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Utilization of taro, (*Colocasia esculenta* L.) Schott and Lemon grass (*Cymbopogon citratus*) in Nile tilapia, *Oreochromis niloticus* (Linnaeus) diets

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ABSTRACT : **In order to evaluate the effect of taro (Colocasia esculenta L.) Schott and Lemon grass (Cymbopogon citratus) in Nile tilapia diets. Five isonitrogenous and isocaloric experimental diets were used in this study containing both animal and plant proteins sources to provide 30% protein and 4.49 kcal/ g. Diets were formulated from commercial ingredients and used to feed triplicate groups of fish (14.50 ± 0.3g) to apparent satiation for 10 weeks. Treatments were the control, and treatments were diets supplemented with taro leaves extract and Lemon grass extract at the rate of 0.2 and 0.4% kg−1 . The experimental treatments on diet supplemented with Lemon grass extract at the level of 0.2% kg−1 diet on the hematological parameters of Oreochromis niloticus was Hemoglobin 7.49, Red blood cells 2.73, Hematocrit 23.14,White blood cells 28.28, which is the best compared with Taro extract and control group. Results of the study showed a good overall growth performances and status of experimental fish. This article describes the bioactive phenolic compounds of ethanolic extracts of taro leaves (Colocasia esculenta) (TLE) and lemon grass (Cymbopogon citratus) (LGE) by using HPLC analysis. Phenolic compounds obtained from the extracts of the two plants contain Rutin as main compound with rate of 65.62 and 34.24 % respectively.**

KEYWORDS: **Colocasia esculenta, Cymbopogon citratus, HPLC, Oreochromis niloticus, Growth performance .**

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I. INTRODUCTION

Medicinal plants are commonly used to obtain phyto therapeutic medicines, food and cosmetics (Martinazzo et al., 2009). However, their use is limited by several factors including: cultivation, harvest period, climatic factors, humidity, brightness, part of the plant, transportation method, storage, drying process and extraction process, which may all modify the composition of the products, directly affecting its safety and efficiency (Calixto, 2000). Phenolic compounds are ubiquitous in the plant kingdom exerting several functions on plant. Many factors influence phenolic accumulation in plants although some are more significant than others. The hydric stress and other exogenous growth factors, exposure to various lights sources and the presence of fungal or predatory pressures are shown to alter plant biosynthesis (Cohen and Kennedy, 2010).

Colocasia esculenta (L.) is an annual herbaceous plant belonging to the Araceae family, commonly known as taro (Praja-pati, et al., 2011). C. esculentais cultivated mainly due to its tuber, an essential food for millions of people, and it is considered the 14th cultivated vegetable/staple around the world (Oscarsson and Savage, 2007). Moreover, taro leaves have been reported to be rich in crude protein and nutrients including, minerals and vitamins such as calcium, phosphorous, iron, vitamin A, C, B1 (thiamine), B2 (riboflavin) and niacin which are important constituents of human and animal diets (Onwuoeme, 1999; Aregheore and Perera, 2003). Few studies have previously addressed the phenolic composition of C. esculenta, anthocyanins (cyanidin and pelargonidin derivatives) and flavones (apigenin and luteolin derivatives) being already described in different varieties (Ferreres et al., 2012).

Cymbopogon citratus Stapf is perennial grass it widespread in tropical and subtropical countries. Due to its pleasant aroma and good taste, this herb is used for cooking and for preparing beverages and teas. Chemical studies of C. citratus showed the presence of essential oils, tri terpenes, and polyphenols in the aerial parts of the plant (Cheel, et al., 2005; Figueirinha, et al., 2010). Experiments with C.citratus extracts in vitro have demonstrated its natural antioxidant and anti-inflammatory properties in macrophages (Tiwari et al., 2010) Various studies have shown that C. citratus is used in herbal medicine worldwide for a wide range of applications, including antibacterial, antifungal, antiprotozoal, anti-carcinogenic, anti- inflammatory, antioxidant, cardio-protective, antitussive, antiseptic, and anti-rheumatic activities (Akande et al., 2011; Gazola et al., 2004). It has also been used in the prevention of platelet aggregation (Tognolini et al., 2006) in the treatment of diabetes (Mansour et al., 2002), dyslipidemia, gastroin-testinal disturbances (Negrelle et al., 2007; Carlini et al., 1986), anxiety [Peigen .1983], malaria (Tchoumbougnang et al., 2005), flu, fever, and pneumonia (Negrelle et al., 2007) , in aromatherapy, and in cosmetology. In addition to its therapeutic uses, C. citratus is also added to non-alcoholic beverages and baked foods as a flavoring and a preservative in confections and cuisines (Negrelle et al., 2007, Lorenzetti ,1991). Several studies evaluating the phytochemical composition of C. citratus have shown the presence of saponins, tannins, anthraquinones, fla-vonoids, phenols, and alkaloids, in addition to terpenes, aldehydes, alcohols, and esters (Negrelle et al., 2007). Furthermore, trace amounts of other components have been detected, including myrcene, geranial, geraniol, limonene, burneol, citronellol, nerol, α-terpineol, elemicin, catechol, luteolin, 6-C and 7-C-glycosides, caffeic acid, apigenin, luteolin, kaempferol, quercetin, chlorogenic acid, and geranyl acetate (Negrelle et al., 2007). Fu-mesol, furfurol, isopulegol, isovaleranic aldehyde, L-linalool, methylheptenone, n-decyclic aldehyde, nerol, terpineone, p-coumaric acid, and valeric esters have also been isolated in some studies (Negrelle et al., 2007, Akhila, 2010; Faruq, 1994). Cheel et al., (2005) have reported the pres-ence of isoscoparin, swertiajaponin, and orientin in C. citratus, along with numerous other phytochemicals reported recently by Bharti et al (Bharti et al., 2013). C. citratus also contains electrolytes and minerals (including sodium, potassium, calcium, copper, magnesium, manganese, selenium, phosphorus, iron, and zinc), vitamins (including folate, niacin, pyridoxine, ribofla-vin, and vitamins A, C, and E), and macronutrients (carbohydrates, proteins), and a small amount of fat (Aftab et al., 2011). Growing evidence suggests that these phytochemical components are responsible for the wide range of biological and therapeutic actions of C. citratus.

In aquaculture, one of the most promising methods of controlling diseases and stressors impact is by enhancing the defense mechanism of fish through immuno- stimulants supplementation (Raa and Roerstad., 1992). Furthermore, plant extracts can be considered as an alternative to other substances (such as antibiotics or chemicals) used to control fish diseases (Harikrishnan et al., 2011).

II. MATERIALS AND METHODS

Plant preparation

Taro (Colocasia esculenta) leaves were collected from the field in Kaluobiya government. Lemon grass (Cymbopogon citratus) was supplied from the local market. Leaves washed well with fresh tap water then distributed on white paper sheets and left to dry in room temperature , then put in drying oven at 40OC to complete dryness, dry plant was crushed and grinded through a food mixer.

Plant Extraction

Dried plant was charged into soxhlet apparatus and extraction was carried out with following solvents. Petroleum ether (60-80ºC), followed by ethyl alcohol (95%). Each extract was then concentrated using rotary vacuum evaporator at 40-50ºC under vacuum then dried residue was collected in an opaque glass bottles for further studies. (El- Mesallamy et al., 2015).

Chromatographic investigation

1-Paper chromatography

Two dimensional paper chromatography (TDPC) was carried out on whatman (IMM) for comparative studies of Taro leaves and lemon grass extracts under investigation using BAW for the first dimension, followed by 15% ACOH for the second dimension.

2-High-performance liquid chromatographic (HPLC)

Quantitative determination of taro leaves and lemon grass extracts fraction contents by high performance liquid chromatography (HPLC) Analysis of the ethanolic fraction of taro leaves and lemon grass extracts were carried out on: Waters 2690 Alliance HPLC system equipped with a quaternary pump, an on-line degasser, an auto-sampler, and equipped with a Waters 996 photodiode array detector. a) Standard preparation: Mix of eight standards in 20 ml mobile phase then sonicated for 20 min, then filtered using 0.22 µm syringe filter then 10 µl were injected. b) Sample preparation: C. esculenta leaves ethanol extract filtered using 0.22 µm syringe filter then 10 µl were injected. c) HPLC analysis conditions: The separation was carried out on a Zorbax SB-C18 column (4.6 mm×250 mm, 5 µm). Mobile phase: Buffer (0.1 % phosphoric acid in water) and Methanol • Mode of elution: using gradient elution: 0–30 min (3%–100% B), There was a 5-min wash with 100% B after each run and equilibrium time was 15 min. Flow rate: 1ml/min • Temperature: Ambient • Wavelength: 254 nm .The results were calculated by measuring the chromatographic peak area. The phenolic compounds identification was made by comparing the relative retention times of sample peaks with those of the reference standards. All solvents and HPLC grade was acquired from Sigma-Aldrich (Bellfonte, PA)

Fish rearing and feeding regime

One hundred and fifty healthy Oreachromis niloticus, obtained from fish hatchery at the central laboratory for aquaculture Research, CLAR, Abbassa, Abo-Hammad, Sharqia. Fish were kept in indoor tank for 2 weeks as an acclimation period to the laboratory conditions. Fish were randomly distributed to 5 groups in three replicates of 10 fish per aquarium. Glass aquaria filled with 120 L of water with continuous aeration. Settled fish wastes with one half of aquaria water were siphoned daily and water volume was replaced by aerated tap water from a storage tank. Fish in all treatments were fed to apparent satiation 6 days/week. Fish were weighed at the beginning of the experiment and then biweekly for 10 weeks experimental period.

Fish diet

Five isonitrogenous and isocaloric experimental diets were used in this study containing both animal and plant proteins sources to provide 30% protein and 4.49 kcal/ g diet. Diets were formulated from commercial ingredients and used to feed triplicate groups of fish (14.50 ± 0.3g) to apparent satiation for 10 weeks. All experimental diets were pelleted via a 2 mm diameter die. The formulation of the control diet was soybean meal 35.0%, fish meal 16.0%, wheat bran 17.0%, corn 23.0%, oil 2.0%, vitamin premix 1.0%, minerals premix 2.0%, and Starch 4.0%.

Parameters of growth performance

Growth performance was calculated as the following equation:

Weight gain (WG) = W1-W0.

Specific growth rate (SGR%/day) = [(Ln W1− Ln W0)/T] × 100.

Where, Ln = natural log, W0 = Initial body weight (g), W1= Final body weight (g) and T= Time (day).

Survival rate (SR %) = 100 x (fish No. at the end/ fish No. stocked at the beginning)

Hematological indices

Blood samples were obtained by puncturing the caudal blood vessels 24 hour after the end of the experiment using a 1 ml heparinized syringe (five samples/replicate). Evaluation of the hemogram involved the determination of red blood cell count (RBC; 106/ml), hematocrit (Hct; %), hemoglobin concentration (Hb; g/dl. All protocols were performed in accordance with the standard recommended by the Guide for the Care and Use of Laboratory Animals and Directive.

III.RESULTS AND DISCUSSIONS

The fresh leaves of dried powdered taro leaves and lemon grass were extracted by successive solvent and the extracts analyzed by HPLC.

Two dimension paper chromatographic (TDPC) investigation

Two dimensional paper chromatography (TDPC) of the extracts revealed the presence of more than 8 phenolic compounds, corresponding spots gave positive response towards FeCl3 spray reagent, some of which appeared under UV light as dark purple spots which turned orange or lemon yellow or reddish orange when fumed with ammonia vapour or when sprayed with Naturstuff spray reagent, a typical character of normal flavones or flavonol derivatives.

HPLC Analysis of Phenolic Acids and Flavonoids

The results pertaining to the HPLC profile of the taro leaves and lemon grass ethanolic extracts revealed their highest peaks for the secondary metabolites. Similar kind of earlier experiments demonstrates the processing of a crude source material to provide a sample suitable for HPLC analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation (Cannell, 1998). The source material, e.g., dried powdered plant, will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. In the case of dried plant material, an organic solvent (e.g., ethanol, petroleum ether) may be used as the initial extracting and following a period of maceration, solid material is then removed by decanting off the extract by filtration (Bhosale et al., 2010, Hernandez et al., 2007).

The filtrate is then concentrated and injected into HPLC for separation. The usage of guard columns is necessary in the analysis of crude extract. In the present study the phenolic compounds contained in the ethanolic extract was achieved through reverse phase HPLC analysis of taro leaves and lemon grass.

Phenolic content of the ethanolic extracts of taro leaves and lemon grass were summarized in Table (1 and 2) and Fig. (1 and 2). Rui et al., (2013) reported that the aqueous extract of C. Esculenta contain apigenin-6-C-pentoside-8-C-hexoside and luteolin-6-C-pentoside-8-C-hexoside. Also, taro leaves contains bio active compounds like pelargonidin-3-glucoside, cyanindin-3-rhamnoside, cyanidin-3-glucoside, orientin, isoorientin, vitexin, isovitexin and luteoin-7-O-sophoroside Sahar A M Hussein, et al.,(2016), Cheel et al., (2005) and Figueirinha et al., (2010) reported the presence of essential oils, triterpenes and polyphenols in the aerial parts of the lemon grass plant. On the other hand, Cymbopogon citratus contains active ingredients like tannins, saponins, flavonoids, alkaloid, phenols, and anthraquinones. Citral, geranial, nerol, myrcene, geraniol, linalool, tumerone, eugenol, isoeugenol, limonene, burneol, citronellol, neral, α-terpineol, α-Caryophyllene are found in the essential oil (Farhang, et al., 2013; Rivera, 2016).

Table (1): Major phenolic compounds (% of total) identified in Taro leaves ethanolic extract by HPLC.

	Peek Name	RT	Area%
1	Gallic acid	11.054	2.03
2	Catechin	14.819	0.35
3	Chlorogenic acid	15.710	1.84
4	Caffeic acid	16.662	12.19
5	Rutin	18.052	65.62
6	Elagic acid	20.892	13.29
7	Quercetin	23.244	2.83
8	Kampeferol	24.995	1.84

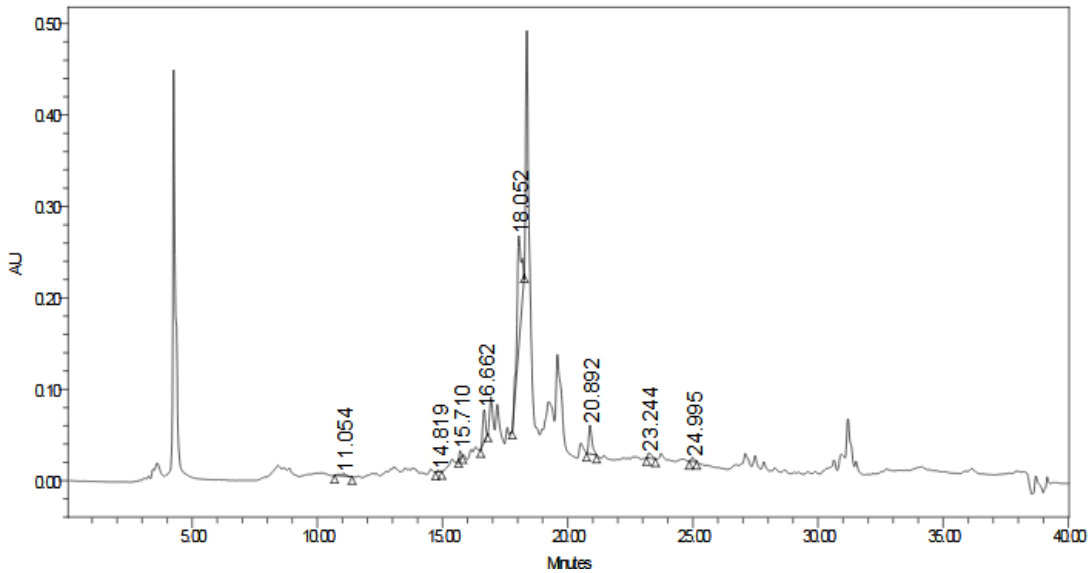

Fig. (1): HPLC chromatogram of Taro leaves

Table (2): Major phenolic compounds (% of total) identified in lemon grass ethanolic extract by HPLC

	Peek Name	RT	Area%
1	Gallic acid	10.458	14.15

2	Catechin	14.690	2.52
3	Chlorogenic acid	15.317	13.22
4	Caffeic acid	16.260	10.18
5	Rutin	18.102	34.24
6	Elagic acid	21.712	1.40
7	Quercetin	23.244	19.59
8	Kampeferol	24.417	4.70

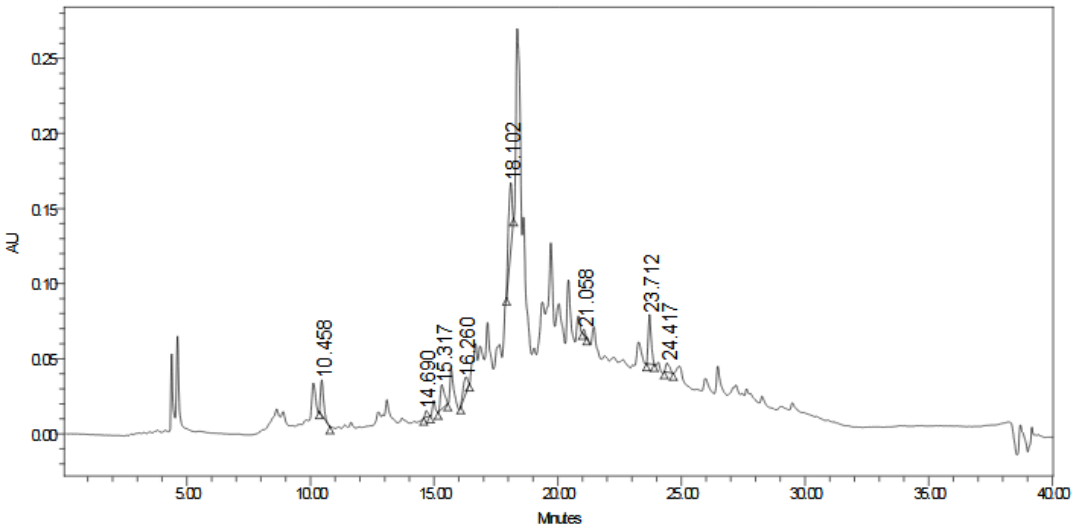

Fig. (2): HPLC chromatogram of lemon grass

Growth performance

Results of growth performance parameters are shown in table (3). Data showed that final body weight (FBW), weight gain (WG) and specific growth rate (SGR %) of Nile tilapia (Oreochromis niloticus) were improved significantly ($P < 0.05$) by dietary supplementation of ethanolic extracts of taro leaves (TLE) and lemon grass (LGE) in fish diet. Fish maintained on diets containing experimental diets were significantly ($P <0.05$) the greatest in growth performance parameters compared to fish fed on control diet. Best FBW, WG and SGR were obtained in fish fed diets containing 0.2 % of LGE, values were 36.8g, 22.23g and1.23 respectively, followed by fish fed diet containing 0.2% of TLE. Moreover, results indicated that fish fed diets contained the lowest level of ethanolic extract of LGE or TLE (0.2%) were higher in values than fish fed diets contained the highest levels (0.4%).

Survival rate at the end of the experiment showed that there were insignificant differences ($P > 0.05$) among treatments, it ranged between 93.3and 100.0 %.

Table (3): Growth performance values (means ± SE) of Nile tilapia fed diets containing taro leaves and lemon grass extract.

Items	Control	Taro leaves extract (TLE)		lemon grass extract (LGE)	
		0.2%	0.4%	0.2%	0.4%
Initial weight	14.57±0.33	14.5±0.00	14.56±0.33	14.57±0.33	14.53±0.33
Final weight	28.93±0.63d	33.1±0.346b	30.93±0.38c	36.8±0.52a	32.7±0.56b
Weight gain (g)	14.37±0.6d	18.6±0.346b	16.36±0.35c	22.23±0.52a	18.2±0.53b
Specific growth rate (SGR)	1.07±0.03d	1.1±0.02b	1.00±0.01c	1.23±0.02a	1.08±0.02bc
Fish Survival (%)	93.3±3.33a	96.6±3.33a	93.3±3.33a	100.0±0.00a	100.0±0.00a

Means with different superscripts in the same row are significantly different (P<0.05).

Hematological analysis

Hematological parameters are presented in Table 4. The mean values of Hb, RBC , Hct % and WBCs increased significantly (P < 0.05) in the groups fed lemon grass extract especially 0.2% group . Additionally, mean values of Hb , RBC, HCT and WBCs were significantly higher in groups fed taro leaves extracts 0.2% values. Interestingly, Taro leaves extract 0.4% had the lowest significant Hct percentage among the experimental groups. WBCs increased significantly in lemon grass 0.2% and taro leaves 0.2% extracts compared with the other concentration and control group.

Table (4): Effects of the experimental treatments on the hematological parameters of Oreochromis niloticus after 10 weeks of feeding.

Parameters	Control	TLE 0.2%	TLE 0.4%	LGE 0.2%	LGE0.4%
Hb (g/dL)	5.72±0.18ab	6.94±0.11a	6.12±0.12ab	7.49±0.19a	6.33±0.16b
RBC(106/mL)	1.93±0.06c	2.35±0.03b	2.07±0.04bc	2.73±0.17a	2.12±0.04bc
Hct %	17.9±0.12d	19.49±0.08c	18.84±0.07cd	23.14±0.09a	21.63±0.14b
WBCS (/mL)	22.6±0.16c	26.3±0.05b	23.2±0.19c	28.28±0.06a	23.7±0.24c

Hb = hemoglobin; HCT = hematocrit; RBC = red blood cell; WBCs = white blood cell.

Means with different superscripts in the same row are significantly different (P<0.05).

V -Conclusion

As a conclusion of this study, it is suggested that ethanolic extract of taro (Colocasia esculenta L.) Schott and Lemon grass (Cymbopogon citratus) in Nile tilapia diets improve the growth performance of Nile tilapia and enhances hematological parametars . The presence of significant amount of respective bioactive phenolic compounds in these plants under study ensures its unequivocal recommendation for the use in the pharmaceutical and nutraceutical sector.

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