeast biomass added to conventional fish feed, regular fish feed, and traditional soil-based technologies. The phytochemical composition of the samples was assessed following microwave-assisted extraction. The evaluation included antioxidant activity (DPPH, FRAP, ABTS assays), as well as total phenolic and flavonoid contents. Among the 14 compounds identified in the UPLC investigation, caffeic acid and rosmarinic acid had the highest content in all the samples.

Key words*: aquaponic culture, antioxidant activity, flavonoid content, Ocimum basilicum, UPLC, total phenolic count.*

INTRODUCTION

Food production depends on the availability of resources and nutrients, which are currently depleting faster than they can be generated for a global population that is estimated to reach 10 billion people by 2050 (FAO, 2017). This growth is expected to occur in a world where climate change, biodiversity decline, and the degradation of arable land are already leaving a mark, which makes providing the appropriate food production levels worldwide an even bigger challenge. In order to combat this situation, alternative ways to soil based plant cultivation are currently being researched and practiced, among which aquaponics systems represent a viable option (Goddek et al., 2019).

Aquaponics systems work on the basis of nutrient exchange between a hydroponic unit and an aquaculture unit with the help of the nitrification process initiated by nitrifying bacteria. Metabolites resulting from the food digestion of various fish species are transformed in a two-step nitrification process. Ammoniac is oxidized to nitrites by *Nitrosomonas* type of bacteria, being further oxidized to nitrates by *Nitrobacter*, which leads to an easier access to nitrogen, an essential component for plant growth and its protein synthesis (Rana et al., 2019).

The simultaneous cultivation of both plants and fish can lead to economic benefits since two types of cultures are obtained and production can increase because of nutrient recycling and the reduced consumption of water. Advantages include a reduced impact on the environment, a continuous plant production throughout the year, as well as the end result of organic cultures with a minimum amount of added chemical substances (Joyce et al., 2019; Greenfeld et al., 2022; Albadwawi et al., 2022).

The foremost characteristics among the fish species used in this type of cultivation system consist of their capacity to tolerate higher densities of population and increased values of solid particles, nitrogen, phosphorus and potassium. Often utilized fish species include Nile tilapia, carp and African catfish (Lennard et al., 2019; Yep & Zheng, 2019).

Plant cultures with a high tolerance towards disease and pests, that develop well in water with high concentrations of nitrogen with

shorter growth periods and without high nutritional requirements are preferable in the case of aquaponics systems. Among the most used plants grown in aquaponics systems, Love et al. (2015) mention *Ocimum basilicum* (81%), *Solanum lycopersicum* (68%), *Lactuca sativa* (68%), *Brassica oleracea* (56%), *Beta vulgaris* (55%), *Brassica rapa* subsp. *chinensis* (51%), *Capsicum annuum* (piper) (48%) and *Cucumis sativus* (45%).

O. basilicum renders itself as a suitable plant for aquaponics, which is an advantage given its potential as a medicinal plant and its historically demonstrated therapeutic effects. Part of the aromatic *Lamiaceae* family, it has a variety of over 65 cultivars, such as sweet basil, purple basil, Genovese basil, holy basil, cinnamon basil, that mostly prefer subtropical climate (Makri & Kintzios, 2008). It is an Asian native plant traditionally used in the treatments of fever, coughing, headaches and digestive problems (Shahrajabian et al., 2020). Its high content in polyphenols and flavonoids determines a wide array of anti-inflammatory, anticancer, antioxidant, antiviral and antibacterial properties. In a 2019 study, Singh et al (2019) found that ethanol extracts from basil leaves inhibited Zika virus replication in Vero E6 cells, with a maximum inhibitory concentration (IC_{50}) of 1:134. Other studies have also demonstrated antiviral activity against viruses, such as herpes, adenovirus, hepatitis B, and enterovirus 71 (Azizah et al., 2023). In a 2022 study, Dharsono et al. (2022) observed inhibition zones of 20-40 mm of essential oils from various basil species against both Gram-positive and Gram-negative bacteria. In a 2021 study, Ciotea et al. (2021) tested basil essential oil obtained by steam distillation against bacteria, which displayed a strong antibacterial activity against *S. pyogenes, S. viridans*, and moderate to low one for the *S. pneumoniae, K. pneumoniae, E. coli, E. faecalis* bacteria.

Basil's anti-inflammatory effects are attributed to compounds like linalool, estragole, methyl cinnamate, and methyl eugenol. Basil essential oil inhibits lipogenesis at low concentrations, while plant extracts suppress nitric oxide (NO) production (Brandão et al., 2022). Additionally, basil extracts exhibit stronger anti-inflammatory activity than aspirin by preventing egg albumin denaturation (Akoto et al., 2020). Plant extracts of *O. basilicum* can act against cancer by inducing cytotoxicity in MCF7 mammary cancer cells (Koolamchal et al., 2022). All these properties display the high nutraceutical potential basil possess.

The present study aims to investigate the phytochemical profile of a lemon flavoured basil grown in an aquaponic culture following a differentiated diet regime for the fish, consisting of a combination between industrial feed and various proportions of *Saccharomyces cerevisiae,* in order to highlight the differences between the antioxidant properties of the various methods of cultivation.

MATERIALS AND METHODS

The aquaponic system was composed of a reservoir with dimensions of 47x37x42 cm and a volume of 73 cm^3 (Figure 1). The biofilter used for the development of nitrifying bacteria consisted of expanded clay granules. The system was complemented by an EHEIM compact 300 pump with a capacity of 150-300 l/h and 5W power, as previously implemented in a 2016 study (Frincu & Dumitrache, 2016). The fish species present in the aquaponic system were *Carassius auratus* and *Hypostomus plecostomus*.

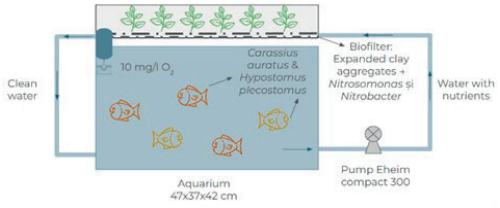

Figure 1. Schematic representation of the aquaponics system

The lemon-flavored basil seeds "Sweet Lemon" were obtained from the Kiepenkerl company and sown using sterilized Jiffy peat pellets with a diameter of 50 mm. Basil samples were obtained through different dietary regimes for the fish: one sample was raised by feeding the fish industrial feed – further utilised in the experiment with the M1 code, another by providing experimental feed with 3% yeast biomass – marked as P1, and a third by feeding with 5% yeast biomass – marked as P2. As a

control, basil plants were grown in soil and marked with the M2 code. The feed and yeast biomass mixture were prepared by incorporating *Saccharomyces cerevisiae* yeast into pellet feed. A total of 1000 g of commercial feed pellets were transformed into dough, pelletized, and dried for 24 hours at 40°C. The fish were fed a daily amount equivalent to 2.5% of their total body weight.

The basil extracts were prepared using the Milestone ETHOS X microwave extraction system (Milestone, Sorisole, Italy). The mature, freeze-dried basil plant was finely ground into a homogenous powder at 4500 rpm using the Grindomix GM mill (Retsch, Dusseldorf, Germany). From each of the four basil samples, approx. 1.5 g of plant material was weighed and mixed with ethanol at varying concentrations (50%, 70%, and 100%) in plant-to-solvent ratios of 1:20 and 1:40. This resulted in a total of 24 hydroalcoholic extracts (as detailed in Table 1). The extraction process involved maintaining a temperature of 100°C for 60 minutes with a power output of 500W. Subsequently, the plant-solvent mixture underwent vacuum filtration using Whatman filter paper No. 1. The resulting extract was then utilized for various analyses, including DPPH, ABTS, FRAP, determination of total phenolic and flavonoid content, and UPLC chromatographic analysis.

Table 1. Hydroalcoholic extracts

Sample code	Plant mass (g)	Plant: solvent ratio	EtOH (%)
P1.1, P2.1, M1.1, M2.1	1.5	1:20	50
P1.2, P2.2, M2.1, M2.2	1.5	1:20	70
P1.3, P2.3, M1.3, M2.3	1.5	1:40	50
P1.4, P2.4, M1.4, M2.4	1.5	1:40	70
P1.5, P2.5, M1.5, M2.5	1.36	1:20	100
P1.6, P2.6, M1.6, M2.6	1.36	1:40	100

The extraction yield of hydroalcoholic extracts was obtained by weighing the mass of the plant material and the solvent before and after filtration.

Total content of phenolic acids

The content of phenolic acids was determined using the Folin-Ciocâlteu method, adapted for microspectrophotometer method according to a previous study published in 2023 by Marchidan et al. The reference standard used was gallic acid and the results were expressed in milligrams of gallic acid equivalents per kilogram of plant material (mg GAE/kg). All extracts were analysed in triplicate by absorbance measurements at 760 nm.

Total flavonoid content

The total flavonoid content was determined using the microspectrophotometer method. (Marchidan et al., 2023) After incubation in the dark for 15 minutes, the absorbance was read at 415 nm and the results were expressed in milligrams of quercetin equivalents pe kilogram of sample (mg EqQ/kg).

Antioxidant activity

The antioxidant activity was assessed using the microspectrophotometer method with DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, as presented in a previous paper by Marchidan et al. (2023), the absorbance of each sample was measured at a wavelength of 517 nm. The inhibition percentage, indicative of antioxidant activity, was calculated using the equation:

$$Inhibition\ percentage = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} x100$$

The antioxidant content was also calculated using the $ABTS^{\cdot+}$ cation radical solution, using the method presented earlier in a study in 2023 (Marchidan et al., 2023). The absorbance was measured at 734 nm and the inhibition percentage was calculated using the following formula:

$$Inhibition\ percentage = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} x100$$

The FRAP (Ferric Reducing Antioxidant Power) solution was prepared using an adapted microspectrophotometer method and the absorbance was measured at 593 nm. The antioxidant capacity was calculated using the following equation:

$$Antioxidant\ capacity = \frac{Sample\ absorbance\ after\ 20\ minutes - Initial\ absorbance}{Positive\ control\ absorbance - Initial\ absorbance} x2$$

The results were expressed in milligrams of ascorbic acid equivalents per kilogram of sample (mg EqAA/kg).

Chromatographic assay

The separation, identification and quantification the polyphenolic compounds

present in the extracts were analysed using Acquity UPLC I Class system (Waters Corporation, Milford, Massachusetts, U.S.), with a Zorbax Eclipse Plus C18 column (100 mm x 4.6 mm, with a 5 µm sized particles) and an injection volume of 10 µl. The temperature was set at 30°C, and the mobile phases consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The elution gradient spanned 0-100% B over 30 minutes, with a flow rate of 0.8 mL/min. 14 reference compounds were used, including caffeic acid, rosmarinic acid, chlorogenic acid, epicatechin, p-coumaric acid, rutin, isoquercetin, ferulic acid, naringin, myricetin, luteolin, quercetin, and naringenin. Detection wavelengths of 280 nm, 320 nm, and 370 nm were employed. Identification and quantification were performed by comparing retention times with reference compounds. Stock solutions of reference compounds were prepared at a concentration of 12 mg/ml, and linear concentrations of 5-150 µg/ml were used to create calibration curves.

RESULTS AND DISCUSSIONS

Microwave-assisted extraction was chosen due to its reduced operating time compared to classical methods such as maceration or ultrasonication, which allows for improved efficiency and cost-effectiveness, as well as reduced degradation of the compounds of interest. Heating the solvents and samples enhances the transition of compounds from the plant matrix into the solvent. Ethanol was selected as the extraction solvent due to its low toxicity, enabling integration of the extract into food or pharmaceutical products. The presence of water in the hydroalcoholic extract alongside the polar solvent determines more efficient heating and facilitates the extraction process (Sonar & Rathod, 2020).

The extraction yield obtained (Table 2) indicate the highest values for the EtOH extraction of 50% and 70% with a plant: solvent ratio of 1: 40 for both concentrations. The extraction yield range varies between 53.98% and 86.17%, the highest yield being observed in the control group cultivated in soil (86.17% for M2.3, 83.75% for M2.4, and 84.59% for M2.1). Following this, the samples obtained through 3% yeast biomass feeding at a 1:40 ratio and using EtOH concentrations of 50% and 70% exhibit yields of 81.68% (P1.3) and 79.90% (P1.4), respectively.

Table 2. Extraction yield

Sample	Plant (g)	Plant:solvent ratio	EtOH (%)	Yield (%)
P1.1	1.50	1:20	50	65.65
P1.2	1.50	1:20	70	68.85
P1.3	1.50	1:40	50	81.68
P1.4	1.50	1:40	70	79.90
P1.5	1.36	1:20	100	62.22
P1.6	1.36	1:40	100	80.53
P2.1	1.50	1:20	50	64.84
P2.2	1.50	1:20	70	55.57
P2.3	1.50	1:40	50	76.29
P2.4	1.50	1:40	70	77.25
P2.5	1.36	1:20	100	63.73
P2.6	1.36	1:40	100	53.98
M1.1	1.50	1:20	50	67.57
M1.2	1.50	1:20	70	63.77
M1.3	1.50	1:40	50	78.73
M1.4	1.50	1:40	70	77.19
M1.5	1.36	1:20	100	61.72
M1.6	1.36	1:40	100	72.74
M2.1	1.50	1:20	50	84.59
M2.2	1.50	1:20	70	75.35
M2.3	1.50	1:40	50	86.17
M2.4	1.50	1:40	70	83.75
M2.5	1.36	1:20	100	62.81
M2.6	1.36	1:40	100	73.63

Total content of phenolic acids

TPC was performed using a calibration curve with a known standard. Based on the absorbances measured at concentrations of 0.20, 0.39, 0.78, 1.56, 3.15, 6.25, 12.50, 25, 50, and 100 µg/ml, a calibration curve was constructed using gallic acid as the reference standard. The total polyphenol content was expressed in milligrams of gallic acid equivalents per kilogram (mg GAE/kg) using the arithmetic mean of the three readings performed for each individual sample. Higher values were observed in the extracts obtained from basil cultivated in aquaponic systems and fish fed with a combination of industrial feed and 3% yeast biomass, as seen in Figure 2.

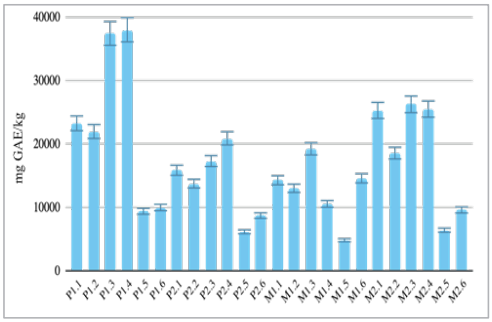

Figure 2. Total polyphenolic content of the basil samples expressed as mg GAE/kg

The best results were achieved through microwave-assisted extraction using 50% and 70% ethanol (EtOH) in a plant:solvent ratio of 1:40. Specifically, the concentrations of active compounds (expressed as gallic acid equivalents, GAE) were P1.3 = 37403 mg/kg and P1.4 = 37970 mg/kg. Notably, relatively high values ranging from 25271 to 26226 mg were also found in the control group of basil grown in soil, particularly at the 1:20 ratio with 50% EtOH concentration and the 1:40 ratio with both 50% and 70% EtOH concentrations. Compared to the highest value obtained for TPC in this study, 37.403 mg/g GAE, Nadeem et al (2022) reported a higher content of 191.2 mg GAE/g in basil extracted with ethanol and lower TPC of 29.7 mg/g in basil extracted with *n*-hexane. In a 2022 study, Romano et al obtained for supercritical CO_2 extracts from "Italiano Classico" and "Genovese" cultivars TPC values of 72.15 and 79.62 mg GAE/g, respectively, while the ethanol extraction method yielded TPC values of 91.66 and 98.99 mg GAE/g for the same cultivars. These findings suggest a possible difference in TPC values due to different extraction methods and different cultivars. According to Albadwawi et al. (2022), total phenols of aquaponic grown basil displayed higher values than soil grown plant, which is different compared to the control TPC values obtained in the present study.

Total flavonoid content

The quantification of total flavonoids was expressed in corelation with a standard calibration curve having quercetin as a reference. The calibration curve was plotted using a series of decreasing concentrations starting with 100 µg/ml and ending with 1.56 µg/ml. Figure 3 indicates the highest value as 2958.40 mg QE/kg obtained for the control basil grown in soil and extracted with EtOH 70% in plant: solvent ratio of 1:40. All the basil samples showed higher values for a EtOH concentration of 70% in a plant solvent ratio of 1:40.

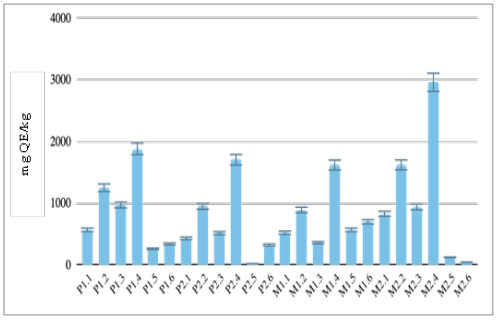

Figure 3. Total flavonoid content of the basil samples expressed as mg QE/kg

Antioxidant activity

The antioxidant activity determined by the inhibition of the DPPH radical was superior for the samples prepared in a 1:20 plant: solvent ratio, as can be observed in Figure 4, regardless of the concentration levels of ethanol (50% or 70%), which suggests that the antioxidant activity is not dependant on the total polyphenolic content in the case of the investigated basil species.

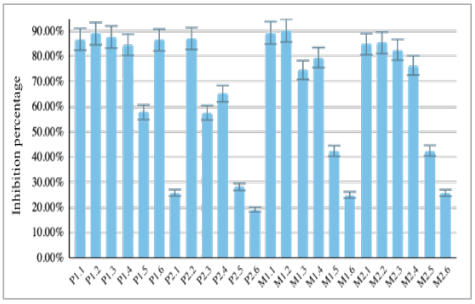

Figure 4. Antioxidant activity obtained following the inhibition of the DPPH radical

Highest values ranged between 86% and 89% for the basil grown with fish fed with a 3% yeast biomass, while the maximum value of 90.21% was registered for the sample grown

with fish fed with just industrial feed, results similar in range to those obtained by DPPH assay by Nadeem et al for ethanolic extracts (82.4%). Figure 4 also indicates lower numerical results for the samples obtained with 100% EtOH and a plant:solvent ratio of 1:40.

The antioxidant potential of the samples was expressed as a percentage of $ABTS^{\bullet+}$ cationic radical inhibition and ranged for almost all samples between 90-100%, as shown in Figure 5, with maximum values of approx. 96% obtained by the sample grown with fish fed with 5% yeast biomass and a plant: solvent ratio of 1:20. Similarly to the results of the DPPH test, the lowest values were found for the 100% EtOH concentration and a 1:40 ratio, where the inhibition percentage ranged from 20-45%. These results suggest that the extraction made with a binary solvent leads to superior results regarding the antioxidant activity. The higher percentage of inhibition for ABTS assay compared to DPPH is similar to the results obtained by in 2022 by Romano et al., suggesting the ABTS assay exhibits distinct reactivity with various components and follows different pathways compared to the DPPH assay.

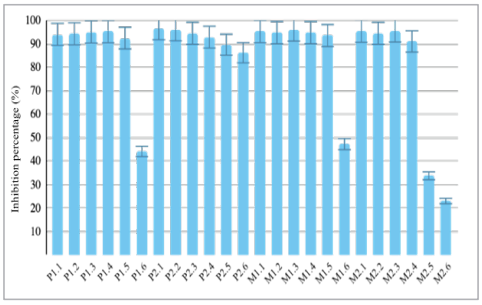

Figure 5. Antioxidant activity obtained following the inhibition of the ABTS radical

For the FRAP assay, the antioxidant potential of basil extracts was expressed in terms of antioxidant activity equivalents, specifically in milligrams of ascorbic acid equivalent per kilogram of plant material. The results are shown in Figure 6. The highest values are observed for sample P1.3 = 38800.07 mg AA/kg and P1.4 = 40233.39 mg AA/kg, corresponding to basil cultivated in an aquaponic system with fish fed a mixture of industrial feed and 3% yeast biomass, a plant: solvent ratio of 1:40 and 50% and 70% EtOH concentrations. Other values fall within the range of 14000 to 28000 mg AA/kg, except for the samples extracted with 100% EtOH which feature lower values.

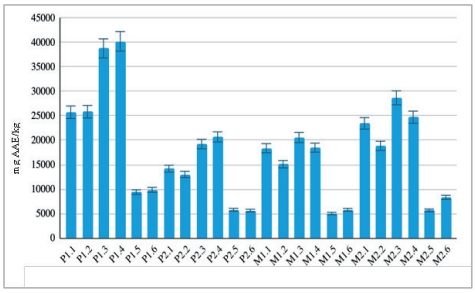

Figure 6. Antioxidant activity resulted from FRAP analysis expressed in mg AAE/kg

Chromatographic assay

To identify and quantify polyphenolic compounds in the hydroalcoholic basil extracts, 14 reference compounds were used, as mentioned in the Materials and Methods subsection. For identification, the retention times of these reference compounds were compared with those obtained during the separation of each basil extract.

The hydroalcoholic basil extracts were analyzed at three fixed wavelengths (280 nm, 320 nm, and 370 nm), selected based on the λ_{max} value obtained from molecular absorption spectra in the 200–400 nm range and relevant literature. Figure 7 represent an exemplification of the chromatograms obtained in case of P1.3 sample, for all three λ values considered.

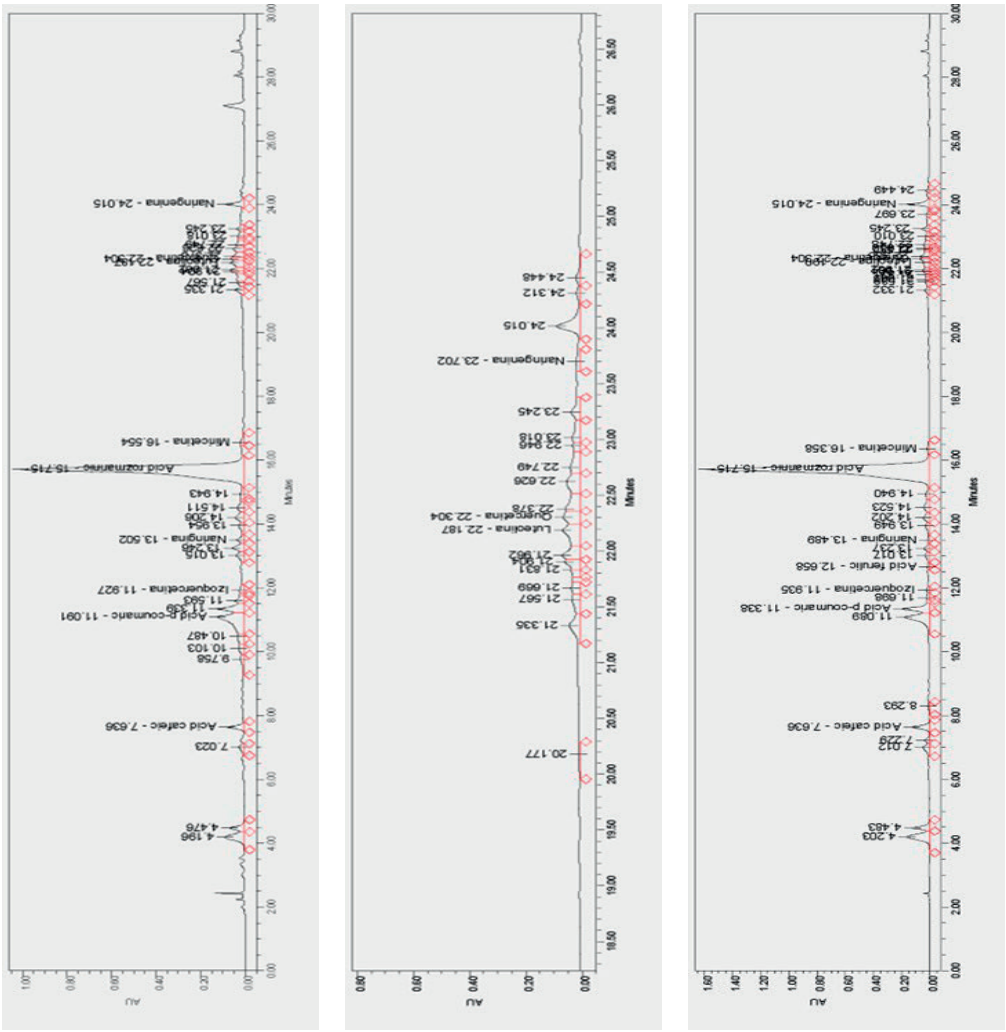

Figure 7. Chromatograms of P1.3 sample at 280, 320 and 370 nm

Table 3. Quantification of compounds in the basil extracts at λ_{max} expressed in mg of compound/kg of plant

Sample	GA	CH	CA	CF	EP	PC	IS	FA	NA	RS	MY	LU	QE	NR
P1.1	910.66	76.83	24.64	4684.55	35.21	325.68	52.99	70.14	380.28	115.05	61.71	163.41	126.79	124.65
P1.2	1145.69	117.83	ND	4454.50	26.49	322.01	31.39	99.01	384.22	108.40	64.79	212.12	119.37	95.03
P1.3	ND	95.06	165.77	6352.63	71.33	599.30	105.25	135.90	495.86	20288.80	146.08	556.88	273.51	123.93
P1.4	ND	107.98	45.70	5708.33	67.45	539.82	58.46	175.91	493.68	107.76	144.24	727.54	266.41	175.15
P1.5	ND	22.95	36.23	36.71	22.58	31.88	14.78	47.70	44.40	22.84	ND	120.11	80.43	40.70
P1.6	47.52	53.19	58.44	123.93	53.60	48.66	32.804	110.59	51.73	3897.44	ND	255.74	196.74	94.97
P2.1	473.73	71.53	19.34	3821.31	27.27	356.28	54.35	56.84	56.70	7883.78	61.94	206.04	111.56	54.46
P2.2	981.68	67.40	ND	2594.03	18.80	195.55	19.60	73.49	24.99	56.15	52.58	156.30	85.66	42.47
P2.3	ND	72.45	47.46	2542.86	55.83	385.95	56.07	103.08	88.77	7688.73	135.33	332.19	211.47	105.24
P2.4	1711.23	91.45	6579.96	1805.22	590.33	387.20	44.99	127.82	218.32	6432.85	138.52	317.12	262.69	102.46
P2.5	ND	21.26	20.90	39.72	21.84	47.47	25.91	43.93	19.12	2265.98	ND	101.30	74.30	39.71
P2.6	45.94	45.47	45.00	50.53	45.76	44.41	39.55	94.74	43.47	48.03	ND	211.90	167.88	204.00
M1.1	ND	26.47	94.21	1196.11	22.18	137.72	24.81	48.87	32.20	11513.20	57.05	228.39	80.70	103.40
M1.2	ND	23.32	25.92	729.32	24.90	99.04	17.80	43.88	25.72	6537.94	55.40	178.22	76.19	77.29
M1.3	ND	49.97	58.44	963.34	43.82	171.57	39.55	95.75	58.22	12348.20	124.53	333.38	165.04	141.31
M1.4	ND	49.08	ND	463.34	40.68	153.13	32.82	101.70	48.92	7954.32	128.13	320.91	164.39	140.95
M1.5	ND	14.09	12.84	17.96	143.30	13.98	ND	30.02	13.42	2774.76	ND	79.07	52.77	29.32
M1.6	35.03	ND	46.99	81.90	32.52	32.33	21.42	71.16	32.70	3246.43	ND	170.64	127.76	75.69
M2.1	2393.43	130.22	ND	3469.53	25.47	477.8	144.7	106.05	54.16	10393.50	77.81	484.14	123.88	748.56
M2.2	ND	92.35	ND	2444.76	353.05	700.47	76.53	81.93	329.38	5506.41	70.03	259.18	134.11	183.22
M2.3	2539.07	78.18	61.2	2430.4	542.74	852.18	134.29	156.83	515.60	10745.8	166.14	481.04	279.73	133.04
M2.4	ND	71.20	ND	1169.64	51.52	862.42	95.54	149.32	392.06	6292.58	161.19	582.82	257.76	172.43
M2.5	22.42	22.49	33.70	33.37	20.34	28.81	13.49	46.07	19.31	1810.47	ND	134.31	77.47	434.55
M2.6	45.82	50.09	70.53	52.85	43.50	43.32	34.24	94.18	43.76	48.24	132.29	251.88	168.06	645.12

ND - unidentified polyphenolic compound. TA - tannic acid; GA - gallic acid; CA - catechin; CF - caffeic acid; CH - chlorogenic acid; EP - epicatechin; PC - p-coumaric acid; FA - ferulic acid; IS - isoquercetin; MY - myricetin; RS - rosmarinic acid; NA - naringin; QE - quercetin; LU - luteolin; NR - naringenin.

The Table 3 displays the maximum concentration for each compound identified in basil extracts obtained from the aquaponic environment through feeding with industrial feed and 3% yeast biomass, the most quantitatively significant being caffeic acid, reaching a maximum value of 6352.63 mg/kg for the sample extracted with EtOH 50% and 5708.33 mg/kg for the sample extracted with EtOH 70% in a plant solvent ration of 1:40 and rosmarinic acid with 20288.75 mg/kg and 3897.44 mg/kg in for the same extraction conditions. Gallic acid was not detected in several samples (P1.3, P1.4, or P1.5), while myricetin was absent in EtOH 100% samples.

For the sample grown with 5% yeast biomass fed fish, high values were obtained for the catechin in the case of the EtOH 70% and 1:40 ratio with a value of 6579.96 mg/kg. Highest value of caffeic acid (3821.31 mg/kg) was found in the EtOH 50%, 1:20 ratio, while rosmarinic acid held high values in the range of 6000-7000 mg/kg for almost all samples resulted from extraction with EtOH 50% and 70%.

In the case of the aquaponic control sample, overall lower values of the compounds were detected, compared to the sample supplemented with yeast biomass, with caffeic acid and rosmarinic acid being the highest in the case of the EtOH 50% and 1:20 ratio and EtOH 50% and 1:40 ratio, respectively.

The sample developed in soil presented the same pattern of higher values in the case of rosmarinic acid and caffeic acid, with almost halved values compared to the 3% yeast biomass fed sample, while catechin remained undetected in more than one case.

The high value of the rosmarinic acid is consistent with another study on lemon flavoured basil conducted by Majdi et al in 2020 and several studies conducted on *Ocimum basilicum* (Makri & Kintzios, 2008; Romano et al, 2022).

CONCLUSIONS

Aquaponic culture is an alternative solution to the current challenges generated by climate change and pollution, presenting several advantages, such as obtaining better production with fewer but better managed resources. Basil, which has many beneficial health properties, is a crop that lends itself to aquaponic production because it grows well in water with high nitrogen concentrations, has low susceptibility to diseases and pests, and has minimal nutritional requirements.

The highest total polyphenol content was obtained from hydroalcoholic extraction assisted by microwaves, using EtOH as the solvent, with a plant:solvent ratio of 1:40 for the sample obtained by feeding fish with 3% yeast biomass.

The highest extraction yield was achieved from basil grown in soil and extracted with EtOH concentrations of 50% and 70%, respectively, with a plant:solvent ratio of 1:40.

Antioxidant activity, determined by DPPH radical inhibition, was superior for samples prepared with a plant:solvent ratio of 1:20, regardless of the EtOH concentration (50% or 70%).

In the ABTS test, all samples showed inhibition percentages in the range of 90-100%, except for samples extracted with 100% ethanol at a plant: solvent ratio of 1:40, where inhibition percentages were in the range of 20-45%.

HPLC analysis identified rosmarinic acid as the major phenolic compound in all basil samples studied. The aquaponically grown basil sample, obtained by feeding fish with 3% biomass, had the highest quantity, consistent with existing literature.

In conclusion, the species grown in the aquaponic system, by feeding the fish with a mixture of industrial feed and yeast biomass (3%), has been proven superior in terms of total polyphenol content, the maximum content being obtained in the case of microwave-assisted hydroalcoholic extraction (70% EtOH) and a plant: solvent ratio of 1:40, and rosmarinic acid with the maximum content in the 50% EtOH, 1:40 plant: solvent ratio sample.

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