UNIVERSITA' DEGLI STUDI DI MILANO
DEPARTMENT OF AGRICULTURAL AND
ENVIRONMENTAL SCIENCES - PRODUCTION,
LANDSCAPE, AGROENERGY

DOUBLE ROLE OF *CORCHORUS OLITORIUS* (L.) CULTIVATED IN FLOATING SYSTEM:
New leafy vegetable for "ready to eat" industry or active food for developing country.

Andrea Giro
R010668

Advisor: Prof. Antonio Ferrante
PhD course supervisor: Prof. Daniele Bassi

This thesis is presented for the degree of Doctor of
Philosophy,
Università degli studi di Milano, Italy

December, 2016

Declaration

I declare that this thesis is my own elaborate of my research and contains as its main content work, which is not previously submitted for a degree at any tertiary education institution.

ABSTRACT

C. olitorius is a traditional African leafy vegetable (ALV). It has interesting nutraceutical properties and is widely consumed among the African and South-East Asia populations. It is commonly cultivated through a conventional way: in the open field. Hydroponic cultivation systems optimize production inputs (water - fertilizer), but they also foster the production and the quality yield. Moreover, hydroponic systems improve shelf life and thus reduce food waste.

For this thesis, a floating system cultivation method has been investigated to grow *C. olitorius,* commercially known as "baby leaf". The study has focused on two aspects, namely nutrient supply efficiency and shelf life qualities, in order to accelerate the commercialization of *C. olitorius* within Europe's leafy vegetable industry. However, both of those improvements were also essential for improving production and quality levels in developing countries. Moreover, the communities which traditionally consumed *C. olitorius* in their native countries have been increasing in Europe. Likewise, public interest about nutritional and healthy food is rapidly rising worldwide, so promoting the discovery of new, nutritionally valuable food plants such as *C. olitorius* could be an interesting step forward. For all these reasons, understanding the nutritional shelf-life of vegetables was crucial claim for meeting customer needs and for boosting innovation in the food industry.

In these previous studies (Chapter 3-4) an Egyptian ecotype of *C. olitorius* has been used as a gold standard. Two different nutrient solutions have been tested to optimize nutrient need during a one year cultivation cycle: a standard solution nutrient in full, then a second one halved to test differences in the growth and qualities response. The harvested biomass has been evaluated as baby leaf at the fresh-cut industry stage. Several quality parameters have been estimated: sugars (total, reducing and sucrose), nitrate, chlorophyll, carotenoids, polyphenols and anthocyanins (Chapter 4). Plant production was similar to other common leafy vegetables cultivated in floating systems in Europe. Analytical determinations displayed good levels of antioxidant compounds and sugars. Mineral content was relevant for Ca, Mg, and Iron in comparison with other common leafy vegetables (Corn salad, rocket and lettuce). Moreover, *C. olitorius*, when cultivated using traditional growing systems in literature, showed less nutrient content than *C. olitorius* cultivated with the floating method, which was an interesting result for bio-fortification in developing countries. Results showed that the plant had healthy characteristics for human nutrition, especially for females and children. High levels of Ca, Mg and Fe, in fact, are very important during pregnancy and childhood. Results obtained from yields and nitrates indicate that this species can be grown with lower mineral concentration in the nutrient solution without decreasing production and quality. The level of sugars measured was higher than in other traditional leafy vegetables which are usually cultivated via floating systems such as rocket, lettuce and

spinach. Higher sugar content could have a positive effect on post-harvest storage and could be an important contribution to the human diet. *C. olitorius* may hence represent a product innovation in the fresh-cut industry in the near future. Moreover, in this thesis, biochemical and physiological differences of several ecotypes of *C. olitorius* worldwide and their growing behavior were studied. These elements were priority aspects for the food industry in order to introduce the best genotype adapted for growing in a Southern European environment (Chapter 5). However, considering the difficulties involved with introducing a new leafy vegetable, it is important to consider not only agronomic and physiological aspects, but also the consumer's point of view.

These days, the European population is interested in the prevention of chronic disease through food consumption. For this reason, the final part of this thesis planned to test the fresh crude extracts of *C. olitorius* in order investigate phenolic and terpene composition. Results confirmed the relevant presence of many active compounds such as quercetin, coumarin, vitamin E and terpenes inside *C. olitorius* leaves (Chapter 6).

TABLE OF CONTENTS

CHAPTER I: INTRODUCTION

1.1 READY TO EAT INDUSTRY

Vegetables are classified into four commercialization classes, depending on the types of processes to which they were subjected. Generally, fresh vegetables are commercialized unprocessed, like bench vegetables, but some of them are stabilized like canned and frozen products like legumes, or otherwise as ready-to-eat vegetables like fresh salads (Baldi and Casati 2009). During the past 10 years, a constant increase of the consumption of ready-to-eat (RTE) vegetables followed lifestyle trends in Western populations and consumers' food habits. Furthermore, the consumer was aware of the strict correlation between vegetable consumption and their own health (Florindo, *et al.*, 2015). A frenetic urban lifestyle increases a demand for products that can be consumed without home labour, like RTE vegetables. In particular, leafy vegetable production was increasing thanks to innovation and new storage technology that increased and extended the quality of RTE foods, delaying their perishing. The increased consumer demand caused companies which produced RTE food to adapt to a dynamic market by optimizing cultivation techniques and focusing on product quality and innovation. However, virtuous market competition has decreased production cost to benefit consumers, increasing the power of purchase (Baldi and Casati, 2009).

RTE vegetables are mainly divided into salads which are cultivated until complete harvest stage and processed (cut), and baby leaves that are collected in very early stage of developing (before they reached 13-15 cm height and have 6-7 leaves). The added value of RTE vegetables consists in cleaning raw material from foreign objects, eliminating damaged parts, cleaning and cutting, packaging and refrigerated transport to stores. Moreover, the consumer does not need to waste any parts of RTEs, since they can be used immediately, and in their entirety, for human consumption. All these supply chain operations could be controlled to avoid nutrient loss as well as organoleptic and aesthetic losses (Colelli and Elias, 2009). For these reasons, it was important to study produce behavior from field to consumers through the supply chain (chapter 2-3). For example, it was essential to understand the response of the produce to chilly injuries, considering common vegetables cold chain 4-5°C.

Consumers are particularly interested in food safety issues surrounding fresh RTE salads. There is a precise regulation in Europe for controlling microbial contaminants EU n. 1441/2007, as well other contaminant like nitrate UE n. 1258/2011. Nitrate is one of the most important inputs for producing leafy vegetables as well as one of monitoring input factors because nitrate must have a limited presence in the human diet, per the advisement of the WHO. Nitrate is not toxic, by itself, for human health. However, it is converted into pro-toxic molecules such as nitrites and other nitrogen oxides. According to a study of the 1976 Spiegelhalder, 5% of nitrate is reduced into nitrite in human digestion. Nitrite is an active compound that can react with ammine forming
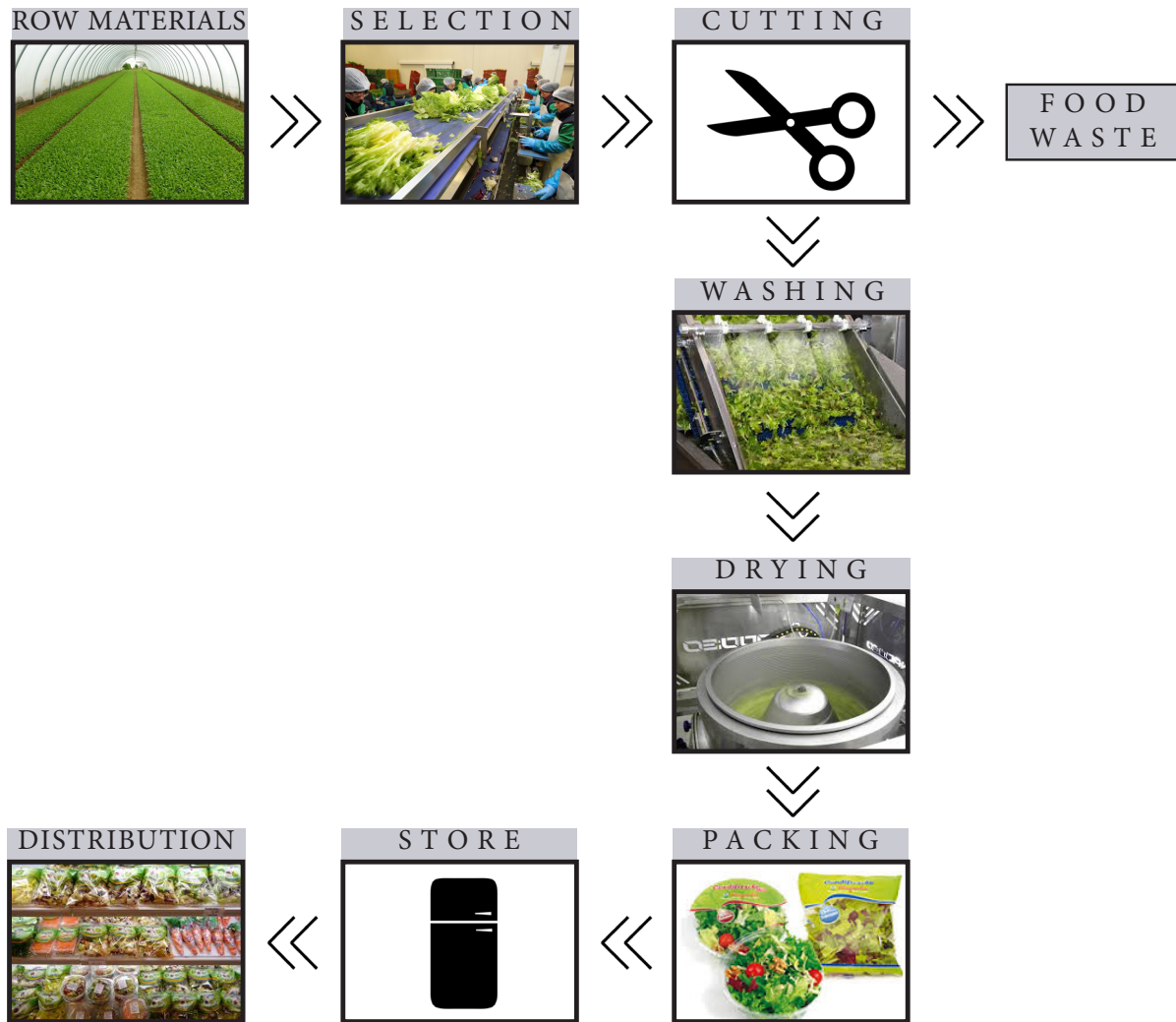
Figure 1. Flow Sheet of Fresh RTE salads.

nitrosamines which are considered to be highly toxic, pro-cancer chemical compounds (Bruning-Fann and Kaneene, 1993). However, the role of nitrate in human nutrition is ambiguous, in fact, according recent studies; nitrates may be involved in the metabolic regulation of the human body. It was suggested that nitric monoxide, generated through the oxidation of nitrate by microbial gut flora, could perform nutraceutical functions. Moreover, it was demonstrated that in the human gut, nitrogen monoxide (NO) plays a protective role against some human microbial pathogens (McKnight et al., 1999; Lundberg *et al.*, 2004; Bryan, 2006). While nitrate supply was controlled mainly in the field during the cultivation cycle, nitrate content in leaves were evaluated in correlation to biomass production and quality levels, during the two first steps of research (Chapter 3-4).

1.2 IMMIGRATION AND THE CONTRADICTION OF "SUSTAINABLE INTENSIFICATION

Corchorus olitorius L. is an interesting nutritional and pharmaceutic plant. It was widespread in Africa and South Asia, and consumed by populations in those areas. *C. olitorius* either grew spontaneously in open fields, or cultivated by humans in a traditional way. However, *C. olitorius* has high genetic variabilities that allow it to be cultivated in different climatic conditions from Africa to Japan, and throughout the Middle East. Cultivating this leafy vegetable in Italy and in other countries in Europe would generate an interesting economic scenario with regard to foreign citizens that were used to eating this leafy vegetable in their native countries.

During the last 10 years, Europe has experienced a large amount of migration from Africa and the Middle East. Famine and war have driven millions of people to move to Europe to improve their living standards. In fact, food prices and water availability are usually catalysts for the very regional tensions that lead to war. In the current political landscape, efficiently controlling agriculture and, in particular, water availability and water access for agriculture, are essential for a good international policy. This strong correlation among water, food and agriculture, enlarged in macro areas like the Middle East, can also be seen in smaller, district areas and in civil war. The increase of competition over the use of water between agriculture and other economic sectors like factories, service and cities, pronounced the risk of a water deficiency for developing countries. This risk of water deficiency is especially acute for areas with a high rate of economic growth, such as Egypt, Kenya and the Sahel. The limited amount of water in many regions where population growth is linked to a high urbanization rate generates difficulties between food production and life in cities.

Figure 2. Water security risk 2010 FAO.

Legend	
Extreme risk	■
High risk	■
Medium risk	■
Low risk	□
No Data	□

Rank	Country	Rating
1	Somalia	Extreme
2	Mauritania	Extreme
3	Sudan	Extreme
4	Niger	Extreme
5	Iraq	Extreme

Rank	Country	Rating
6	Uzbekistan	Extreme
7	Pakistan	Extreme
8	Egypt	Extreme
9	Turkmenistan	Extreme
10	Syria	Extreme

A balance between agriculture, food production and water utilities in urban centers must be found through the reduction of cost and the increase of sustainability. It is also essential to increase the variety of diets in order to guarantee food security for all world communities. Food security is one of the most important items on the global agenda. In order to solve these critical issues, it is necessary to discover more sustainable methods of food production. "Sustainable Intensification" (SI) could be essential for a correct global response to food insecurity and globalization, but SI opened discussions on the conflict between food production and environmental protection. It was suggested by many authors that "sustainable intensification" should focus on increasing productivity more than quality production. In chapters 3 and 4, *C. olitorius* were tested in response to hydroponic cultivation, which is a technique that increases water use efficiency, enhances production and improves food quality. In fact, the floating system is a high performance cultivation method that, in particular, increased the quality and yield of salad production. In general, a standard nutrient solution which was used in the floating system was rich with fertilizers, even after a cultivation cycle. However, if it was reused in other production cycles, and not released from the floating system into the local environment, efficiency could increase significantly. Nutrient solution, if released on the open field, can induce strong pollution, such as nitrate accumulation, in drinkable water and groundwater. For these reasons, a reduced NS has been studied to evaluate *C. olitorius* growth responses in order to reduce fertilization input and preserve food quality. Moreover, the floating system accelerated cultivation cycles, improved food safety against microbes and allowed control of the plants' uptake for fortification, such as oligo-element fortification, which is essentials for human nutrition.

This study on the conduct of *C. olitorius* in floating systems showed an important increase of food production and water use efficiency. Moreover, nutritional levels were higher in comparison with the conventional cultivation methods (Chapter 3). The cultivation protocols used in these studies (Chapter 3-4) could be used for the improvement of quality and yield, and the reduction of water use and fertilizer input in many countries in which C. olitorius is cultivated and consumed.
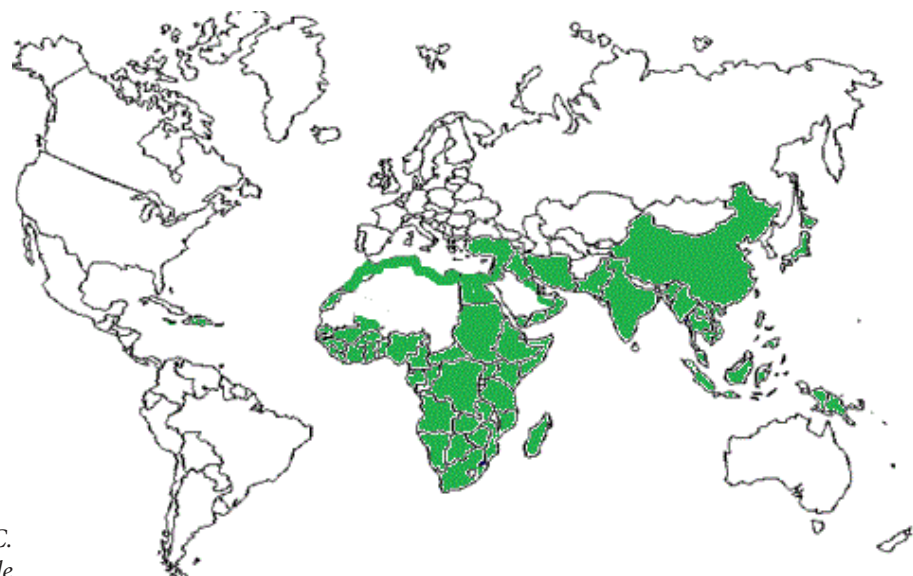

Figure 3. Diffusion of C. olitorius (L.) as leafy vegetable

1.3 FOOD SECURITY: *C. olitorius* potential role

Food security is a central theme of the new millennium and must be faced at national and international levels, taking into consideration the multidisciplinary nature of the field which involves socio-cultural, political and environmental as well as agronomic and economic aspects (Deaton, 1998). From an economic and environmental point of view, food security was defined as a situation in which people may have safe and appropriate food with the nutritional requirements for leading an active and healthy life (WFS - Plan of action, 1996). Food security was based on three pillars: food availability, access and utilization, as reported in FAO guidelines (Matushke *et al.*, 2009). Human nutritional requirements change from childhood to old age, particularly with regard to oligo elements and vitamins (Ogunlesi *et al.*, 2010). For these reasons, *C. olitorius*, as shown in chapter 3, has interesting nutrient characteristics which enable it to function as a super food against malnutrition. Inside *C. olitorius* leaves are 17 nutraceutical compounds (Islam, 2013). The leaves are commonly used as food, having higher nutritional values than fruits and stems (Ndlovu, *et al.*, 2008) (Chapter 6).

Table 1. Leaves, stems and seeds composition. Different letters underlined statistical difference $p \leq 0.05$. Data in media±SD; n=3 (Ndlovu, et al., 2008).

	Leaves (g kg⁻¹ Dw)	Fruits (g kg⁻¹ Dw)	Stems (g kg⁻¹ Dw)
Lipids	17.20±2.9a	42.30±6.0b	69.00±1.3c
Fibres	20.30±1.0a	35.50±1.5b	88.20±4.8b
Proteins	162.60±3.37a	76.50±3.20b	51.00±1.80b
Total carbohydrates	695.00±32.4a	788.00±28.70b	802.00±18.2b
Iron (Fe)	0.228±0.01a	an.	0.0428±0.005b
Calcium (Ca)	0.347±0.02a	0.197±0.012b	0.0761±0.008c
Zinc (Zn)	0.051±0.006a	0. 028±0.005b	0.0340±0.001b
Magnesium (Mg)	0.560±0. 02a	0.449±0.020b	0.3340±0.010c
Phosphorus (P)	0.258±0. 03a	0.156±0.02b	0.0380±0.003c

Moreover, several studies underlined its ability to adapt in different environments. In fact, *C. olitorius* is generally quite tolerant to drought and can be cultivated in soils with low fertility levels (Chapter 5). In developing countries, particularly in Africa, the diet of poor communities is mainly based on rice, potato, and cassava. These foods are full of carbohydrates, but they lack oligo elements and vitamins, hence *C. olitorius* can be added to solve this deficiency. Chronic undernutrition is a hidden risk for millions of people all around the world (FAO).

For example, vitamin B9 content inside *C. olitorius* is high in comparison with other vegetables that are rich in vitamin B9, as shown in 1981 by Chen et al. Per the literature in chapters 3 and 4, the carotenoids levels in *C. olitorius* leaves has been measured during summer and spring and is higher than common leafy vegetables. Moreover, vitamin C and polyphenols are found within *C. olitorius*, and they can positively affect human health, preventing chronic disease. However, it is important to remember that *C. olitorius* is traditionally boiled before consumption to prevent biological contamination.

Corchorus spp. has a good correlation between patterns of amino-acids and their amount inside leaves. Both characteristics can be used as dietary supplements in developing countries, as suggested by Kamga et al., in 2013. The protein percentage inside leaves ranged between 3-4% (Kamga *et al.*, 2013). Protein uptake is generally considered to be a main components of food security during childhood. Threshold protein deficiency could seriously compromise the healthy growth of a baby and cause the mother to experience malnutrition during pregnancy and lactation. *C. olitorius* can also be a supplement for threshold elements like iron, zinc and potassium, as underlined in several studies (Ndlovu and Afolayan, 2008). In chapter 3, threshold elements were analysed in two NSs to see the difference in uptake in *C. olitorius* as a fortifying food.

1.4 SCOPE OF THE REASERCH

The aim of this Ph.D. thesis was to investigate:
- the behaviour of *C. olitorius* cultivated in floating systems as healthy vegetable for human nutrition in Europe and Africa;
- the possibility of producing *C. olitorius* as an ethnic vegetable in South Europe considering migration trend from Africa in the next 20 years;
- the use of *C. olitorius* as new product for RTE industry, considering the reduction of cost.

The objective of this study was to determine:
- growth performance and nutrient contents of *C. olitorius* using different nutrient solutions;
- *C. olitorius* behaviour during storage at 4° C after harvest;
- different worldwide ecotypes and their growing and physiological performances in Italy
- active compounds of *C. olitorius* inside leaves .

The above objectives was been studied in four major experiments and they were elaborated in the following chapters.

CHAPTER II: *C. olitorius (L.)* as a traditional subtropical leafy vegetable for human health: a review

2.1 INTRODUCTION

C. olitorius belongs to the genus *Corchorus (L.)* under the family of Malvaceae. *Corchorus* spp. are generally spread in tropical and subtropical regions. Jute is a common cash crop for fibre, but it has been used for food and drugs in traditional medicine for millennia. *C. olitorius* goes by various national names from Africa to Asia, but it was commonly referred to as Mulukhiyah, Jew's mallow or Nalta jute. Its original name, Jew's mallow, came from its association with the traditional Jewish culture of biblical times. However, the introduction of the cultivation of *C. olitorius* in Africa and in Asia can be dated around 3000 B.C., even though its use as a spontaneous crop might predate the Neolithic era (Wraing et al., 2012). While it primarily was considered as a cash crop, it was also recognized as a TLV (traditional leafy vegetable). Thus, its genome was sequenced in 2010 for many economic reasons. It is commonly used as food in traditional dishes in most of the regions of Africa. It is obtained through cultivation and after spontaneous growth (van Jaarsveld *et al.*, 2014). Interest in *C. olitorius* has increased over the last 10 years. This increase in interest is due, in part, to FAO's goal of finding ways in which TLVs are useful for increasing food security in developing countries. Moreover, the interest in these TLVs and, in particular, *C. olitorius*, led to further investigations into its phytochemical properties, as suggested by the Japanese Resources Council of Science and Technology Agency (2000). TLVs are considered important sources of phytochemicals, vitamins and trace elements like iron, manganese and copper, especially during pregnancy and childhood. Hence, *C. olitorius* can be used as supplement for human nutrition. Moreover, the utilization of C. olitorius as a traditional herb could improve the income of small communities, as was suggested by High and Shackleton, 2000. Since *C. olitorius* is commonly used and cultivated in many communities in West Africa and South Asia, it could possibly address the needs of people who suffer from many different nutrient deficiencies. The high genetic plasticity of *Corchorus* spp. and *C. olitorius* provides it with the capacity to adapt to a wide variety of environmental conditions (i.e., tropics, Sahel, Mediterranean environments and Desert areas). For example, C. tridens was also consumed in semi-arid lands as a supplement during arid seasons (Freiberg *et al.*, 1998). Indonesian's *C. olitorius* (Salayut) is cultivated in tropical, humid areas and, as an indigenous leafy vegetable, was considered generally more nutritious than common leafy species like *Lactuca sativa*, *Chicorium endivia* and *Spinacia oleracea*. However, it is also important to consider anti-nutritional factors inside all of these species (Ndlovu and Afolayan, 2008) (Musa *et al.*, 2010).

2.2 AGRONOMIC NEED

C. olitorius grows in temperatures ranging from 28° to 33° C and in humidity conditions as high as 74%. Some varieties can also adapt in semi-arid land (Freiberg *et al.*, 1998). However, Jew's mallow generally grows in environments with annual rainfalls ranging from 600mm to over 1000mm, according to the Jew's Mallow Productive Guide Line, written by the South African Department of Agriculture (2012) and by Olaniyi (2008). Generally, seed germination did not occur in temperatures lower than 25° C. However, pre-chilled seeds demonstrated a better vitality (Nkomo and Kambizi 2009).

Seeds showed signs of germination at the temperature of 35° C. This may be made possible by the fact that *C. olitorius* grows better when day temperatures average 30° C or above (Nkomo and Kambizi 2009). Hence, 30 °C was chosen for the study of seed germination responses in the two *C. olitorius* varieties. (Mguis *et. al.,* 2014). Moreover, *C. olitorius* was a candidate as a cash crop in soil in high salinity soil, showing salt tolerance, particularly during germination. It was considered a stress tolerant plant, with a negative linear regression response to salinity (Mguis *et. al* 2014). These results indicated that, in saline stress conditions, Jew's mallow may germinate, which could be a key feature of these species which enables them to colonize saline areas like deserts. These properties could be adapted in all Sahel areas where the soil salinity causes desertification to be a relevant emergency, like the areas near Lake Chad (Ndehedehe *et al.*, 2016). Moreover, desertification may be accelerated by soil tillage. *C. olitorius* had an emergence rate of 87% at depth ranges between 0 to 2 cm, but it progressively decreased in relation to depth. No emergence was recorded at a depth of 8 cm (Chauhan and Johnson 2008). These characteristics could be essential for minimum tillage or sod seeding, as both techniques could prevent soil erosion and desertification. Taking into consideration jute's nutrient requirement, in terms of effects of nitrogen (N) and phosphorus (P): 45 kg for N and 30 kg for Pha1 respectively.

2.3 NUTRIENT VALUES AND ANTIOXIDANT

C. olitorius is used as a leafy vegetable. Leaves of *C. olitorius* contained, on average, 85-87 g H2O, 5-6 g protein, 0.7 g oil, 5 g carbohydrate, 1-5 g fibre, 250-266 mg Ca, 4-8 mg iron, 3000 iu vitamin A, 0.1 mg thiamine, 0.3 mg riboflavin, 1.5 mg nicotinamide and 53-100 mg ascorbic acid (per 100g Dw) (Oke, 1964, 1968). Vitamin C ranged from 30 to 89 mg x 100g Dw. Zn ranged 21 µg g^{-1} Dw. to 51 µg g^{-1} DW (Ndlovu and Afolayan, 2008) 57 or 47 µg g^{-1} DW or 0.46 µg g^{-1} FW (Giro and Ferrante 2016).

C. olitorius was considered a healthy plant because it has a high value of phenolic compounds (Giro and Ferrante 2016). The chemicals inside *C. olitorius* leaves demonstrated inflammatory and antitoxic effects: Dewanjee *et al.*, 2013 evaluated antitoxic effects of the leaves against lead intoxication. Moreover, a positive effect in controlling dioxin was also discovered by Nishiumi *et al.*, 2006. Akinwumi *et al.*, in 2016, demonstrated that *C. olitorius* extract had an active influence in

reducing potassium dichromate toxicity. Moreover, *C. olitorius* phenols play a role in controlling human body temperature, as demonstrated by Zakaria *et al.*, 2005. A study conducted by Wang *et al.*, 2011 underlined the beneficial properties of *C. olitorius*, such as the positive correlation between jute consumption and decreased obesity. Most of these studies underlined that the consumption of *C. olitorius* benefits human organs, particularly kidney and liver tissue, opening the possibility for further studies related to the activity of *C. olitorius* extract to treat kidney and liver diseases. Moreover, *C. olitorius* proved to have a dramatic effect in decreasing prostaglandins, which are mediators of inflammation signals in human cells, as a consequence of the strong scavenging capacity of the leaves' phenolic extract (Yan *et al.,* 2013).

In literature, the values of phenols range considerably depending on environmental conditions such as light, temperature and fertilisation. One of the most prevalent phenols detected was 5-coffeolyquinic acid ranging from 1.2 mg g^{-1} Fw to 3.8 mg g^{-1} Fw. However, the levels of total phenols ranged from 3 mg g^{-1} Fw as a Gallic equivalent to 289 mg g^{-1} Dw, depending on the varieties and methods of analysis. Thus, *C. olitorius* can be considered as a good phenolic supplement in the human diet, since it is full of active compounds, as shown by Morsy *et al.*, 2015. Although one has to consider the complexity of the interaction between diet and health, *C. olitorius* extract has demonstrated some protective effects in the gastric apparatus. In fact, the consumption of jute was shown to have a protective effect against inflammation in the mouse gut (Owoyele *et al.*, 2015).

References	Phenolics content of C. olitorius leaves
Azuma *et al.*, 1999	1 mg / 100 g Fw (Prevalent phenols quercitin)
Zeghichi et al., 2003	38 mg /100 g Fw
Das et. al, 2009	Flavonoids 26.2 mg/g Dw ; Phenolic 39.2 mg /g Dw
Oboh et al., 2012	839 mg /100 g Dw
Acho et al., 2015	244 mg /100g Dw

According to literature, the gastro protective effect of *C. olitorius* is related to the reduction of activity of the gut's hydrogen pump. According to Owoyele *et al.*, 2015, its active compounds mimic cimetidine drug action. Similar studies were conducted by Parvin *et al.*, 2015, to evaluate the analgesic activity of jute. Moreover, *C. olitorius* methanolic extract interacts with insulin receptors and was shown to decrease sugars content in blood. Hence, these preliminary results suggested the presence of anti-diabetic properties which could be useful as a food supplement (Parvin *et al.*, 2015; Wang *et al.*, 2011; Morsy *et al.*, 2016; Oboh G. *et al.*, 2012; Phuwapraisirisan *et al.*, 2009).

2.4 PHARMACEUTICAL ACTIVITIES: antibiotics effect, anti-cancer actions and anti-inflammatory

C. olitorius has different biological activities and it is used in traditional medicines in Africa and in Asia. This traditional pharmacology, in general, is linked to one or many phytochemicals. *C. olitorius* was used against various synthons, particularly those linked to the gut apparatus (Islam, 2013). Human health is considered a priority all around the world and especially in developing countries in which the access to pharmaceutical drugs, particularly antibiotics, is denied in most cases. Moreover, the increase of super bacteria in developed countries as a consequence of an excess of the excessive use of antibiotics opened an interesting opportunity to find new active compounds to control bacteria. Many studies underlined the antibiotic activity of *C. olitorius* extract against several gut diseases, including microbes like *E. coli*, *Enterobacter* spp., and *Pseudomonas* spp. Moreover, *C. olitorius* showed an interesting controlling effect against *S. aureus* and *B. cerasus* and *C. diphtheria* (Zakaria *et al.*, 2006). Even though the main bacteria controlled was Gram-negative bacteria like *Klebsiella* spp., (Nwakaeze *et al.*, 2014), several Gram-positive bacteria, such as *Streptococcus*, are controlled (Ilhan, 2007). Moreover, *C. olitorius* extract has control over the proliferation of human pathogenic fungi like *B. candidum*, albeit most fungi were resistant against all extracts (Ilhan, 2007).

To discover new active antibiotic compounds inside *C. olitorius* leaves could be an essential step forward for pharmaceutical research. However, some other interesting active compounds against cancer cells were characterized inside leaves and seeds. As was noted previously, anti-inflammatory effects of *C. olitorius* extract could prove to be beneficial against cancer cells (Yan *et al.*, 2013). In fact, the relation between inflammation and chronic disease like gut cancer is well known (Dreher, and Junod, 1996). Moreover, antinociceptive activity was discovered by Zakaria in 2005. All these aspects demonstrated that *C. olitorius* extracts reduced cell stress and affected animal organs, such as those with heavy metal intoxication disease (Das *et al.*, 2010; Dewanjee *et al.*, 2013; Akinwumi *et al.*, 2016; Ibrahim *et al.*, 2016). The reduction of cancer in humans through food prevention is a worthy goal for the new millennium. *C. olitorius*, as a nutraceutical plant, has already demonstrated a high antioxidant capacity, as previously mentioned. While oxidants were considered one of the teratogen agents inside human cells, reducing the oxidants inside the cell increased tissue health, preventing DNA damages (Dreher, and Junod, 1996). A reduction of NO production in mice by using chorcorifatty acids A-B-C-D-E has been reported (Yoshikawa *et al.*, 1998). However, *C. olitorius* has several compounds inside its tissue that have had cytotoxic activities against human melanoma (İşeri, 2013). Moreover, corchurosid-D also demonstrated a controlling effect in human melanoma cells (Mallick *et al.*, 2013). Activity against tumor promoter-induced Epstein-Barr was reported by Furumoto *et al.*, 2002.

2.5 CONCLUSION.

C. olitorius as a leafy vegetable is widely consumed amongst African and South-East Asian communities, and it demonstrated interesting nutraceutical properties. *C. olitorius* has high levels of pharmaceutical compounds such as phenols and antibiotics. *C. olitorius* cultivation is easy to manage and its genetic plasticity made it suitable for many environments. *C. olitorius*' biological cycle was around 30-40 days in optimal environmental conditions, thus opening up an opportunity for the pharmaceutical and cosmetic industries, as well.

CHAPTER III: Yield and quality of Corchorus Olitorius (L.) baby leaf grown in a floating system

3.1 INTRODUCTION

The minimally processed leafy vegetables sector has been growing over the last few years. Besides common leafy vegetables, new species are identified and evaluated in order to give wider produce availability to the consumer. The potential new species must have specific requirements, such as adaptability to the growing conditions in the commercialization area, a good shelf life, and high content in nutraceutical proprieties. Jute mallow (*Corchorus olitorius*) belongs to the Malvaceae family. The botanic genus of *Corchorus* includes about 60 species and 30 of them are found in Africa (Emongor *et al.*, 2004), where it is considered a traditional leafy vegetable. It is also used in India and in Bangladesh, primarily as a cash crop for making fibres. It is generally cultivated in all tropical areas as a green vegetable, but it also occurs in the wild as a spontaneous plant. *C. olitorius* is rich in carotenoids, calcium, iron, phenols and flavonoids (Azuma *et al.*, 1999; Zeid, 2002; Khan *et al.*, 2006). For this reason, it can be a potential vegetable of ready-to-eat meals. Jute mallow is also used as traditional pharmaceutical drugs in Africa and Asia for treating different pathologies thanks to its high level of antioxidants (Yan *et al.*, 2013).

According to literature, several glycosides are inside seeds like strophanthidin glycoside (Ohtani *et al.*, 1995). These glycosides have a positive effect on the human vascular apparatus and could be used as cardiac drugs for heart failure treatment. In Japan, the young leaves of *C. olitorius* were introduced as healthy food not only for their nutritional characteristics (Resources Council, Science and Technology Agency, Japan 2000) but also for two different active compounds, phytol and monogalactosyldiacylgly-cerol, which have been shown to counteract tumor development induced by Epstein-Barr virus (EBV) (Furumoto *et al.*, 2002; Nishiumi *et al.*, 2006). There is no published information on jute mallow performance as a cultivated vegetable in hydroponic systems or as a ready-to-eat vegetable production. Therefore, the study of the behavior of the *C. olitorius* in a floating system will provide useful information regarding cultivation protocols for growers and as a vegetable for the ready-to-eat industry. The aim of this work was to evaluate the yield and quality of this potential new leafy vegetable by varying the nutrient solution concentration commonly used for leafy salad grown in floating systems.

3.2 MATERIALS AND METHODS

3.2.1 Plant material and growing system

An Egyptian accession of Jute (*C. olitorius*) was grown in 8 hydroponic floating system modules. Seeds were directly sown on plateaus containing perlite. Three growing cycles were performed and leafy vegetables were harvested at the commercial stage: the first during spring from March 2014 to April 2014, the second from May 2014 to June 2014 and the third cycle from October 2014 to November 2014. After germination, the plateaus were placed on 12 L tanks for each treatment. Two different nutrient solutions were compared: a standard solution (NS100%) containing mineral elements expressed in mM (N-NO$_3$ 12; N-NH$_4$ 3.8, P 2.8, K 3.8, Ca 3.5, Mg 1.4, Fe 0.04) and micronutrients expressed in µM (4.2 B, 2.1 Cu, 12 Mn, 1.4 Mo, 3.8 Zn). The second nutrient solution was halved (NS50%) to respect the standard.

3.2.2 Nitrate determinations

About 1 g of fresh leaves was ground in 5 mL of distilled water. The extracts were centrifuged at 5000 rpm x 5 minutes. After the centrifugation, the supernatant was collected for the colorimetric determinations. Four biological samples for both treatments were analysed in each biochemical assay. Nitrate content was determined in leaves using the salicylic acid method (Cataldo *et al.*, 1975). Twenty µL of samples were collected and 80 µL (5% weight/volume) of salicylic acid in concentrated sulfuric acid were added. After the reaction, 3 mL of NaOH 1.5 N were added. Samples were cooled to 25 °C and reactions were read at 410 nm. Nitrate concentrations were calculated using a calibration standard curve.

3.2.3 Chlorophyll and carotenoids determination

Chlorophyll and carotenoids were extracted from fresh tissue using methanol 99.9% as solvent. Samples were kept in a dark room at 4 °C for 24 hours. Quantitative chlorophyll determinations were carried out immediately after extraction. Absorbance readings were measured at 665.2 and 652.4 nm for chlorophyll pigments and 470 nm for total carotenoids. Chlorophyll and carotenoid concentrations were calculated by Lichtenthaler's formula (Lichtenthaler, 1987).

3.2.4 Sugars determinations: sucrose, reducing and total sugars

For the determination of sucrose, about 1 g of leaves was extracted by homogenization in a mortar with 5 mL of distilled water. The samples were centrifuged at 10 000 g for 5 min. The sucrose assay was performed by mixing 0.2 mL of crude extract with 0.2 mL of 2 M NaOH and incubated in a water bath at 100 °C for 10 min, then 1.5 mL hot resorcinol buffer (containing 30% hydrochloric acid, 1.2 mM resorcinol, 4.1 mM thiourea 1.5 M acetic acid) was added to samples and incubated in a water bath at 80 °C for another 10 min. After cooling at room temperature, the optical density was determined spectrophotometrically at 500 nm, using a sucrose standard curve (0, 0.5, 1, 1.5 and 2mM) (Rorem *et al.*, 1960).

The reducing sugar analysis was performed using 0.2 mL of crude extract that was added to 0.2 ml of a solution containing 62.6 mM dinitrosalicylic acid (DNS) and 1.52 M potassium sodium tartrate. The reaction mixture was heated at 100 °C for 5 min, then 1.5 ml of distilled water was added and absorbance readings were taken at 530 nm. The reducing sugars were expressed as glucose equivalent using a glucose standard curve (0, 1, 2, 3 and 4 mM) (Miller, 1959). The total sugars leaves were determined spectrophotometrically following the anthrone method (Yemm and Willis 1954) with slight modifications. The anthrone reagent (10.3 mM) was prepared dissolving anthrone in ice-cold 95% H_2SO_4. The reagent was left to stand for 30–40 min before use, 0.5 ml leaf extract was placed on top of 2.5 ml of anthrone reagent incubated in ice for 5 min and then vigorously vortexed. The reactions were heated to 95 °C for 10 min and left to cool in ice. Readings were performed at 620 nm. Calibration curve was carried out using a glucose solution.

3.2.5 Mineral content determination

About 400 mg DW were mineralized at 120 °C in 5 mL 14.4 M HNO_3, clarified with 1.5 mL 33% H_2O_2 and finally dried at 80 °C. The mineralized material was solubilized in 5 mL 1 M HNO_3 and filtered on a 0.45 μm nylon membrane. Mineral content was measured by inductively-coupled plasma techniques (ICP-MS; Varian 820-MS, ICP Mass Spectrometer). Three biological replicates were used for the determination of the following elements: sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), phosphorus (P), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), molybdenum (Mo) (Nocito *et al.*, 2011).

3.2.6 Phenolic compounds and anthocyanins determination

Phenols were spectrophotometrically determined in fresh samples following two different approaches: the direct measure of the methanolic extract absorbance at 320 nm (phenolic index) and the Folin-Ciocalteu method (Ke and Saltveit, 1989; Singleton *et al.*, 1999). Phenolic index was expressed as $ABS_{320 nm}$ g^{-1} FW, while the Folin-Ciocalteu method expresses total phenols as μg g^{-1} FW Gallic acid equivalent (GAE). For anthocyanins determination, samples of frozen tissue (30-50 mg) were ground in pre-chilled mortar and extracted into methanolic HCl (1%). Samples were then incubated overnight at 4 °C in the dark. The concentration of cyanidin-3-glucoside equivalents was determined spectro-photometrically at 535 nm (Klein and Hagen, 1961).

3.2.7 Statistical Analysis

The data reported are means ± standard errors (SEM). Data were subjected to two-way ANOVA analysis and differences between treatments were determined using Bonferroni's post-test (P<0.05). Significant differences are highlighted using different letters.

3.3 RESULTS

3.3.1 Yield and nitrate content

The *C. olitorius* plants showed a good performance in both nutrient solutions. The yield was similar between treatments, considering the shoots' biomass was harvested when plants reached 12-15 cm in height with 4-5 fully expanded leaves. This corresponds to the typical development stage of produce commercially harvested for the baby leaf fresh-cut industry. The yield during springtime was 1.9 kg FW m^{-2} and 1.7 kg FW m^{-2} in the NS100% and NS50% treatment, respectively. During summer (May-June), the yield was similar to that obtained during spring. The NS50% provided 2.4 kg FW m^{-2} of produce but no differences were found between treatments. In autumn, the yield decreased in both treatments and it was 0.819 kg FW m^{-2} in the NS100% and 0.794 Kg m^{-2} in the NS50% (Figure 1 (A)). The percentage of dry matter (DM) was measured during spring period and results showed a similar trend between the two nutrient solutions. In the NS100% treatment the DM was of 11.3% and 10.4% in the NS50%. Leaf nitrate content was measured at harvest and no difference was found between NS100% and NS50% treatments during summer and autumn cycles (Figure 1 (B)). On the contrary, significant differences were observed in the spring cycle. Plants harvested from nutrient solution with higher nutrients showed higher accumulation of nitrate. Leaf nitrate content was 5235 mg Kg^{-1} FW in the NS100% treatment and 2612 mg Kg^{-1} FW in the NS50% treatment (Figure 1 (B)). In summer, leaves harvested from plants grown in the NS100% nitrate content was 3881 mg Kg^{-1} FW and 3098 mg Kg^{-1} FW in the NS50% treatment. In autumn, the leaf nitrate content was higher in both treatments, in the NS100% leaves showed 5959 mg kg^{-1} FW and 5191 mg kg^{-1} FW in the NS50% treatment.
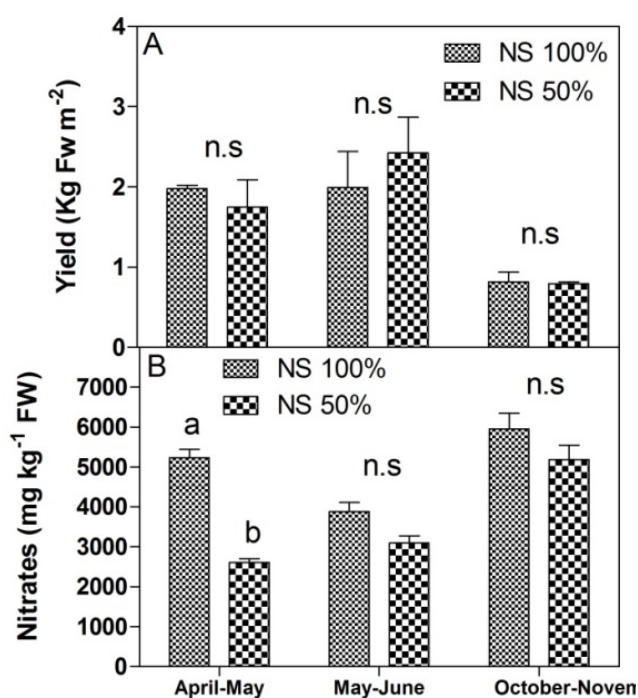

Figure 1. Yield, expressed as fresh baby leaf commercial stage (A) and leaf nitrates content (B) in C. olitorius grown in floating systems with full nutrient solution (NS100%) and reduced (NS50%) in three different seasons: spring, summer and autumn. Values are means with standard errors (n=4). Data were subjected to two-way ANOVA analysis and differences among means were determined using Bonferroni's Post-test. Different letters indicate significant statistical difference for P<0.05.

3.3.2 Sugars concentrations

Sugars were not significant between treatments in the different growing seasons, except for total sugars in the spring cycle (Figure 2 (C)) and sucrose in summer (Figure 2 (B)). The levels of reducing sugars ranged from 4.39 mg g-1 FW to 5.89 mg g-1 FW in the NS100% and from 4.12 mg g-1 FW to 4.67 mg g-1 FW in the NS50% (Figure 2 (A)). However, NS 100% showed an increased trend during summer for sucrose and reducing sugar contents. Sucrose content ranged from 0.65 mg g-1 FW to 1.04 mg g-1 FW for NS100% and from 0.62 mg g-1 FW to 0.78 mg g-1 FW in the NS50%. In spring the total sugar content in leaves harvested from the NS100% treatment was 9.91 mg g -1 FW and 16.73 mg g-1 FW in the NS50%. In summer, the total sugars content between treatments ranged from 8.48 and 8.59 mg g-1 FW and in autumn from 11.11 to 13.43 mg g-1 FW.
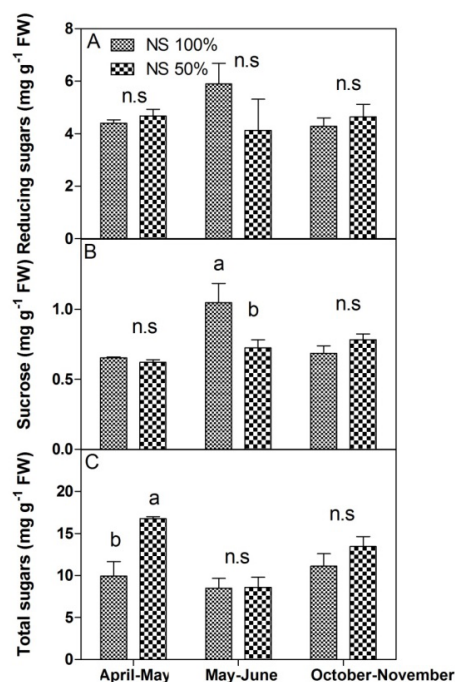
Figure 2. Reducing sugars (A), sucrose (B) and total sugars (C) determined in leaves of C. olitorius grown in floating systems with a full nutrient solution (NS100%) and reduced (NS50%) in three different seasons: spring, summer and autumn. Values are means with standard errors (n=4). Data were subjected to two-way ANOVA analysis and differences among means were determined using Bonferroni's Post-test. Different letters indicate significant statistical difference for P<0.05.

3.3.3 Mineral contents

The mineral content was statistically different between the leaves between plants cultivated in NS100% or NS50% treatments. The mineral concentrations were significantly higher in NS50% than NS100%. Leaf magnesium (Mg) concentration was 3.494 g kg-1 DW in the NS100% treatment and 4.505 g kg-1 DW in the NS50%. The potassium (K) content was 51.400 g kg-1 DW and 56.703 g kg-1 DW, respectively for NS100% and NS50%. Calcium (Ca) content in leaves of plants grown in the NS100% was 22.743 g kg-1 DW and 26.337 g kg-1 DW in the NS50% (Table 1). Iron (Fe) content was 139.4 mg kg-1 DW and 148.3 mg kg-1 DW in the NS100% and NS50%, respectively. When the *C. olitorius* plants grown in a floating system are compared with traditional methods, differences among macro-elements can be observed (Suppl. Table 1). The Mg and Ca contents are 8-100 fold higher in floating systems.

*Table 1. Mineral and trace elements content in C. olitorius leaves determined at harvest in plants grown in a floating system with a full nutrient solution (NS100%) and reduced (NS50%) in Spring 2014. Data are means with standard errors (n=3). Asterisks indicate significant differences between means obtained for plants grown on the two nutrient solution (P<0,001 = ***, P < 0.0001 = ****)*

Mineral content

	Na	Mg	K	Ca	P
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
NS 100%	0.42±0.54	3.49±0.00	51.40±0.03	22.74±0.01	12.69±0.10
NS 50%	0.64±0.80	4.51±0.06***	56.70±0.88****	26.34±0.39****	12.96±0.02

Trace elements content

	Mn	Fe	Ni	Cu	Zn	Mo
	mg kg^{-1}	mg Kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
NS100%	125.0±0.1	139.4±1.6	3.9±1.26	21.3±0.481	41.2±1.0	1.5±0.0
NS50%	510.0±2.2	148.3±2.4	6.5±0.307	24.9±4.6327	45.8± 1.0	0.9±0.0

3.3.4 Chlorophyll and carotenoids

There were no significant differences between NS100% and NS50% in all cycles for chlorophyll value (Table 2). In the NS50% total chlorophyll (Chla+Chlb) ranged during all seasons (spring, summer and autumn) from 2.84, to 1.58 mg g^{-1} FW. In the NS100% the chlorophyll values were 3.12, 2.53 to 2.28 mg g^{-1} FW in spring, summer and autumn, respectively. Carotenoids during the different growing seasons ranged from 0.18 mg g^{-1} FW to 0.75 mg g^{-1} FW in the NS50% and from 0.27 mg g^{-1} FW to 0.69 mg g^{-1} FW in the NS100%.

Table 2. Chlorophyll (a and b) content and total carotenoids determined in leaves of C. olitorius grown in a floating system with a full nutrient solution (NS100%) and reduced (NS50%) in three different seasons: spring, summer and autumn. Values are means with standard errors (n=4).

2014	April-May			May-June			October-November		
	Chl a (mg/g)	Chl b (mg/g)	Carotenoids (mg/g)	Chl a (mg/g)	Chl b (mg/g)	Carotenoids (mg/g)	Chl a (mg/g)	Chl b (mg/g)	Carotenoids (mg/g)
NS 100%	2.4±0.12	0.79±0.02	0.65±0.06	1.9±0.02	0.66±0.03	0.69±0.07	1.5±0.20	0.64±0.07	0.27±0.04
NS 50%	2.0±0.06	0.5±0.01	0.6±0.03	2.1±0.10	0.6±0.01	0.75±0.01	1.1±0.12	0.4±0.09	0.18±0.04

3.3.5 Anthocyanin and phenolic compounds content

The anthocyanins content in *C. olitorius* leaves was different in plants grown in the two nutrient solutions in trials of spring and summer. In plants grown in the NS100%, the anthocyanins ranged from 46.66 mg 100 g^{-1} FW in spring to 18.38 mg 100 g^{-1} FW in autumn, while those grown in the NS50% ranged from 63.95 mg 100 g^{-1} FW to 15.12 mg 100 g^{-1} (Figure 3 (A). Total phenols were evaluated using two determination methods (Figure 3 (B),(C)). Both methods reported the same trend, but the phenolic index showed significant differences between NS100% and NS50% in the summer cultivation.

The phenolic index ranged from 26.3 to 9.0 ABS320nm g^{-1} FW, and lower values were found in leaves harvested from plants grown in NS100% (Figure 3 (B)). The total phenols expressed as GAE (Gallic Acid Equivalent) were not able to highlight differences between nutrient solutions but changed with seasons. The levels of phenols ranged from 2754.39 µg g^{-1} FW to 2233.88 µg g^{-1} FW during spring (Figure 3 (C)).
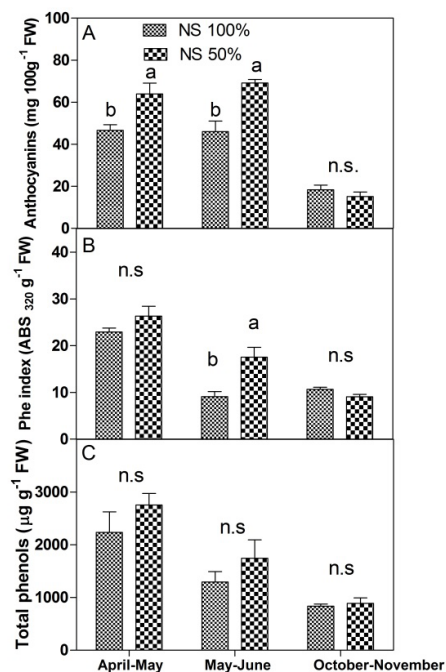
Figure 3. Anthocyanins expressed as cynidin-3-glucoside (A), Phenolic index (B) and total phenols expressed as Gallic acid equivalent (C). All parameters are referred to fresh weight and were determined in leaves of C. olitorius grown in a floating system with a full nutrient solution (NS100%) and reduced (NS50%) in three different seasons: spring, summer and autumn. Values are means with standard errors (n=4). Data were subjected to two-way ANOVA analysis and diffe-rences among means were determined using Bonferroni's Post-test.

3.4 DISCUSSION

The yield of jute mallow is an important parameter for understanding the behavior and productivity of these tropical plants in European countries using floating systems as a cultivation strategy. Other leafy vegetables commonly used for fresh cut industry have similar yields from floating systems. Spinach grown in different nutrient solutions provided yield ranging from 1.0-1.5 kg m^{-2}, lettuce ranged from 1,5 -2.0 Kg m^{-2}, and lamb lettuce's ranged from 1.7-3.5 Kg m^{-2} (Fallovo *et al.*, 2009). The plant's biomass is strongly influenced by nitrate availability (Chen *et al.*, 2004), but high levels of nitrates inside vegetables can have negative effects on human health. Therefore, nitrates must be low and represent an important quality parameter for leafy vegetables. Epidemiological studies have demonstrated that high levels of nitrates in diet were correlated with gastrointestinal cancer (Bruning-Fann and Kaneene, 1993). Therefore, the nitrate content in leafy vegetables must respect the limits imposed by EU regulation n. 1258/2011. The significant differences between nutrient solutions observed during the first cycle can be explained considering the higher light availability and optimal growing temperature (Suppl. Figure 1) conditions (Fritz *et al.*, 2006). Sugars and nitrates are both involved in the osmotic regulation activity, and nitrate reductase activity is light dependent and influenced by season (Solomonson and Barber, 1990; Santamaria, 2006; Fallovo *et al.*, 2009).

Macro elements were higher in the floating system, particularly for P, Mg and Ca. Zinc (Zn) and Iron (Fe), for each of which concentrations were in accordance with values available in literature (Ndlovu and Afolayan, 2008). *C. olitorius* leaves have lower content of Ca, Fe and Zn than other common leafy vegetables such as spinach. However, they also have less phytate content. Therefore, leaf minerals might be better bioavailable (Ndlovu and Afolayan, 2008). Several studies carried out on *C. olitorius* mineral content demonstrated that this species can be a source of these elements in the human diet, in particular for females and children (Orech *et al.*, 2007; Uusiku *et al.*, 2010; Kamga, *et al.*, 2013; van Jaarsveld *et al.*, 2014).

Form a global point of view, trace elements deficiency is recognized as having a negative impact on human health, especially among populations in developing countries. These preliminary findings demonstrated that *C. olitorius* could potentially enhance the mineral contribution in the diets of poor populations. (Achigan-Dako *et al.*, 2011).

At the level of plant physiology, the higher macro-elements obtained in plants grown in the NS50% treatment could be a response to a moderate nutrient deficiency stress (Chapin 1980), while the lower macro element concentrations inside NS100% could be also link to the higher nitrogen availability (Musa et al., 2010). Leafy vegetables after the harvest undergo a sugar content reduction due to respiration and senescence. Leaf sugars content is another parameter that changed during the growing seasons. These concentrations of sugars found in *C. olitorius* leaves are similar to those of traditional European leafy vegetables such as lettuce or rocket (Ali *et al.*, 2002).

Chlorophyll is an important quality parameter since they contribute to leaf colour and have a direct effect on the consumer. The green colour of vegetables is influenced by light and nitrogen levels (Wu and Kubota , 2008). The levels of chlorophyll in the *C. olitorius* were higher than other leafy vegetables (Bouayed *et al.*, 2011). Carotenoids in *C. olitorius* were higher with the maximum level of 75 mg 100 g^{-1} during the summer season in NS50% conditions, hence higher than the different *Brassica* varieties (Kaulamnn *et al.*, 2014). Carotenoids have an important role on human nutrition because they work as oxidant-preventing compounds (Maiani, *et al.*, 2009).

The anthocyanins content found in *C. olitorius* are similar to other coloured leafy vegetables such as Radicchio and Red Cabbage (Kaulamnn *et al.*, 2014). The increase of anthocyanins during spring and summer can be associated with higher solar radiation intensity (Marin *et al.*, 2015). A strong relationship between UV light and anthocyanin biosynthesis has also been reported in several studies (Li and Kubota, 2009; Tsormpatsidis *et al.*, 2007). Total phenols including anthocyanins have antioxidant activity and, with carotenoids, contribute to the antioxidant capacity of *C. olitorius* (Wang *et al.*, 2011; Obho *et al.*, 2012; Yan *et al.*, 2013). If compared with other leafy vegetables, *C. olitorius* had similar phenols content (Fallovo *et al.*, 2009; Marin *et al.*, 2015). Total phenols are not only interesting for the human nutritional

point of view as active compounds against ROS (Shukla *et al.*, 2009; Rose *et al.*, 2005), but also for the postharvest performance of the produce itself (Bergquist *et al.*, 2005; Larsson *et al.*, 1986). A positive correlation between light intensity and phenolic levels has been reported (Neill *et al.*, 2003). Phenols biosynthesis depends from PAL activity that could also be induced by lower nitrogen content in the NS50% (Kováčik and Bačkor, 2007). As shown in Figure 3 during summer cultivation, the range of phenols decreased in comparison to spring. Similar trends were reported by Ribeiro (2001).

3.5 CONCLUSION

C. olitorius showed good performances in floating system cultivation and can be a potential new leafy vegetable. Yield and nitrates indicate that this species can be grown with a reduced nutrient solution without compromising the biomass accumulation and the quality of the produce. The high sugar content can have a positive effect on the shelf life and on the human diet. The *C. olitorius* may represent, in the near future, a worldwide produce innovation in the fresh cut industry. During spring, a relation between the reduction of nitrate and a higher percentage of nitrogen inside NS50% leaf suggested a higher efficiency in nitrogen assimilation. This hypothesis could be justified by anthocyanin accumulation in NS50% during spring and summer as the result of an increased amount of phenylalanine. Study will have to be conducted on this previous result.

Additional information

Carbon and Nitrogen % of C. olitorius leaf cultivated in NS 50% and in 100% during spring.

Figure Suppl. 1. Total % of nitrogen (N) and organic carbon (C) was performed by using an elemental analyser (ThermoQuest NA 1500 N; Thermo Electron, Milan, Italy). Analyses were carried out with whole leaf (n = 3 per plot). Data were subjected to t-test. Statistical significance determined using the Hold Sidak method, with α = 5.000%.
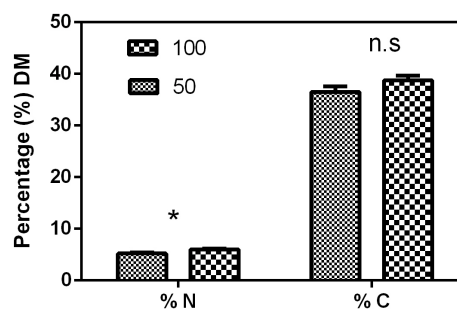
CHAPTER IV:
Seasonal storage behavior of several quality parameters of *C. olitorius (L.)* Cultivated as baby leafy in different nutrient supply solutions

4.1 INTRODUCTION

C. olitorius is considered a traditional leafy vegetable by FAO. The increase of public interest in nutritional and healthy food has been rapidly increasing worldwide (Cawley *et al.*, 2015). Therefore, the identification of new potential high quality leafy vegetables has been promoted by fresh cut industries for commercialization as ready-to-eat food. *C olitorius*, commonly known as jute or Jew's mallow, is grown as a leafy vegetable in Africa and Asia. It is cultivated for its potential benefits for human health due to its high levels of nutritional components such as phenols and carotenoids (Yan, *et al.*, 2013). It is also an important source of amino acids, such as methionine (Freiberger *et al.*, 1998). Moreover, the jute leaves are excellent sources of vitamin C, minerals and sugars (Ogunlesi, *et. al*, 2010). New technologies are applied to minimally process leafy vegetables, because of the physiological characteristics of plant tissues. These characteristics are specific for every vegetable, requiring specific technologies for storage (Medina *et al.*, 2012).

Preventing degradation of nutrient contents is always a priority for the food industry and for consumers (Tulio *et al.*, 2002). However, consumers mostly evaluate the freshness and taste of vegetables more than their nutritional content. Greenness, for example, is one of the first visual parameters evaluated during the purchasing of leafy vegetables. This visual appearance is considered the most important quality index for commercialization in supermarkets (Roura *et al.*, 2000). Moreover, health-oriented consumers prefer to buy ready-to-eat (RTE) vegetables as a source of phytochemicals and antioxidant compounds, which can prevent certain diseases (Cocetta *et al.*, 2014). The floating system technique allows for the control of nutrient supply and plant uptake, leaving mineral contents inside leaves which positively influence quality, optimizing cultivation inputs and reducing waste. Moreover, during storage, nutrients and active compounds might be affected by both agronomic aspects (Scuderi *et al.*, 2011), as noted by Favollo *et al.*, 2009. In fact, nutrient solutions influenced several quality components such as minerals, nitrates and chlorophyll. The aim of this work was to evaluate the performance of *C. olitorius* as baby leaf produce for the fresh cut industry. Interactions between growing nutrient solutions and the quality retained during storage were studied and characterized.

4.2. MATERIALS AND METHODS

4.2.1 Plant material and growing system

An Egyptian accession of Jute (*C. olitorius*) was grown in 8 hydroponic floating system modules. Seeds were directly sown on plateaus containing perlite. Three cultivations cycles were performed: two during spring and summer (one from March 2014 to April 2014 and another from May 2014 to June 2014) and one during autumn (from October 2014 to November 2014) in the greenhouse of the University of Milan. Solar radiation and temperature were recorded throughout the entirety growing cycles (suppl. Figures 1-2).

In order to create a small floating system module, the plateaus (20x15cm) were placed on 12 L tanks (25x15x35cm) after germination. Four modules of each type of nutrient solution were compared: a standard solution (NS100 %) containing mineral elements were expressed in mM (N-NO3 12; N-NH4 3.8, P 2.8, K 3.8, Ca 3.5, Mg 1.4) and micronutrients expressed in µM (4.2 B, 2.1 Cu, 12 Mn, 1.4 Mo, 3.8 Zn). The second one halved (NS50 %). The quality of jute leaves stored was evaluated during 10 d from harvest (0, 1, 3, 7, and 10 d) stored in a cool chamber at 4 °C. Forty g of *C. olitorius* leaves were enclosed in plastic boxes and sampling was performed in quadruplicates during their shelf-life.

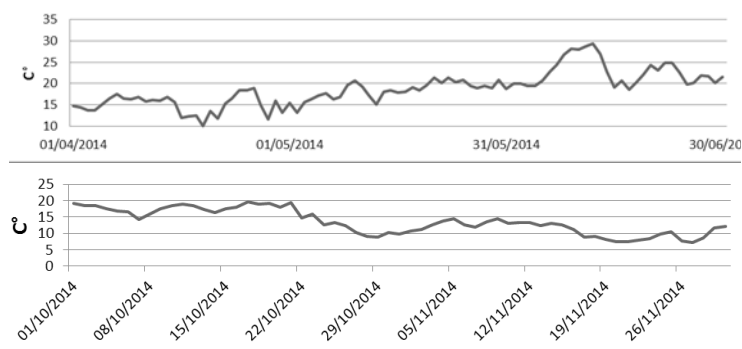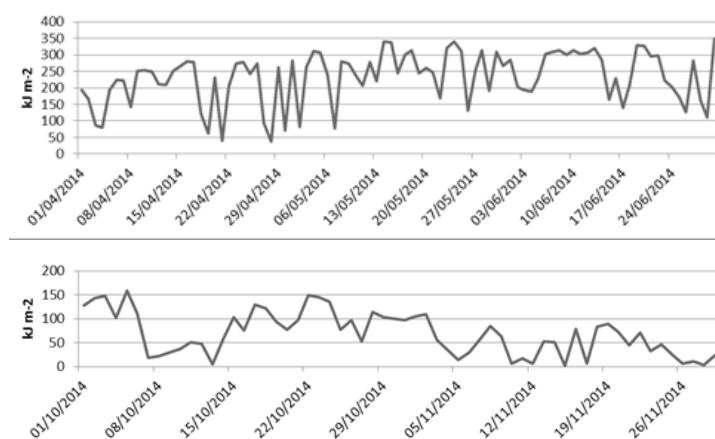*Figure Suppl. 1. Air temperature during the growing seasons.*

Figure Suppl. 2. Solar radiation during the growing seasons.

4.2.2 Nitrate determinations

About 1 g of fresh leaves was ground in 5 mL of distilled water. The extracts were centrifuged at 10,000 g for 5 minutes. After centrifugation, supernatant was collected for the colorimetric determinations. Four biological replications were analysed for each biochemical assay. Nitrate content was determined in leaves using a salicylic acid method (Cataldo *et al.*, 1975). 20 µL of samples were collected and 80 µL (5 % weight/volume) of salicylic acid in concentrated sulfuric acid were added. After the reaction, 3 mL of NaOH 1.5 N were added. Samples were cooled at 25 °C and they were read 410 nm using Evaluation 300 BB Thermo and quartz UV light cuvette. Nitrate concentrations were calculated using a calibration standard curve.

4.2.3 Chlorophyll and carotenoids determination

Chlorophyll was measured by using spectrophotometer methods. Three leaf disks (5 mm diameter) of fresh leaves were sampled using 5 mL of methanol (99.9 %). Samples were stored in a dark room at 4 °C for 24 h. After 24 h, the samples were read at 470, 652 and 665 nm. The chlorophyll and carotenoid concentrations were determined using Lichtenthaler's formulas (1987).

4.2.4 Sugars determinations: sucrose, reducing and total sugars

For the determination of sucrose, about 1 g of fresh leaves was extracted by homogenization in a mortar with 5 mL of distilled water. The samples were centrifuged at 10,000 g for 5 min. The sucrose assay was performed by mixing 0.2 mL of crude extract with 0.2 mL of 2 M NaOH and incubated in a water bath at 100 °C for 10 min, then 1.5 mL hot resorcinol buffer (containing 30% hydrochloric acid, 1.2 mM resorcinol, 4.1 mM thiourea 1.5 M acetic acid) was added to samples and incubated in a water bath at 80 °C for another 10 min. After cooling at room temperature, the optical density was determined spectrophotometrically at 500 nm, using a sucrose standard curve (0, 0.5, 1, 1.5 and 2mM) (Rorem *et al.*, 1960). The reducing sugar analysis was performed using 0.2 mL of crude extract that was added to 0.2 mL of a solution containing 62.6 mM dinitrosalicylic acid (DNS) and 1.52 M potassium sodium tartrate. The reaction mixture was heated at 100 °C for 5 min, then 1.5 mL of distilled water was added and absorbance readings were taken at 530 nm. The reducing sugars were expressed as glucose equivalent using a glucose standard curve (0, 1, 2, 3 and 4 mM) (Miller, 1959).

The total sugars were determined spectrophotometrically following the anthrone method (Yemm and Willis 1954) with slight modifications. The anthrone reagent (10.3 mM) was prepared dissolving anthrone in ice-cold 95% H_2SO_4. The reagent was left to stand for 30–40 min before use, 0.5 mL leaf extract was placed on top of 2.5 mL of anthrone reagent incubated in ice for 5 min and then vortexed vigorously. The reactions were heated to 95°C for 10 min and left to cool in ice. Readings were performed at 620 nm. The calibration curve was carried out using a glucose solution.

4.2.5 Phenolic compounds and anthocyanins determination

Phenols were spectrophotometrically determined in fresh samples following two different approaches: the direct measure of the methanolic extract absorbance at 320 nm (phenolic index) and the Folin-Ciocalteu method (Keng and Saltveit, 2003). Phenolic index was expressed as $ABS_{320\,nm}$ g^{-1}, while total phenols were measured using the Folin-Ciocalteu method and expressed as g kg^{-1} gallic acid equivalent (GAE). For anthocyanins determination, samples of frozen tissue (30-50 mg) were ground in pre-chilled mortar and extracted into methanolic HCl (1 %). Samples were then incubated overnight at 4 °C in the dark. Samples were determined spectrophotometrically at 535 nm using Evaluation 300 BB Thermo and quartz UV light cuvette. The concentration was expressed in cyanidin-3-glucoside equivalents using direct absorption (Klein and Hagen 1961).

Statistical Analyses

The data reported were means ± standard errors (SEM). The data were subjected to two-way ANOVA analysis and differences between treatments were determined using Bonferroni's post-test (P<0.05).

4.3. RESULTS

4.3.1 Nitrate and total sugars content

Among quality parameters, nitrates and sugars are particularly important because they can have positive and negative effects on human health. In our study, nitrates were significantly different from plants grown in the different nutrient solutions in spring (Fig 1 A), while no differences were observed in the summer and autumn cycles (Fig. 1C). At harvest, nitrate concentration in leaves of plants grown in spring was on average 18.89 % less in NS50 % (4.2 g kg^{-1} FW) compared to NS100% (5.2 g kg^{-1} FW). During storage, nitrate concentration did not change in the leaves harvested in spring (Fig. 1A), while it declined after 4-10 days in the experiments performed with leaves harvested in summer (Fig. 1B). In autumn, nitrate reduction was found after 10 days only in the leaves of plants grown with NS50% (Fig. 1C). During storage, nitrate content results were statistically different between samples harvested from the two nutrient solutions over the course of 7 days.

Total sugars in jute leaves grown with NS100% in spring produced statistically different results from NS50% at harvest and during the first day of storage, with values that ranged from 12 to 9 g kg-1 FW, in NS100% and NS50%, respectively (Fig. 1D). In summer and autumn, the harvested leaves did not show significant differences between the two nutrient solutions. Total sugars measured at harvest among seasons and between nutrient solutions ranged from 16 to 8.59 g kg-1 FW (Fig. 1 D,E,F). In all storage experiments, the total sugars declined, starting from the first or fourth day of storage (Fig. 1 D, E, F).

Figure 1. *Storage behavior at 4 °C of leaf nitrate (A,B,C) and Total sugars (D,E,F). contents of Corchorus olitorius plants grown during spring (A,D), summer (B,E) and autumn (C,F) on either full (circles) or half strength (squares) nutrient solutions and stored at 4 °C for up to 10 d. Given are means ± standard error (n=4). Asterisks indicate significant differences between means obtained for plants grown on the two nutrients (P<0.05 = *, P < 0,01 = **; P < 0,001 = ***). Different letters indicate statistical differences among different days of storage.*
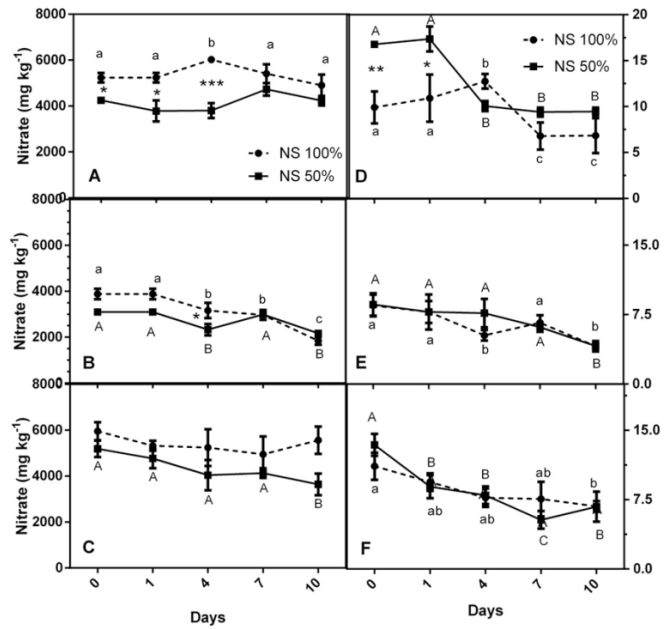
4.3.2 Sucrose and reducing sugar content

At harvest, the sucrose content was statistically different only in the summer and spring cycles, with 1.2 g kg^{-1} in leaves harvested from NS100% +44.3 % and 0.72 g kg^{-1} in leaves harvested from NS50 %. During storage, sucrose content declined after 4 days in leaves harvested from NS100% (Fig. 2B) and in leaves harvested in autumn from NS50% treatment stored for 10 days (Fig. 2C). There was no significant difference regarding the reducing sugars between the nutrient solutions at harvest and during storage in all seasons. However, during postharvest, the reducing sugars decreased in storage experiments. In leaves obtained from the spring cultivation, there was a 10% reduction of reducing sugars, passing from 4.0 g kg^{-1} at harvest to 3.6 g kg^{-1} after 10 days of storage. In summer, the highest reduction was observed in NS100% with 89.2 % (Fig. 2E). In autumn, however, the highest reduction of 62 % was found in leaves harvested from N50 % with values of 3.2 g kg^{-1} at harvest and 1.2 g kg^{-1} at the end of storage (Fig. 2F).

Figure 2. *Storage behavior at 4 °C of leaf sucrose (A,B,C) and reducing sugars (D,E,F). Contents of Corchorus olitorius plants grown during spring (A,D), summer (B,E) and autumn (C,F) on either full (circles) or half strength (squares) nutrient solutions and stored at 4 °C for up to 10 d. Given are means ± standard error (n=4). Asterisks indicate significant differences between means obtained for plants grown on the two nutrients (P<0.05 = *, P < 0,01 = **; P < 0,001 = ***). Different letters indicate statistical differences among different days of storage.*
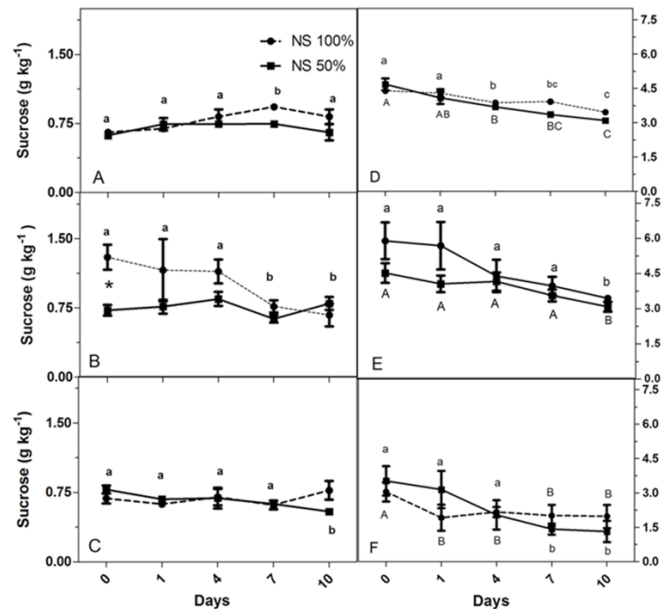
4.3.3 Carotenoids and total chlorophyll content

Chlorophyll content was highly stable inside *C. olitorius* leaves during shelf life. No differences were found from harvest until the end of the storage period (Table 1). Between NS100% and NS50% treatments, the differences in chlorophyll content was observed after 4 and 10 days of storage in samples harvested in spring.

Chlorophyll content at harvest ranged from 3.2 g kg^{-1} for NS100% to 2.9 g kg^{-1} for NS50%. During summer, chlorophyll content ranged from 2.7 g kg^{-1} for NS 100 % to 2.8 g kg^{-1} for NS 50 %. During autumn, the chlorophyll content inside the leaf during storage decreased after seven days of storage by 51 % for NS100 %, while in NS50% the chlorophyll loss was of 17.7 % after seven days, but the difference was not statistically different. Total carotenoids were different between nutrient solutions only in the autumn growing cycle, with concentrations of 0.27 g kg^{-1} in NS100% and 0.18 g kg^{-1} in NS50% (Tab. 2). During storage, no significant changes were observed in the experiments carried out in spring and summer. The only statistical differences in the storage experiments were found after 10 days in the leaves harvested from both nutrient solutions.

Table 1. Chlorophyll (a+b) content (g kg-1) storage behavior at 4 °C determined in leaves of C. olitorius grown in a floating system with a full nutrient solution (NS100 %) and reduced (NS50 %) in three different seasons: spring, summer and autumn. Values were means with standard errors (n=4). Asterisks indicate differences among different NS treatments. Different letters indicate statistical differences among different days of storage.

spring	harvest	1 d	4 d	7 d	10 d
NS 100%	3.1±0.16	3.2±0.16	3.2±0.16	2.6±0.10	2.8±0.06
NS50%	2.9±0.30a	2.5±0.14a	2.6±0.28a	2.4±0.05a	1.8±0.07b
Diff. treatments	ns	ns	*	ns	**
summer					
NS 100%	2.7±0.27	2.2±0.39	2.5±0.12	2.2±0.04	2.2±0.06
NS50%	2.8±0.05	2.6±0.13	2.3±0.12	2.1±0.05	2.3±0.02
Diff. treatments	ns	ns	ns	ns	ns
autumn					
NS 100%	2.2±0.27a	2.2±0.41a	2.0±0.27a	1.0±0.1b	0.8±0.34b
NS50%	1.5±0.21	1.5±0.28	1.2±0.14	1.3±0.02	1.0±0.06
Diff. treatments	ns	ns	ns	ns	ns

Table 2. Carotenoids content (g kg-1) storage behavior at 4 °C determined in leaves of C. olitorius grown in a floating system with a full nutrient solution (NS100 %) and reduced (NS50 %) in three different seasons: spring, summer and autumn. Values were means with standard errors (n=4). Asterisks indicate differences among different NS treatments. Different letters indicate statistical differences among different days of storage.

spring	harvest	1 d	4 d	7 d	10 d
NS100%	0.60±0.06	0.65±0.03	0.66±0.04	0.64±0.017	0.73±0.23
NS50%	0.65±0.03	0.64±0.02	0.60±0.03	0.58±0.03	0.54±0.02
Diff. treatments	ns	ns	ns	ns	ns
summer					
NS100%	0.69±0.07	0.67±0.09	0.76±0.03	0.65±0.01	0.65±0.03
NS50%	0.75±0.01	0.75±0.02	0.60±0.03	0.60±0.00	0.67±0.01
Diff. treatments	ns	ns	ns	ns	ns
autumn					
NS100%	0.27±0.04a	0.27±0.05a	0.31±0.04a	0.15±0.03a	0.09±0.05b
NS50%	0.18±0.04A	0.31±0.05A	0.19±0.02A	0.18±0.00A	0.08±0.11B
Diff. treatments	*	ns	ns	ns	ns

4.3.4 Phenols content and anthocyanin

Phenolic content was not significantly influenced by nutrient solutions and decreased during shelf life. Considering, the high percentage of chance of random interaction between storage time and nutrient solutions, none interaction were observed overall. Phenols content decrease started from harvest. However, it did not decrease in a linear way. High losses in phenolic contents arose from day one to day four as occurred for reducing sugars. Jute grown in the NS100 % treatment in the spring cycle, lost its phenolic content from 2.2 g kg^{-1} to 2.1 g kg^{-1} with a 5.6 % of reduction and in the NS50 % phenolic content decreased from 1 d to 4 d, from 2.7 g kg^{-1} to 2.1g kg^{-1} with 21.8% of loss. However, *C. olitorius* leaves grown in the NS100% treatment and harvested in the summer cycle, lost their phenols between day four and day seven, and passed from 1.0 g kg^{-1} to 0.7g kg^{-1}, with a 27.7 % loss. During summer, *C. olitorius* leaves harvested from NS50 % maintained the same trend observed during spring, in fact the higher losses were found between day one and day four with a loss percentage of -37.7 %. In this period, leaves harvested from the NS50 % treatment reduced the phenols from 1.7 g kg^{-1} to 1.0 g kg^{-1}. *C. olitorius* harvested from both NS50 % and NS100 % did not show significant differences during autumn at harvest as well as during shelf-life (Table 3). Anthocyanin content showed similar trend during shelf life in all seasons. During spring and summer, in jute harvested from NS50 % the anthocyanin significantly increased (Table 4). Inside N50 % leaves cultivated in autumn, anthocyanin decreased significantly during shelf-life. During spring and summer anthocyanin did not show a significant reduction from harvest to 10 d; however, their values during shelf-life suggested a slowly reduction trend starting after one day.

The critical decrease point occurred between day one and day four, in fact, an anticipated anthocyanin loss (15.8 %) was observed from 0.6 g kg^{-1} to 0.5 g kg^{-1} (Table 4). On the contrary, during spring, inside NS100 % jute leaves anthocyanin decreased rapidly later on: after four days from harvest, from 4 d (0.4 g kg^{-1}) to 7 d (0.4 g kg^{-1}) with a similar loss percentage (13.7 %) (Table 4). During summer, higher anthocyanin reduction was found between 1 d to 4 d with 8.5 % loss in leaves cultivated in NS50 % while the NS100 % jute leaves did not show significant anthocyanin loss content during entire shelf life. During autumn, anthocyanin content in leaves of jute plants grown in the NS100 % and NS50 % decreased with the same trend during entire shelf life. Jute leaves grown in the NS50 % showed higher decrease after four after harvest till 7 d with 38 % of content loss. NS100 % decreased later than seven days till the end with a 31 % of anthocyanin loss.

Table 3. Storage behaviour at 4 °C of phenolic phenols expressed as gallic acid equivalent (GAE) g kg-1 in C. olitorius grown in floating system with full nutrient solution (NS100 %) and reduced (NS50 %). in three different seasons: spring; summer and autumn. Values were means with standard errors (n=4). Data were subjected to two-way ANOVA analysis and differences among means were determined using Bonferroni's Post-test. Indicated significant statistical.

spring	harvest	1 d	4 d	7 d	10 d
NS100	2.2±0.34	2.2±0.39	2.1±0.24	2.2±0.97	2.1±0.17
NS50	2.6±0.19	2.7±0.21	2.1±0.26	2.3±0.13	2.0±0.17
Diff. treatments	ns	ns	ns	ns	ns
summer					
NS100	1.4±0.23	1.2±0.19	1.3±0.27	1.0±0.4	1.1±0.16
NS50	1.6±0.35	1.7±0.21	1.0±0.22	0.7±0.7	1.0±0.15
Diff. treatments	ns	ns	ns	ns	ns
autumn					
NS100	0.8±0.40	0.8±0.03	0.6±0.07	0.6±0.050	0.7±0.13
NS50	0.8±0.10	0.7±0.13	0.6±0.03	0.6±0.13	0.7±0.08
Diff. treatments	ns	ns	ns	ns	ns

Table 4. Anthocyanin contents (g kg-1) expressed as cynidin-3-glucoside in C. olitorius grown in floating system with full nutrient solution (NS100 %) and reduced (NS50 %). in three different seasons: spring; summer and autumn. Values were means with standard errors (n=4). Data were subjected to two-way ANOVA analysis and differences among means were determined using Bonferroni's Post-test. Indicated significant statistical differences for: P<0.05 = *, P < 0,01 = **; P < 0,001 = ***. Asterisks indicate differences among different NS treatments. Different letters indicate statistical differences among different days of storage.

spring	harvest	1 d	4 d	7 d	10 d
NS100	0.4±0.26a	0.4±0.26a	0.4±0.59a	0.4±0.19b	0.4±0.52a
NS50	0.6±0.51	0.6±0.53	0.5±0.53	0.4±0.29	0.4±0.47
Diff. treatments	**	**	ns	ns	ns
summer					
NS100	0.4±0.50	0.4±0.42	0.4±0.49	0.4±0.48	0.4±0.80
NS50	0.6±0.17	0.6±0.15	0.6±0.33	0.5±0.12	0.5±0.69
Diff. treatments	**	**	ns	ns	ns
autumn					
NS100	0.1±0.21a	0.1±0.23a	0.1±0.19a	0.1±0.09a	0.9±0.10b
NS50	0.1±0.22A	0.1±0.20A	0.1±1.3A	0.9±0.07B	0.6±0.07B
Diff. treatments	ns	ns	ns	ns	ns

4.4. DISCUSSION

The nutraceutical components of fruits and vegetables are represented by several bioactive molecules such as anthocyanins, carotenoids and phenols. These compounds contribute to the antioxidant potential of the produce. The concentrations of these compounds vary among species and old or ancient varieties usually have higher concentrations of these bioactive compounds (Dasgupta and De, 2007, Francini *et al.*, 2016). Besides these health promoting compounds, there are other compounds as well such as nitrates, which must be lower that regulation limits because they may be dangerous for human heath since they are correlated with gastrointestinal cancer induction (Cavaiuolo and Ferrante, 2014). Therefore, nitrate content is an important quality parameter that has to be controlled in leafy vegetables. It is well known that nitrate accumulation is inversely correlated with the availability of solar radiation, which is responsible for the activation of nitrate-reductase, a key enzyme for nitrate assimilation in plants (Cram, 1976). These findings explain the lower nitrate concentrations during summer (Supplementary file). Under low light intensity conditions, nitrates and oxalate ions have an osmotic regulatory function in the cell in substitution for organic acids and sugars. Therefore, the nitrates are accumulated in the leaf vacuole to counteract the low sugar contents due to lower photosynthetic activity (Raven and Smith, 1976 and Blom-Zandstra, 1989). Nitrates during shelf life or storage are partially reduced, but significant quantities remain stable inside leaves. The stability of nitrates during postharvest is due to the lack of electron flux from photosynthetic machinery and due to the low temperatures, which slow down enzyme activity.

Chlorophyll in leafy vegetables strongly depend on nitrate content and light intensity (Kasim *et al.*, 2012). However, nutrient solutions did not influence chlorophyll content, indicating that both contain enough nutrients for the adequate biosynthesis of these compounds. Carotenoids content also followed the chlorophyll trend, because they shield chlorophyll against photo-oxidation. Carotenoids, in addition to phenols and anthocyanins, are considered to be important antioxidants for the human diet (Martin *et al.*, 2013). Carotenoids in C. olitorius leaves were higher compared with other Mediterranean leafy vegetables like rocket (0.08 g kg^{-1}) and Dandelion (0.07 g kg^{-1}) (Sindhu and Kantharaj, 1995; Giro and Ferrante, 2016). During storage, carotenoids did not statistically decrease in *C. olitorius* leaves in spring and summer, guaranteeing a high concentration for the consumer. Instead, higher reduction was observed in autumn after seven days of shelf life: -10 % for both nutrient solutions. However, this aspect does not compromise the quality for the consumers because the commercialization of leafy vegetables is limited to seven days.

Sugar content in leaves depends on environmental conditions rather than nutrient solutions (Favollo *et al.*, 2008). After harvest, sugars content inside leaves represent the substrate for respiration and the maintenance of basal metabolism. Sugars availability affect the storability and the shelf life of produce (Sanchez-Mata, *et al.*, 2003).

In fact, during storage, sugars reduction is influenced by storage temperature, which affects the respiration rate of the product (Cao et *al.*, 2013; Der Agopian *et al.*, 2011). The high content of total sugars inside *C. olitorius* leaves, in particular, represents a good energetic reserve, which can be used for quality retain during storage. However, the sucrose content in *C. olitorius* leaves, if compared with other varieties of baby leaf, such as lettuce, is retained. In lettuce baby leaf, the sucrose content strongly declined after 5 days of storage at 5 °C (Spinardi and Ferrante, 2012; Tudela *et al.*, 2013). Reducing sugars decreased after four days of storage. In fact, unlike sucrose, reducing sugars were metabolic sugars, firstly used as a substrate for cell respiration. Moreover, reducing sugars and sucrose content were less influenced by nutrient solutions and storage conditions. These results can be explained considering that total sugars during postharvest can be hydrolysed in simple sugars keeping reducing sugars and sucrose stable. Sugars cover important nutritional functions for human health, not only as energetic compounds but also like a probiotic for human gut microbiota (Goh and Klaenhammer, 2015). During spring, significant differences between nutrient solutions were measured at harvest within one day for total sugars. The significant differences can be explained considering the higher light availability and optimal growing temperature conditions during springtime (Fritz *et al.*, 2006). In both NS100 % and NS50 %, nitrates did not change during shelf life. Significant differences were found among seasons according to different light intensities (Favollo *et al.*, 2009). Total phenols and anthocyanins did not show high variability during postharvest. The main changes can be due to seasons' effect on the phenolic metabolism (Samuolienė *et al.*, 2012). The phenols content might also be correlated to nitrogen availability and to phenylalanine ammonia-lyase activity (Kováčik *et al.*, 2007). In fact, when there's low nitrogen availability, the plants activate the phenylalanine ammonia-lyase enzyme to obtain nitrogen, removing the amine group from the phenylalanine. This is a futile cycle that is transiently activated by plants to cope with nitrogen deficiency. This can explain the lower anthocyanins concentration in the leaves of *C. olitorius* grown in the NS100%.

4.5. CONCLUSION

Our results indicated that reduced NS did not influence postharvest performance of this crop and can be advised for the reduction of fertilizer input during cultivation. The storage at 4 °C preserved the most important quality factors such as chlorophyll, carotenoid and sucrose. It can be concluded that *C. olitorius* baby leaf maintained high-quality characteristics in particularly antioxidant compounds during shelf life. Therefore, this new leafy vegetable can be effectively suggested as a new ready-to-eat vegetable for the fresh cut industry, providing high nutraceutical components to the consumer.

CHAPTER V:
C. olitorius cultivated in Italy: biochemical, physiological characterization among different

5.1 INTRODUCTION

Jute (*C. olitorius* n=24) cultivation has a great economic value regarding worldwide fibre use, but it is also used as a leafy vegetable in Africa and in Asia. Moreover, several communities, particularly in Asia, use *C. olitorius* as a nutraceutical plant (Resources Council, Science and Technology Agency, Japan 2000). There are several studies which confirm that *C. olitorius* originates from Africa. In particular, Kandu *et al.*, 2013, identified West Africa as the centre of origin for *C. olitorius*. However, archeo-botanical evidence shows that *C. olitorius* was domesticated in the Indo-Burma region (Wright *et al.*, 2011) like *C. capsicaris* (L.). Despite *C. olitorius*'s economic benefit to many countries, including India, Bangladesh and China, limited knowledge of the plant's morphologic and physiologic aspects has stopped its genetic improvement (Kandu *et. al.*, 2013). Moreover, the lack of relationship between geographic origin and molecular classification can be due to genetic exchanges among species nearby border limits, thus reducing differences among ecotypes. Moreover, a correlation between genome size and phonological traits was reported by Benor *et al.*, 2013, although the same study also confirmed the difficulties with finding a significant relationship between genome size and geographic origins. This morphological plasticity allows *C. olitorius* to adapt to a large amount of environmental conditions from Africa to Japan. Hence, transpiration and gas exchanges behaviour can be essential for selecting the best varieties in different geographic areas. These parameters are linked with physiological conditions belonging to morphology and genetic traits. It is interesting to see that a positive correlation between genome size and the microclimatic level was already discovered among Ethiopian accessions (Benor *et al.*, 2011). Moreover, this correlation is supported by several studies: Bancheva and Greilhuber 2006, Šmarda and Bureš 2006 and Achigan-Dako *et al.*, 2008. Our study can represent a step forward in understanding *C. olitorius* plasticity through analysing several geographic accessories worldwide. Physiological, morphological and biochemical parameters were evaluated to establish differences and correlations.
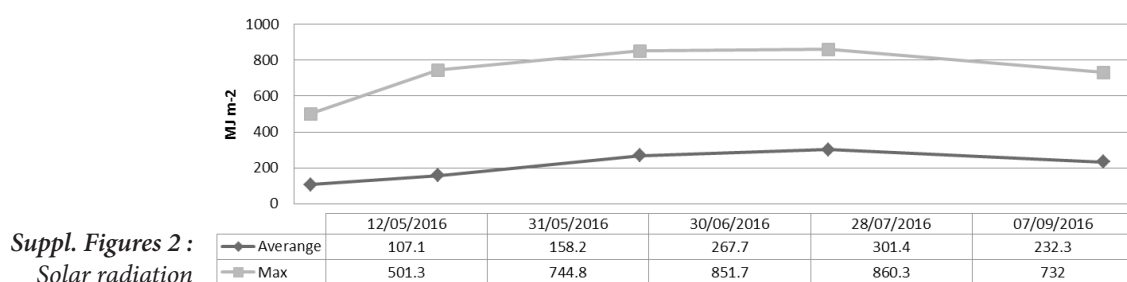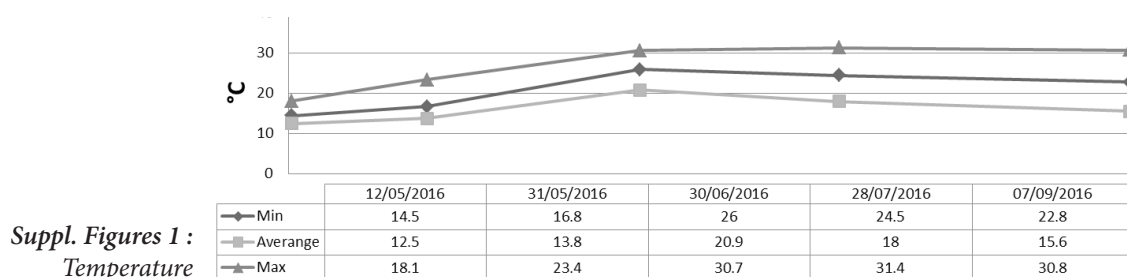
Sugars are one of the main metabolites for life cells. Sugar content is particularly important for vegetables' taste and storage (Kader, 2002). High levels of sugars inside tissue can increase their shelf life and influence flavour during the ripening process. In leafy vegetables and baby leaves in particular, the photosynthetic pathway is working during post-harvest treatments. For these reasons, some physiological parameters

can be used as a stress marker (Ferrante and Maggiore, 2007). Moreover, polyphenols are secondary metabolites; they are considered nutraceutical compounds for humans thanks to their antioxidant activity. The accumulation of these important compounds inside leaves are linked to several biotic and abiotic stresses, which increase ROS inside their tissue. In particular, photorespiration and photo-inhibition generate ROS. Hence, different combinations of polyphenols characterize each vegetable in response to oxidant stress. (Parida, *et al.*, 2002). These healthy compounds are prevalent inside *C. olitorius* baby leaves (Giro and Ferrante 2016). Different varieties have different growing behaviour and different attitudes that influence polyphenols biosynthesis and accumulation (Favollo *et al.*, 2009) in response to environmental conditions. *C. olitorius* has different active compounds inside leaves like carotenoids. Carotenoids are secondary compounds which are essential for all photosynthetic tissues because they have photo protection against chlorophyll oxidation. It is well known that chlorophyll content, carotenoids and photosynthetic efficiency are all factors that influence plants growth (Tsormapatsidis *et al.*, 2008, Wu and Kubota 2008, Zeid 2002). For these reasons, this study can indicate variability among accessories coming from different geographical areas for cultivating *C. olitorius* in South Europe.

5.2 MATERIALS AND METHODS

5.2.1 Plant material and growing system

Ecotypes were acquired by IPK Gene Bank of Leibniz Institute using their *C. olitorius* seed availability. *C. olitorius* were directly sown in plastic conic box 10L (0.28x0.28 m) using peat as substrate (class 3), pH 6 , EC 0.3 dS, density 170 kg m^{-3}. Four biological replicates of each ecotype were cultivated in a protected green house during germination (March and April 2017), after which time the plants, bearing 4-6 leaves, were moved outside for the eco-physiological measurements. Light intensity and temperature were measured during the cultivation cycle (suppl. Figures 1 and 2). An automatic irrigation system was used for water supply in order to guarantee the optimum water content availability inside the substrate. Mature leaves were collected for the biochemical analysis.

Suppl. Figures 1 :		12/05/2016	31/05/2016	30/06/2016	28/07/2016	07/09/2016
	Min	14.5	16.8	26	24.5	22.8
Temperature	Average	12.5	13.8	20.9	18	15.6
	Max	18.1	23.4	30.7	31.4	30.8

Suppl. Figures 2 :		12/05/2016	31/05/2016	30/06/2016	28/07/2016	07/09/2016
Solar radiation	Average	107.1	158.2	267.7	301.4	232.3
	Max	501.3	744.8	851.7	860.3	732

Statistical Analysis

Data were statistically analysed using One-way ANOVA method and the differences among means were determined using Bonferroni's Post-test.

Table 1. Ecotypes origin list delivered by IPK (Gene Bank of Leibniz Institute).

Ecotype	Region
C1	India
C2	China
C3	Libya
C4	Libya
C5	Libya
C6	Libya
C7	Tunisia
C10	Egypt
C11	Japan

5.2.2 Chlorophyll and carotenoids determination

Chlorophyll and carotenoids were extracted from fresh tissues using methanol 99.9% as solvent. Samples were kept in a dark room at 4 °C for 24 hours. Quantitative chlorophyll determinations were carried out immediately after extraction. Absorbance readings were measured at 665.2 and 652.4 nm for chlorophyll pigments and 470 nm for total carotenoids. Chlorophyll and carotenoid concentrations were calculated by Lichtenthaler's formula (Lichtenthaler, 1987).

5.2.3 Sugars determinations: sucrose, reducing and total sugars

For the determination of sucrose, about 1 g of leaves was extracted by homogenization in a mortar with 5 mL of distilled water. The samples were centrifuged at 10 000 g for 5 min. The sucrose assay was performed by mixing 0.2 mL of crude extract with 0.2 mL of 2 M NaOH and incubated in a water bath at 100 °C for 10 min, then 1.5 mL hot resorcinol buffer (containing 30% hydrochloric acid, 1.2 mM resorcinol, 4.1 mM thiourea 1.5 M acetic acid) was added to samples and incubated in a water bath at 80 °C for another 10 min. After cooling at room temperature, the optical density was determined spectrophotometrically at 500 nm, using a sucrose standard curve (0, 0.5, 1, 1.5 and 2mM) (Rorem et al., 1960). The reducing sugar analysis was performed using 0.2 mL of crude extract that was added to 0.2 ml of a solution containing 62.6 mM dinitrosalicylic acid (DNS) and 1.52 M potassium sodium tartrate. The reaction mixture was heated at 100 °C for 5 min, then 1.5 ml of distilled water was added and absorbance readings were taken at 530 nm. The reducing sugars were expressed as glucose equivalent using a glucose standard curve (0, 1, 2, 3 and 4 mM) (Miller, 1959). The total sugars leaves were determined spectrophotometrically following the anthrone method (Yemm and Willis 1954) with slight modifications. The anthrone reagent (10.3 mM) was prepared dissolving anthrone in ice-cold 95% H_2SO_4. The reagent was left to stand for 30–40 min before use, and 0.5 ml leaf extract was placed on top of 2.5 ml of

anthrone reagent incubated in ice for 5min and then vortexed vigorously. The reactions were heated to 95 °C for 10 min and left to cool in ice. Readings were performed at 620 nm. Calibration curve was carried out using a glucose solution.

5.2.4 Phenolic compounds and anthocyanins determination

Phenols were spectrophotometrically determined in fresh samples following two different approaches: the direct measure of the methanolic extract absorbance at 320 nm (phenolic index) and the Folin-Ciocalteu method (Ke and Saltveit, 1989; Singleton et al., 1999). Phenolic index was expressed as ABS320nm g^{-1} FW, while the Folin-Ciocalteu method expresses total phenols as $\mu g\ g^{-1}$ FW Gallic acid equivalent (GAE). For anthocyanins determination, samples of frozen tissue (30-50 mg) were ground in pre-chilled mortar and extracted into methanolic HCl (1%). Samples were then incubated overnight at 4 °C in the dark. The concentration of cyanidin-3-glucoside equivalents was determined spectrophotometrically at 535 nm. (Klein and Hagen, 1961).

5.2.5 Leaf physiological parameters

Gas exchange measurements were performed with a portable photosynthesis system (CIRAS-2, PP System, USA). Measurements were taken on young, fully expanded, intact leaves of C. olitorius plants every week for 3 months form mid-May to mid-July 2016. Net CO_2 assimilation rate, stomatal conductance and transpiration were assessed setting the CO_2 concentration of the instrument at 400 μmol mol-1, 50% relative humidity, 500 mL min-1 airflow and a photon flux density of 1000 μmol photons m-2 s-1. The instrument was stabilized according to manufacturer guidelines. Chlorophyll a fluorescence was measured from randomly selected dark-adapted leaves in from mid-May to mid-June and using leaf-clips and a portable Handy Plant Efficiency Analyser (PEA, Hansatech, UK). Fluorescence parameters were automatically calculated: Fv/Fm or (Fm-Fo)/Fm. The JIP-test was performed for the determination of the following indexes (Force et al., 2003). Fluorescence monitoring system was used for the measurement of photosynthetic quantic efficiency of light adapted leaves and electron transport flux.

Glossary of JIP-test terms

- Dissipation in a PS II cross-section (DI0/CS): total dissipation measured over the cross-section of the sample that contains active and inactive RCs. Dissipation occurs as heat, fluorescence and energy transfer to other systems.
- Effective antenna size of an active RC (ABS/RC): total number of photons absorbed by chl-molecules of all RCs divided by the total number of active RCs. It is influenced by the ratio of active/inactive RCs.
- Electron transport in PSII cross-section (ET0/Cs): the re-oxidation of reduced Q A via electron transport over a cross-section of active and inactive RCs.
- Electron transport in an active RC (ET0/RC): the re-oxidation of reduced QA via electron transport in an active RC. Only reflects the activity of active RCs.
- Maximal trapping rate of PS II (TR0/RC): the maximal rate by which an exciton is trapped by the RC resulting in the reduction of QA. This is a situation synonymous with measuring the trapping rate in the presence of DCMU-3-(3,4-dichlorophenyl)-1,1-dimethylurea.
- FV/FM: maximum quantum yield of PS II photochemistry.
- Pi: photosynthetic index.

5.3 RESULTS

5.3.1 Sugars contents and canopy growth

Among *C. olitorius*, canopy dried matter ranged from 10.45 % to 15.13% confirmed significant sugar variation (Figure 1 and table 2); this result could be linked to the different photosynthetic activity among ecotypes. The level of total sugars significantly ranged among *C. olitorius* ecotypes. Sugar values ranged from 4.2 mg g^{-1} Fw to 48 mg g^{-1} Fw (Fig. 1). Moreover, sucrose showed the same trend of total sugars among *C. olitorius* varieties, underlined differences in biological activity of carbon fixation and sugar transport inside leaves. However, the levels of sucrose ranged from 0.6 mg g^{-1} Fw to 2.1 mg g^{-1} Fw. The higher values of total sugars were found inside C2 leaves (48 mg g^{-1} Fw), while C6 and C11 had similar values: (32 mg g^{-1} Fw and 35 mg g^{-1} Fw) and also C1 (28 mg g^{-1} Fw) had higher values in comparison with C5 and C4 that had 26 and 22 mg g^{-1} Fw respectively. Lower values were detected in C7 and C10: 4.2 and 7.1 mg g^{-1} Fw respectively. Sucrose inside C. olitorius leaves was more clustered than total sugars. Higher levels were observed among C6, C2, C5 with values of 1.9, 2.1 and 1.6 mg g^{-1} Fw, respectively. Another group C4, C1, C11 had sucrose values of 1.4, 1.5 to 1.6 mg g-1 Fw respectively. C3 and C7 had similar sucrose contents: 1.2 and 1.0 mg g^{-1} Fw respectively. The lowest amount of sucrose inside *C. olitorius* leaves was found inside C10: 0.6 mg g^{-1} Fw.

Table 2. Accession leaves dried matter percentage

Samples	leaf DW%
C1	15.13
C2	10.92
C3	11.83
4	12.33
C5	10.78
C6	10.45
C7	11.78
C10	11.45
C11	12.23

Figure 1. Sugar contents among ecotypes. Total sugars, sucrose and reducing sugars contents of Corchorus olitorius (L.) plants. Given are means ± standard error (n=4). Different letters indicate statistical differences among different ecotypes.

43
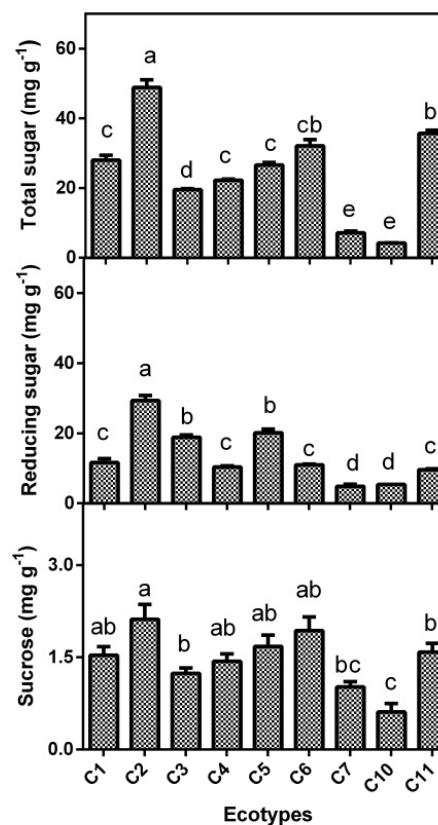
5.3.2 Chlorophyll content, leaf gas exchanges capacity, and PSII

Higher levels of chlorophyll a were found inside C6, C10 and C11. Values ranged from 1.4 mg g^{-1} Fw to 1.5 mg g^{-1} Fw. C1 and C4 showed intermediate chlorophyll content. Chlorophyll b did not show significant differences among ecotypes with values that ranged from 0.17 to 0.68 mg g^{-1}. Chl a inside C3, C5, C7 ecotypes had values ranged from 0.8, 0.9 and 0.7 mg g^{-1}. C4 had the second amount in Chla: 1.1 mg g^{-1} Fw; the lowest was C7 with an amount of 0.7 mg g^{-1} Fw. Chl b did not have significant differences. However, some variations of values were higher from C2 (0.17 mg g^{-1}) to C10 (0.68 mg g^{-1} Fw) of 75% difference. A positive correlation was found between Chl a and Chl b values and also between Chl a and carotenoids.

Figure 2.
Chlorophyll content among ecotypes. Chlorophyll a and b contents of Corchorus olitorius plants are expressed in µg/mg of fresh weight. Given are means ± standard error (n=4). Different letters indicate statistical differences among different ecotypes. Direct correlation among chlorophyll and carotenoids are reported.
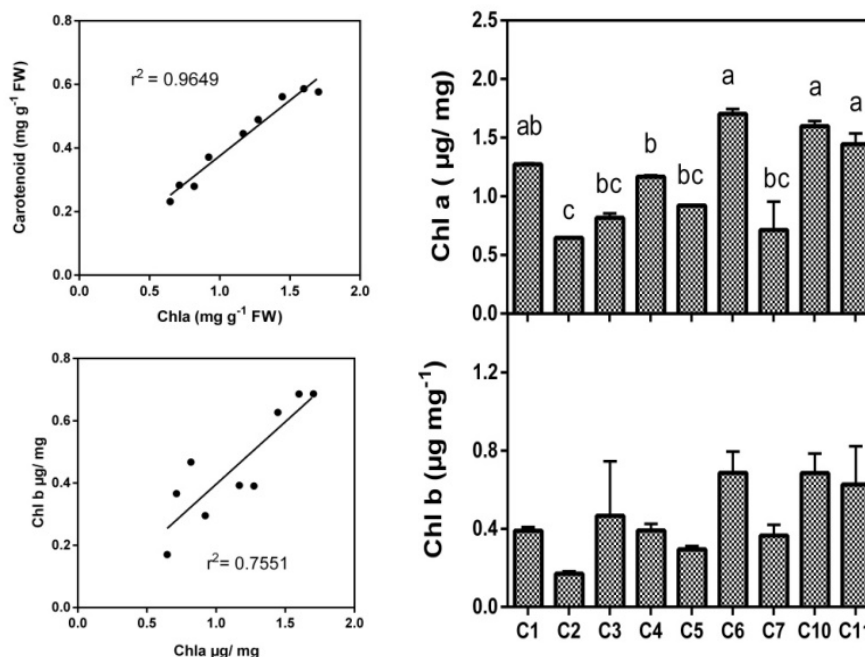
5.3.4 Leaf gas exchange capacity efficiency

Leaf gas exchange parameters, like stomatal conductance and transpiration, changed significantly among ecotypes during cultivation and summer time (Figure 3 D). However, stomatal conductance showed significant differences among accession mainly in June and July. Some statistical differences among ecotypes for the CO_2 intercellular concentrations were evaluated during summer period in June: C10 and C11 had lower levels of stomatal conductance. This aspect was linked to CO_2 assimilation that could change among ecotypes that could influence the photosynthetic rate (Pn) parameter. On the contrary, in June, C10 and C11 had significantly higher levels of photosynthesis. However, higher average levels of transpiration were found in ecotypes with lower levels of photosynthesis rate in June.

Leaf gas exchange parameters, like stomatal conductance and transpiration, changed significantly among ecotypes during cultivation and summer time (Figure 3 D). However, stomatal conductance showed significant differences among accession mainly in June and July. Some statistical differences among ecotypes for the CO2 intercellular concentrations were evaluated during summer period in June: C10 and C11 had lower levels of stomatal conductance. This aspect was linked to CO2 assimilation that could change among ecotypes that could influence the photosynthetic rate (Pn) parameter. On the contrary, in June, C10 and C11 had significantly higher levels of photosynthesis. However, higher average levels of transpiration were found in ecotypes with lower levels of photosynthesis rate in June.
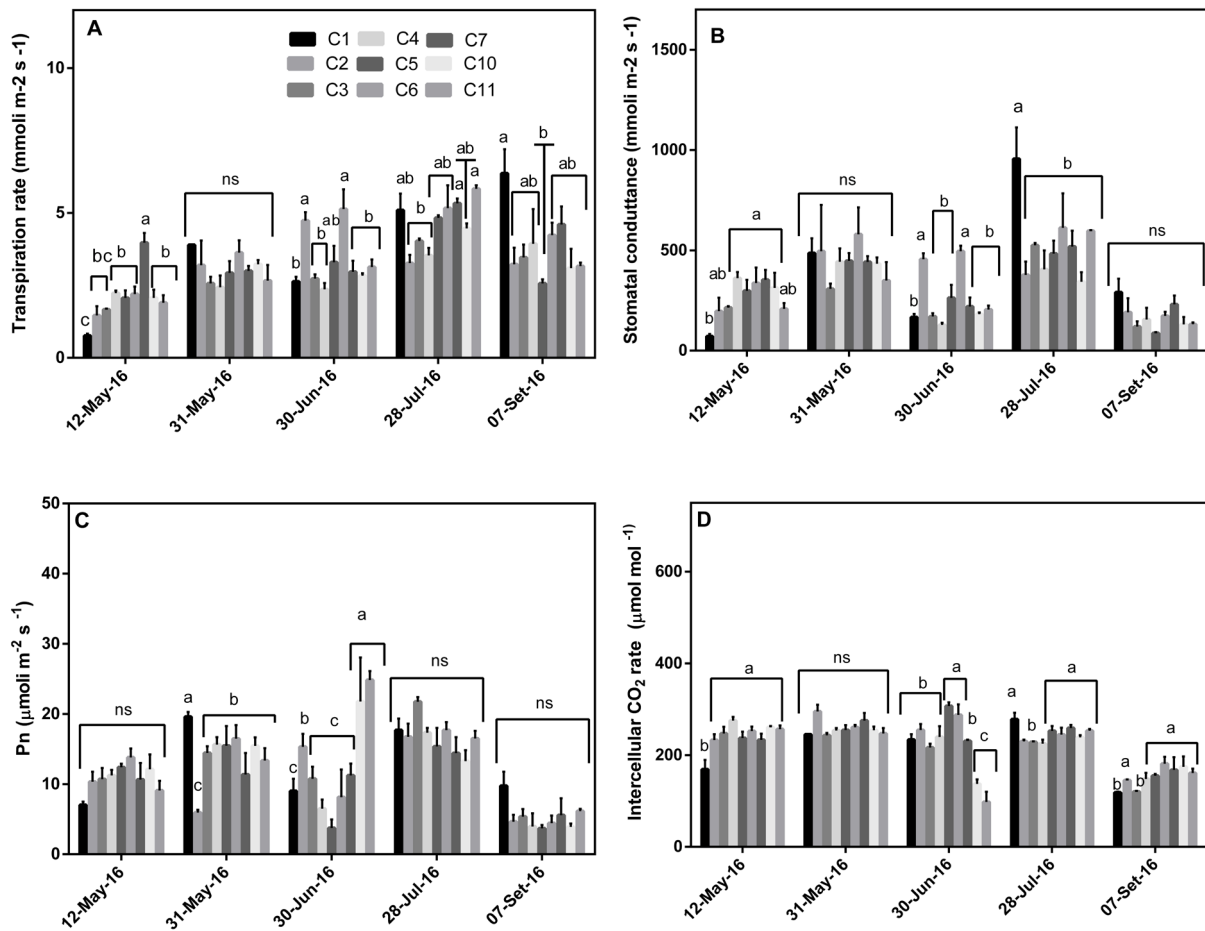

Figure 3. CIRAS: Gas exchanges analysis during the cultivation in different C. olitorius ecotypes. Values are means with standard errors (n=3). Data were subject to one-way ANOVA and differences among means were highlighted using Bonferroni's post-test. Different letters indicate statistical difference for p<0.05.

5.3.5 PSII efficiency

A few significant differences were found in relation to the efficiency of PSII among ecotypes and a general decreasing efficiency trend was observed during the cultivation cycle in summer time (Fig 4). The performance index (Pi) changed significantly among accession only during June. Fv/Fm ratio underlined a general sub stress condition among ecotypes with all levels that were below 0.8 (Fv/Fm). Performance index (PI) (Fig. 4C) decreased during summer with the higher values reached on September. DIo/RC, dissipated energy (Fig. 4F) increased with the increase of solar irradiation in summer (July), with significant differences among accessions.

Only on May 31st and June 30 th were significant differences evaluated in the C3 ecotype beside others. In addition to the performance index, the quantum yield of electron transport (ETo/CS) (Fig. 5A) also decreased in all ecotypes during summer period. A similar trend was found for the averange of antenna size and absorbed energy (ABS/RC) (Fig. 4E). In fact, on May 31st and June 30th significant differences were measuered among ecotypes linked to solar radiation during cultivation. On May 31st, the C3 accession showed an expected parameter, there was a lower gas exchange rate (Fig. 4 B) and there was a lower ET0/RC, TR0/RC, FV/FM, ABS/RC as well.
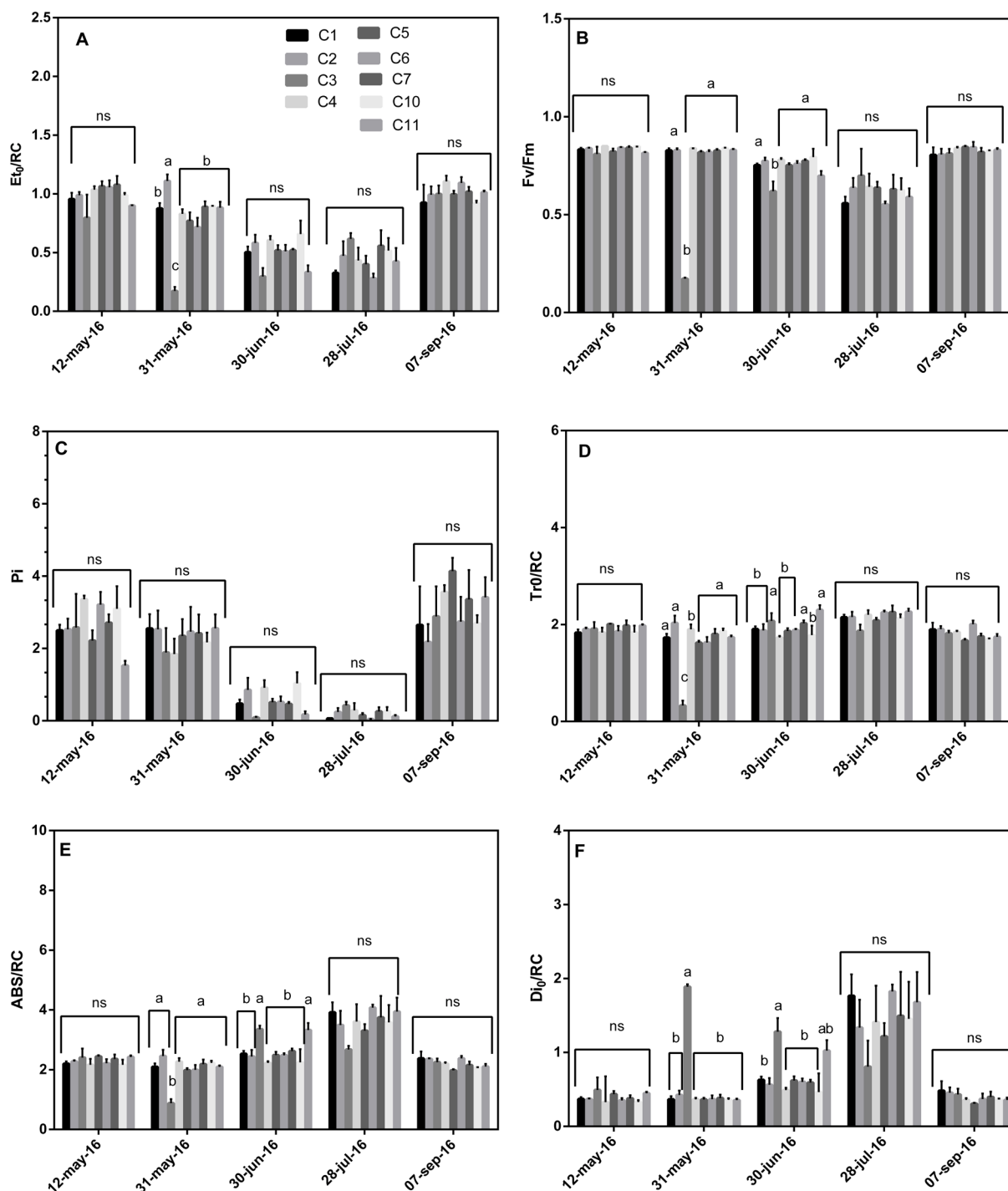
Figure 4. PEA (Advanced Continuous Excitation Chlorophyll Fluorimeter). Values are means with standard errors (n=8). Data were subject to one-way ANOVA and differences among means were highlighted using Bonferroni's post-test (p< 0.05). Different letters indicate statistical difference. A (Electron transport flux); B (Quantum efficiency); C (Photosynthetic index); D (Trapping); E (Absorption); F (Dissipated energy flux per reaction center).
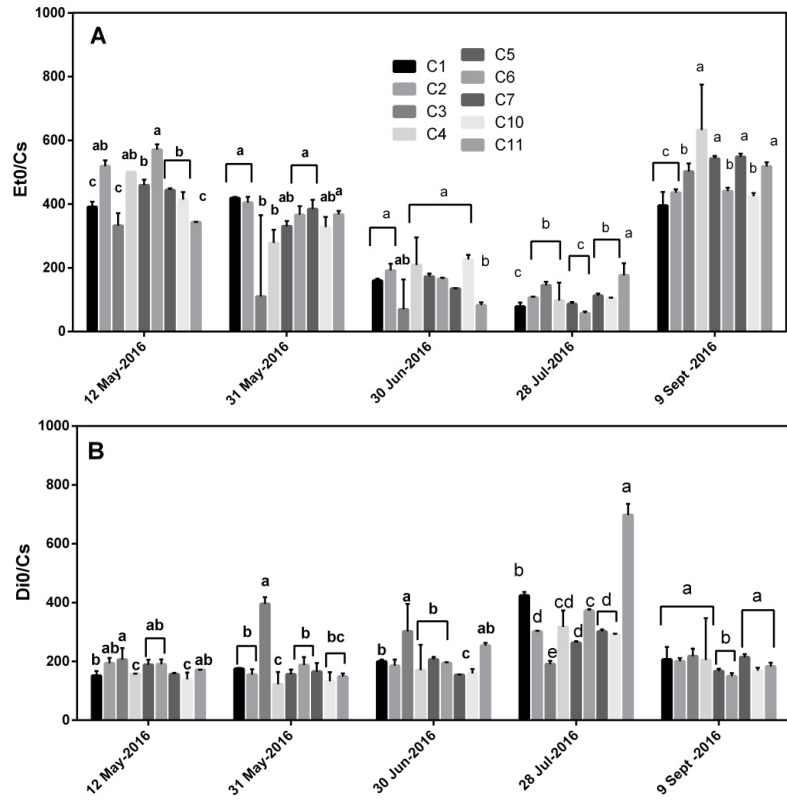
Figure 5. Quantum yield of electron transport (ETo/CS), Dissipation (DIo/CS). Standard error has been considered. Significant difference among values for all accessions at p< 0.05 is indicated by letters.

5.3.6 Fluorescence monitoring system

Electron transport flux measurements confirmed the previous analysis of Et0/RC with a decreasing trend during the summer period. However, electron transport flux increased during July 28th for C3, C7 and C10 compared to C1, C2 and C11. Moreover, quantic photosynthetic efficiency percentage showed the main differences among accessions during summer measurements of June 30th and July 28th (Fig.6), confirming data of performance index (Pi) that decreased during summer, as well.
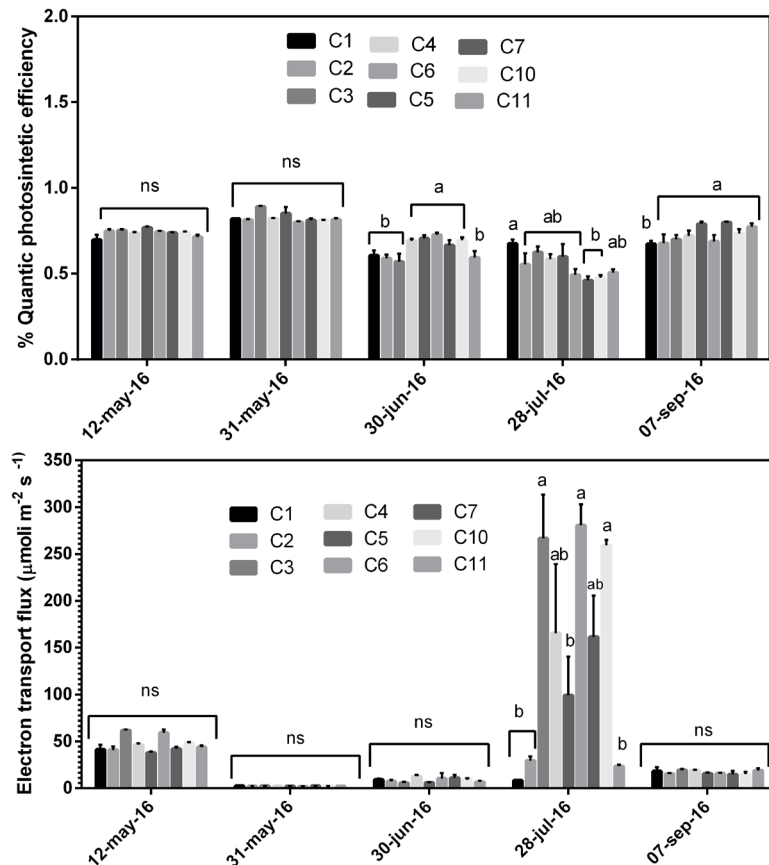

Figure 6. Fluorescence monitoring system. Standard error has been considered. Significant difference among values for all accession at p< 0.05 is indicated by letters.

47

5.3.7 Active compounds: Polyphenols, total anthocyanins and total carotenoids

Phenolic index (absorbance of methanol extract at ABS 320 nm) underlined significant differences among *C. olitorius* accessions' phenolic amount. The high values of polyphenols were measured in ecotype C1 and C11 (India and Japan) with absorbance values of 54.2 and 58.31 $ABS_{320\,nm}$ g^{-1} Fw. Lower values were measured for the varieties C2 and C3 (China and Libya) with values of 24.1 $ABS_{320\,nm}$ g^{-1} Fw and 20.1 $ABS_{320\,nm}$ g^{-1} respectively. Ecotypes C5, C6, C7, and C10 ranged from 31.3, 33.0, 32.5 to 28.1 $ABS_{320\,nm}$ g^{-1} Fw. Moreover, the same trends were analysed inside the phenolic content (GAE) inside leaves. The ecotypes, C1 and C11, showed higher amounts of phenols with values 5994 µg g^{-1} Fw and 6402 µg g^{-1} Fw. C4 had 4354 µg g^{-1} Fw. The C5, C6 and C7 range showed values of 3506, 3680, 3633 µg g^{-1} Fw, respectively. The C10 ecotype had an intermediate content similar to C5 groups 3214 µg g^{-1} Fw and the lower amount: 2779 and 2343 found in C2 and C3, respectively (Fig. 7).
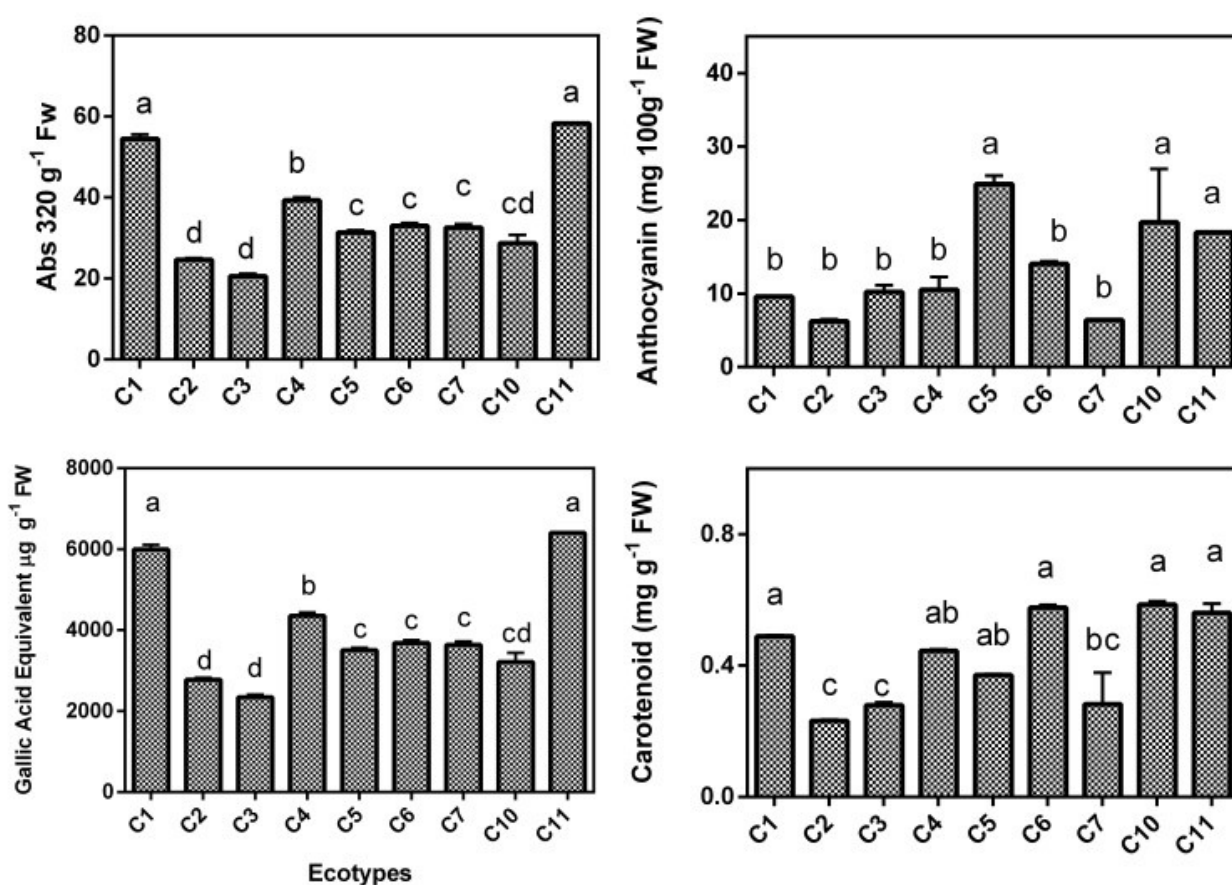

Figure 7. *Anthocyanins expressed as cynidin-3-glucoside, Phenolic index (Abs320) and total phenols expressed as gallic acid equivalent, carotenoid, all parameters are referred to fresh weight and were determined in leaves of C. olitorius accession. Values are means with standard errors (n=4). Data were subjected to one-way ANOVA analysis and differences among means were determined using Bonferroni's Post-test.*

Anthocyanin had variances among the ecotypes, however, anthocyanin contents were clustered in only two main groups. The high anthocyanin content was measured in C5, C10 and C11 with values ranged from 18.3 to 24.9 mg 100 g^{-1} Fw; other ecotypes C1,C3,C4,C7 and C6 ranged from 6.3 to 14.6 without significant differences among them. The lower levels were detected inside C2 leaves: 6.2 mg 100 g^{-1} Fw. Moreover, carotenoids and chlorophyll levels inside *C. olitorius L.* leaves changed significantly among ecotypes.

In fact, carotenoids amount inside *C. olitorius* leaves changed in correlation with chlorophyll according to their physiological action of supplementary pigments. However, significant differences in content were less underlined than chlorophyll among ecotypes. In fact, carotenoids level inside C1 leaves was 0.48 mg g^{-1} Fw similar to C6, C10 and C11. The higher values of carotenoids ranged from 0.58 to 0.56 mg g^{-1} Fw. In other ecotypes C2, C3, C4, C5 and C7, carotenoids content were 0.23, 0.27, 0.44, 0.37 and 0.28 mg g^{-1} Fw. The C2 ecotype showed lower amounts of carotenoids and anthocyanins.

5.3.8 Water use efficiency parameters

Water use efficiency showed differences during seasons among accessions as well as sugar accumulation in leaves (Figures 1-8). Significant variations among accessions occurred during the growing season, particularly during summer time. However, considering the mean of the entire period, there were no significant value differences for both measurements: photosynthetic water efficiency (p WUE) and intrinsic water use efficiency (i WUE), as shown in Supplementary Table One. However, total biomass per planta (aerial part + roots) showed some statistical differences among accessions. In particular, C4 had the higher biomass production during production cycle (suppl. Figure 1). Considering the general trend of *C. olitorius* ecotypes during the growing season, it was a clear progressive reduction on pWUE from May to September with a 76% water use efficiency reduction
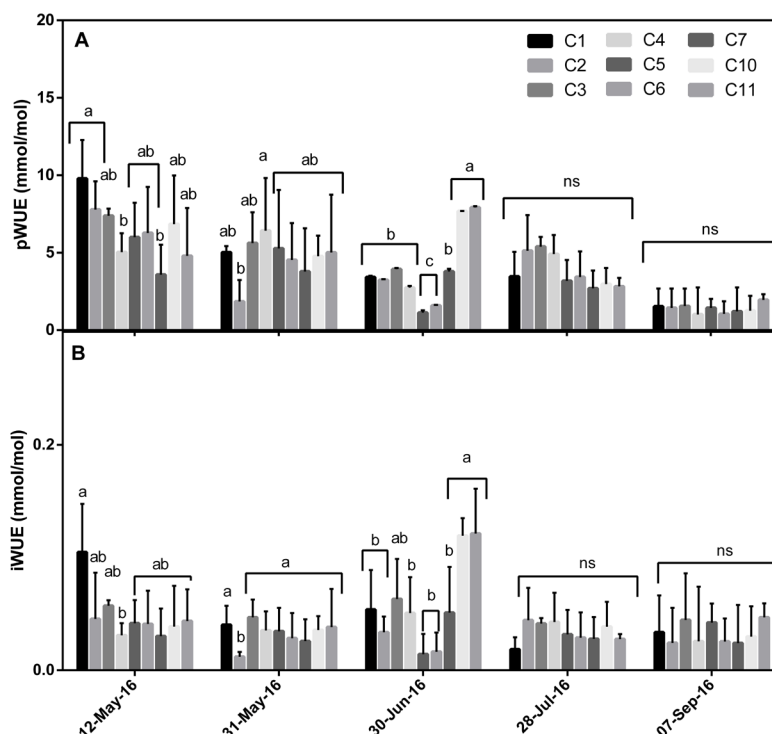
Figure 8. Accessions specific mean values of photosynthetic water use efficiency WUE (Pn/E) and intrinsic water use efficiency WUE (Pn/gs) in 2016. Standard error has been considered. Significant difference among values for all accessions at p< 0.05 is indicated by letters.

5.4 DISCUSSION

Ecotypes of *C. olitorius* show significantly different variations in several compounds like sugars, phenols, anthocyanins, chlorophyll and carotenoids. Ecotypes C1 and C11 have particularly interesting antioxidant compounds compared to the other accessions.

These antioxidant compounds changed in relation to photoperiod that influenced in response of eventually stresses linked to peculiar genetic traits. In particular, phenols are influenced by solar irradiation that increases the accumulation of these compounds in leaf tissues (Larsson *et al.*, 1986).

Chlorophyll are important leaf pigments involved in light use efficiency. The C6, C10, C11 and C1 ecotypes had statistically higher Chl a content. Chl a is considered an essential protein for photo-absorption in leaf thylacoid. However, plants have the ability to regulate their biosynthesis and action in order to reduce photo oxidation and light burn blast. Significant decreasing trends among some species between ABS/RC and chlorophyll levels are reported, like C1 during the season. Moreover, our analysis showed that content of Chl a and Chl b influenced ABS/RC ratio among varieties like those in C2. In fact, the content of pigments influenced the absorption capacity of the leaves. Moreover, energy absorption by thylacoid receptors also influenced carotenoid content. In fact, carotenoids are secondary compounds which are able to protect leaves against photoreception and dissipate expected energy. It is important to consider photosynthesis as a biochemical process, which is strongly correlated to plant plasticity and different environmental conditions.

Sugar contents ranged strongly among ecotypes of *C. olitrius*, probably linked to different metabolism and sugar translocation. Although this variation could be suggested by a correlation to respiration and photosynthesis, few physiological measurements underlined this correlation between sugars accumulation and ecotypes. In fact, inside the plant C1, C2, C3, C4 and C5 the level of sugars was higher than expected, considering measurements of carbon fixation rates, which were in line with other accessions like C11 or C10. *Corchorus* species are C3 plants, which means that they lose about 30%-50% of their photosynthetic products during photorespiration, as suggested by sub optimal Fv/Fm ratio (Figure 4). It's likely that C1, C2, C3, C4 and C5 plants had a lower photorespiration rate that can increase triose sugar deposits in leaves. In fact, triose sugars could be stored in chloroplast. This data could be supported by the differences among total sugars amount in leaves *C. olitorius* among accessions. Sucrose, for example, does not have, in general, high significant variations among ecotypes. However, sucrose is significantly higher inside ecotypes with lower respiration rates like C2. This aspect can be explained by a faster metabolism which consequently transports triose sugars in the form of sucrose to be exported in vascular tissues tissues as suggested by the yield of dried matter (Kaiser and Heber 1984; Ward *et al.*, 1997).

Physiological activity of photosystems II is a key factor in the leaf during season. High solar irradiation causes biochemical changes in photosynthesis patterns that could inhibit electron transport flux (Et0/RC), causing reduction of carbon fixation by the saturation of the system (Figure 4-5). Moreover, stomatal conductance is an interesting parameter with which to understand water potential per photosynthetic rates (Pn) to estimate water use efficiency.

Generally, during photo inhibition, DI0/CS increases and Et0/CS decreases, as was the case during summer measurements (Figure 7). C3 accession previously showed an increase of ET0/RC compared to the others. A similar trend is suggested by the C3, C7 and C10 accession that increased electron transport flux July 28th and is linked to the reduction of quantic efficiency. This is probably a consequence of the dissipation of energy (DI0/RC), as suggested by C10 accession, which had a higher content level of carotenoids.

These stresses could influence photorespiration, while photosynthetic stresses and dissipation, particularly during the summer, can produced O2 which increases photorespiration and the consumption of sugars. In our case, there are significant differences in the photosynthetic quantic efficiency status of photosystem II (Figure 8.) among accessions on summer. Taking into consideration DI0/Cs and Et0/Cs differences among accessions, sugars leaf levels could suggest different sugar store behaviour and the use of primer amid. Although sucrose and reducing sugars showed different amounts inside *C. olitorius* L. accessions, both parameters were not influenced by photosynthetic rate because sucrose is a transport sugar and reducing sugars are metabolic substrata in cytosol. Moreover, variations of gas exchange rate suggested that there is a relation between photosynthetic rate and stomatal conductance. However, all plants which had higher mean values also had a high respiration rate parameter. This combination of factors generated a higher use of sugars in leaves tissue, probably causing respiration, as suggested in C7 and C10 leaves. Data suggested that plants C10, C11, C7 and C6 had high carbon fixation but generally less efficient use of sugars compared to the C1, C2, C3 and C4 metabolism. Moreover, Figure 7 suggested that ecotypes coming from Libya had less photo-inhibition response during the summer period, showing a good adaption to high temperature. However, at the same time, during the summer period (June-July), C4 (Libya) water efficiency uses decreased dramatically compared to the other ecotypes. This aspect could be explained as a form of adaptation to North African temperatures, thanks to a higher water transpiration rate. In fact, during June and July, measurement C4 photosynthetic quantic efficiency was not influenced by the high temperature. Moreover, C4 turned out to be one of the better ones, considering photosynthetic quantic efficiency (Figures 5-8).

Moreover, the increase of temperature could produce a vapour pressure deficit (VPD) that had a negative direct effect on WUE (Kamiski *et al.*, 2015). In fact, VPD influenced leaf transpiration with higher values of transpiration rate. Intrinsic water use efficiency value had a lower decreased compared to pWUE during the test with a 26% decrease in mean from May 12th to September 7th. According to the mean values of pWUE of many crops like potatoes and cereals, C. olitorius had interesting values like potatoes in many case like C1 or C3 (Kamiski *et al.*, 2014). Moreover, plant biomass was generally allocated to the upper part of the plant and not to the roots. In general, C3 like wheat allocated more than 30% of biomass to roots. However, C. olitorius accessions had lower roots biomass percentage with values ranged from 10 to 20% that are similar to the C4 plant (Kalapos *et al.*, 1996).

Water limitation and water use efficiency will be a major issue in the future of agriculture, due to the increase of global warming. The increase of temperature will raise the water vapour concentration difference between leaf and air, although relative humidity in the atmosphere is foreseen to be constant, globally (Willet *at al.*, 2007). Accessions C10 and C11 showed high intrinsic WUE at high temperatures.

Unfortunately, all accessions had values of less than 1% of photosynthetic quantic efficiency during the entire growing cycle. Generally, typical crop plants performances ranged between 1-2% of photosynthetic efficiency. Despite electron transport flux mean values ranged under 50 $\mu mol\ m^{-2}\ s^{-1}$ in all data collected in the July measurement of North Africa accessions, C3, C4, C5, C6, C7, and C10 have mean values ranged from 100 $\mu mol\ m^{-2}\ s^{-1}$ to 300 $\mu mol\ m^{-2}\ s^{-1}$. Considering the high values of temperature and light during July in Milano, the increment of electro transport flux could be an adapting response to stress linked to the native North African origin of these plants. However, this increase of electron transport flux does not generate higher photosynthetic rate, while it could be a response of energy dissipation (Ruban and Horton 1995).

5.5 CONCLUSION

To understand the difference in growing response, it is important to find the best ecotype that is adapted to European environmental conditions in order to introduce the highest performing and most valuable plant. Results show that varieties C1 and C11 had high values in antioxidant compounds and sugars. Photosynthetic rate results underline an interesting light use efficiency of C1 ecotype in comparison with C11, although a difference in iWUE has been reported. According to our results, further investigations about the ecology of African accessions should be done, considering the good production level of C4 ecotype thanks to the response to the high temperature climate.

Suppl. table 1. Accessions specific mean values of photosynthetic WUE (Pn/E) and intrinsic WUE (Pn/gs) in 2016. Biomass expressed in Kg of dried matter. Standard error has been considered. Significant difference among values for all accessions at p< 0.05 is indicated by letters.

Accessories	pWUE				i WUE				Total biomass			
	Mean	Error	N	Differences	Mean	Error	N	Differences	Mean	Error	N	Differences
C1	4.52	1.29	25	ns	0.049	0.012	25	ns	0.155	0.081	3	b
C2	3.74	1.05	25	ns	0.033	0.006	25	ns	0.136	0.025	3	c
C3	4.58	0.86	25	ns	0.049	0.003	25	ns	0.127	ns	3	c
C4	4.02	0.96	25	ns	0.037	0.004	25	ns	0.208	0.028	3	a
C5	3.40	0.98	25	ns	0.033	0.005	25	ns	0.141	0.015	3	b
C6	3.37	0.96	25	ns	0.028	0.004	25	ns	0.141	0.030	3	b
C7	2.83	0.47	25	ns	0.032	0.004	25	ns	0.157	0.057	3	b
C10	4.51	1.12	25	ns	0.052	0.015	25	ns	0.142	0.006	3	b
C11	4.50	1.03	25	ns	0.056	0.015	25	ns	0.106	0.010	3	c
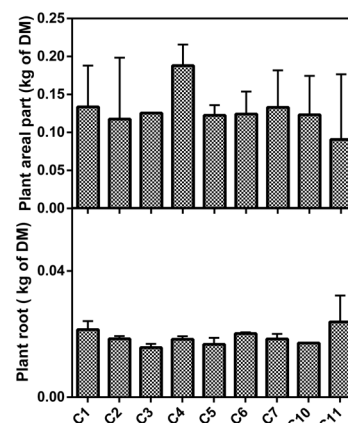
Suppl. Figure 3. Seasonal production of Corchorus olitorius (L.) dried weight per plant.

CHAPTER VI:
Ethno-pharmacology possible role of several secondary compounds of *C. olitorius* detection and quantification

6.1 INTRODUCTION

C. olitorius is a traditional leafy vegetable that is cultivated and consumed among different tropical areas in Africa and Asia. It has putative anti-cancer properties in traditional medicine (Taiwo *et al.*, 2016). Moreover, the leaf extract is rich in polyphenols and shows interesting activity against several pathogenic gut microbes (Zakaria *et al.*, 2006; Nwakaeze et al., 2014). Polyphenols are considered the primary active compounds inside C. olitorius (Das *et al.*, 2009; Oboh *et al.*, 2012¸ Acho *et al.*, 2014¸ Morsy *et al.*, 2015). However, terpene could also have important biological properties. Moreover, several specific compounds inside its tissue shows in vitro and in vivo activity against cancer (Yan *et al.*, 2013). According to the literature *C. olitorius*, extracts show a high capacity to reduce pro-inflammatory stress compounds like oxidant NO, H_2O_2, O^{-} (Yoshikawa *et al.*, 1998; Dewanjee *et al.*, 2013; Islam *et al.*, 2013).

The beneficial properties of *C. olitorius*, such as its, antioxidant activities and inflammatory actions, could be related to a specific class of compounds like terpenes or flavonoids (Talhouk *et al.*, 2007). Further investigation into the chemical composition of C. olitorius, by screening its chemical composition using proper HPLC-MS and GS-MS, could be an important step for finding new pharmaceutical compounds and for understanding the basis of its traditional uses in medicine.

6.2 MATERIALS AND METHODS

One g of leaves of lyophilized powder of *C. olitorius* (Chinese accession from IPK seed bank) was dissolved in a 10 ml of MeOH and stirred for 24 h in a cold room of 4°C. The extract and the solution was centrifuged and concentrated at 2,147g and 45 °C until all of the volatile solvents were evaporated in a Centrivap concentrator connected to a cold trap (Labconco, Kansas City, MO). Chloroform was added to the concentrated mixture of 1 gram of leaves of lyophilized powder, and this solvent fraction was used to analyse the profiling of the compounds present in the extract by GS/MS. The crude MeOH extract was fractionated in four fractions: phenolic acids (F1), anthocyanins (F2), flavonols (F3) and procyanidins/polymeric anthocyanins (F4) by solid-phase extraction using C18 cartridges as previously described18 (Figure 1) by Noratto et al., fractionation procedure reported later.

The aqueous extract was adjusted to pH 7.0 with 5 N NaOH. A total of 1 mL of Jute leaves extract was loaded in SEP Pack C18 cartridge (55–105 µm, Waters Corp., Milford, MA, USA) previously conditioned to pH 7.0 with 20 mL of 100% methanol and 50 mL of nano-pure water (pH 7.0). The neutral phenolic compounds were absorbed in the cartridge, whereas the phenolic acids were not. The cartridge was washed with 20 mL of water (pH 7.0). The water from the wash contained phenolic compounds that were not adsorbed in the cartridge. This fraction was adjusted to pH 2.0. This mixture of compounds was loaded into a second cartridge previously conditioned at pH 2.0 with 20 mL of 100% methanol and secondarily washed by 20 mL of nano-pure water at pH 2.0. Phenolic acids bound to the matrix of the second cartridge (F1) were later eluted with 50 mL of 100% methanol. Next, the first cartridge was adjusted to pH 2.0 and the elution of anthocyanins was accomplished by passing 20 mL of 16% acetonitrile solution at pH 2.0 (F2). Next, flavonoids were eluted form the first cartridge using 20 mL of 100% ethyl acetate (F3). The last elution was performed using 20 mL of 100% methanol for the anthocyanin polymers (F4).

Fractions were concentrated with low boiling point solvents (F1, F3, and F4) using Rotavapor. After these procedures, all the fractions were completely evaporated at 45 °C using a Centrivap (Labconco, Kansas City, MO). *C. olitorius* fractions were analysed by HPLC-MS. Identification of the phenolic profile was performed at 200–600 nm by a comparison of retention times and the MS/MS.
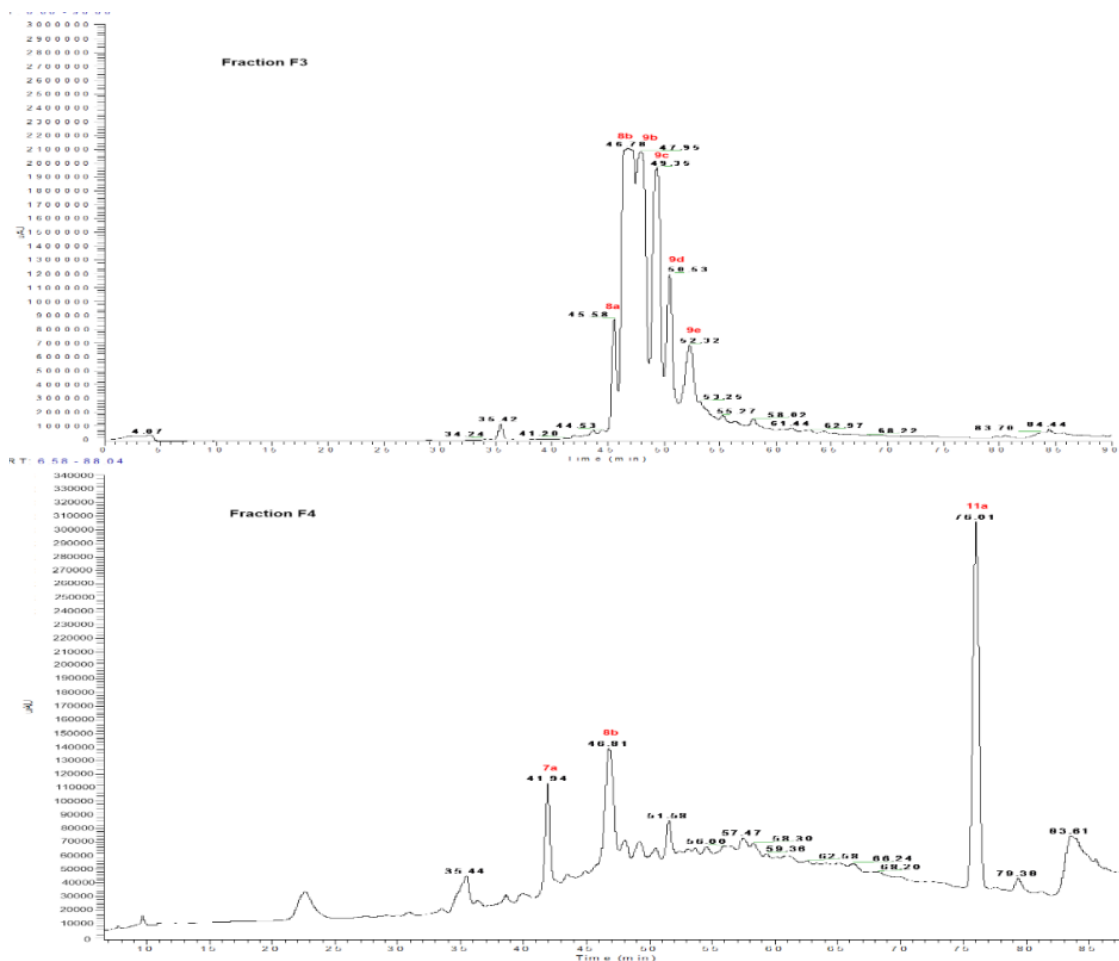
6.3 RESULTS
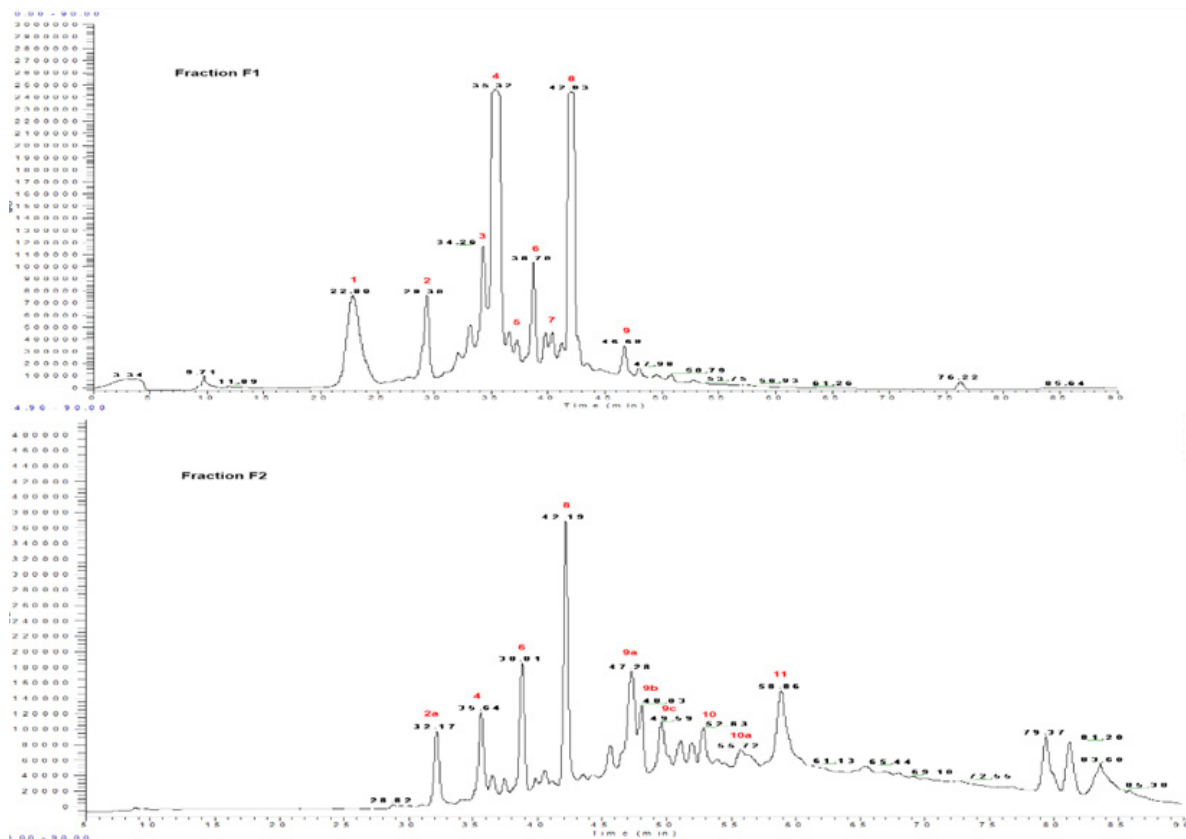
Figure 1. C. olitorius phenolic crude extract HPLC of fractions

Table 1. Identification of chemical components in fraction F1.

Peak No	R.T	UV	[M-H]-	[M+H]+	MS fragments	Identification
	Identification					
1	28.2	240, 300sh, 328	353	355	191, 179, 173, 135	1-caffeoylquinic acid
2	29.3	240, 300sh, 328	353	355	191, 179, 173, 135	3-caffeoylquinic acid
3	34.2	240, 300sh, 328	353	355	191, 179, 173, 135	4-caffeoylquinic acid
4	35.3	240, 300sh, 328	353	355	191, 179	chlorogenic acid
5	36.5	240, 324	367	369	193, 191, 149	5-feruloylquinic acidb
6	38.7	240, 300sh, 328	631	-	353, 191, 179	caffeoylquinic acid glycoside
7	41.2	240, 300sh, 328	515	-	353, 191, 179, 135	3,4-dicaffeoylquinic acid
8	42.0	240, 300sh, 328	645	-	601, 397, 223, 191, 179	3-sinapoyl-5-caffe-oyl-1-methoxyoxaloyl-quinic acid
9	38.7	240, 300sh, 328	559	-	397, 223, 191, 179	3-sinapoyl-5-caffeoylqui-nic acid

Table 2. Identification of chemical components in fraction F2.

| Peak No	R.T	UV	[M-H]-	[M+H]+	MS fragments	Identification
	Identification					
2b	32.2	240, 300sh, 328	-	449	287	Cyanidin-3-glucoside
4	35.3	240, 300sh, 328	353	355	191, 179, 173, 135	3-caffeoylquinic acid
6	38.7	240, 300sh, 328	631	-	353, 191, 179	caffeoylquinic acid glycoside
8	42.0	240, 300sh, 328	645	-	601, 397, 223, 191, 179	3-sinapoyl-5-caffe-oyl-1-methoxyoxaloyl-quinic acid
9a	47.3	255, 295	339	-	161, 145	Chicoriine
9b	48.03	255, 290	353	-	191, 145	Scopolin-7-O-glucoside
9c	49.6	254	177	-	145	4,7 dihydroxy coumarin
10	52.8	254, 280	543	-	353,191, 179	3-O-Dimethoxycinna-moyl-4-O-caffeoylquinic acid
10a	55.7	254, 290	513	-	353, 191, 179	3-O-p-Coumaroyl-4-O-feruloylquinic acid
11	58.9	254, 295	573	-	223,191, 179	3-O-Sinapoyl-5-O-feruloylquinic acid

Table 3. Identification of chemical components in fraction F3.

Peak No	R.T	UV	[M-H]-	[M+H]+	MS fragments	Identification
	Identification					
8a	45.5	255, 360	463	465	301	Hyperoside
8b	46.8	255, 360	463	465	301	Isoquercitrin
9b	47.9	255, 365	447	449	285	kaempferol-3-O-β-D-glucopyranoside
9c	49.4	255, 365	447	449	285	kaempferol-3-O-β-D-galactopyranoside
9d	50.5	360	549	551	463, 301	Quercetin 3-O-(6''-O-malonyl)-β-D-glucoside
9e	52.3	360	549	551	463, 301	Quercetin 3-O-(6''-O-malonyl)-β-D-galactoside

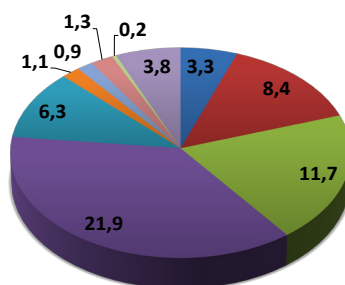*Table 4. Identification of chemical components in fraction F4.*

| Peak No	R.T	UV	[M-H]-	[M+H]+	MS fragments	Identification
	Identification					
7a	41.9	255, 360	315	317	301, 191, 175	isorhamnetin
8b	46.8	255, 360	463	465	301	Isoquercitrin
11a	76.0	235, 265	145	-	117, 89	coumarin
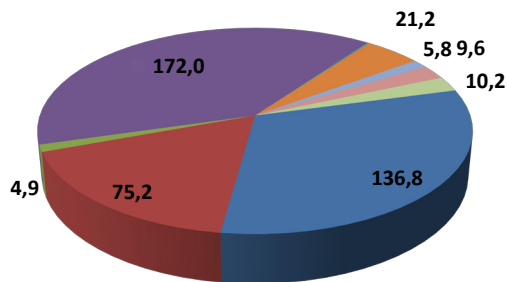
Fraction 1: compound content expressed in mg 100 g-1 of leaf

- 1-caffeoylquinic acid
- 3-caffeoylquinic acid
- 4-caffeoylquinic acid
- chlorogenic acid
- 5-feruloylquinic acid
- caffeoylquinic acid glycoside
- 3,4-dicaffeoylquinic acid
- 3-sinapoyl-5-caffeoyl-1-methoxyoxaloylquinic acid
- 3-sinapoyl-5-caffeoylquinic acid

Fraction 2: compound content expressed in mg 100 g-1 of leaf

- Cyanidin-3-glucoside
- 3-caffeoylquinic acid
- caffeoylquinic acid glycoside
- 3-sinapoyl-5-caffeoyl-1-methoxyoxaloylquinic acid
- Chicoriine
- Scopolin-7-O-glucoside
- 4,7 dihydroxy coumarin
- 3-O-Dimethoxycinnamoyl-4-O-caffeoylquinic
- 3-O-p-Coumaroyl-4-O-feruloylquinic
- 3-O-p-Coumaroyl-4-O-feruloylquinic
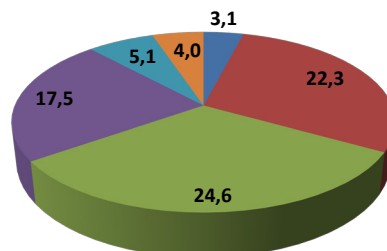
Fraction 3: compound content expressed in mg 100 g-1 of leaf

- Hyperoside
- Isoquercitrin
- kaempferol-3-O-β-D-glucopyranoside
- kaempferol-3-O-β-D-galactopyranoside
- Quercetin 3-O-(6"-O-malonyl)-β-D-glucoside
- Quercetin 3-O-(6"-O-malonyl)-β-D-galactoside
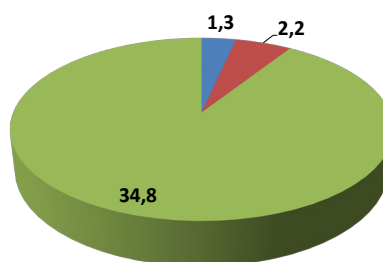
Fraction 4: compound content expressed in mg 100 g-1 of leaf

- isorhamnetin
- Isoquercitrin
- coumarin

Different fractions were analysed with different standards in order to evaluate the concentration inside *C. olitorius* L. leaves. The standards used for the quantification were quercetin, chlorogenic acid, coumarin and cyanidin in order to cover all the chemical species found inside the leaves. Inside the first fraction, the prevalent compounds found were chlorogenic acid, 1.72 mg g^{-1} and 1-caffeolyquinic acid, 1.36 mg g^{-1}. The third most abundant compound present was 3-caffeolyquinic acid, 0.75 mg g^{-1}. Other chlorogenic acid derivatives were detected and measured as caffeoylquinic acid glycoside, 0.21 mg g^{-1}, 3-sinapoyl-5-caffeoylquinic acid 0.10 mg g^{-1}, 3-sinapoyl-5-caffeoyl-1-methoxyoxaloylquinic acid, 0.096 mg g^{-1}, 3,4-dicaffeoylquinic acid, 0.04 mg g^{-1}, 4-caffeoylquinic acid, 0.049 mg g^{-1}, 3,4-dicaffeoylquinic acid, 0.058 mg g^{-1} and 5-feruloylquinic acid 0.0072. The second fraction is mainly composed by flavonoids compounds. However, several chlorogenic acids derivatives and coumarin were present in this fraction.

Moreover, these derivatives were the major constituents of the second fraction: 3-sinapoyl-5-caffeoyl-1-methoxyoxaloylquinic acid, 0.21 mg g^{-1} and caffeoylquinic acid glycoside 0.11 mg g^{-1} and 3-caffeoylquinic acid, 0.84 mg g^{-1}. Other compounds were found as reported in Table 2 in trace: cichoriine, 0.063 mg g^{-1}, cyanidin-3-glucoside, 0.033 mg g^{-1}, 3-O-p-Coumaroyl-4-O-feruloylquinic, 0.038 mg g^{-1}, Scopolin-7-O-glucoside, 0.01 mg g^{-1}, 4,7 dihydroxy coumarin, 0.009 mg g^{-1}, 3-O-p-Coumaroyl-4-O-feruloylquinic, 0.002 mg g^{-1}. Inside the third fraction, several quercetin and kaempferol derivatives were detected: kaempferol-3-O-β-D-glucopyranoside, 0.24 mg g^{-1}, isoquercitrin, 0.22 mg g^{-1}, kaempferol-3-O-β-D-galactopyranoside, 0.17 mg g^{-1}, quercetin 3-O-(6"-O-malonyl)-β-D-glucoside, 0.05 mg g^{-1}, quercetin 3-O-(6"-O-malonyl)-β-D-galactoside, 0.04 mg g^{-1} , hyperoside, 0.03 mg g^{-1}. The fourth fraction is mainly composed by coumarin. However, two flavonoids were detected from the previous fraction (fraction number 3) and some flavonoids were detected in this fraction: coumarin, 0.34 mg g^{-1}, Isoquercitrin. 0.02 mg g^{-1}, isorhamnetin, 0.01 mg g^{-1}.
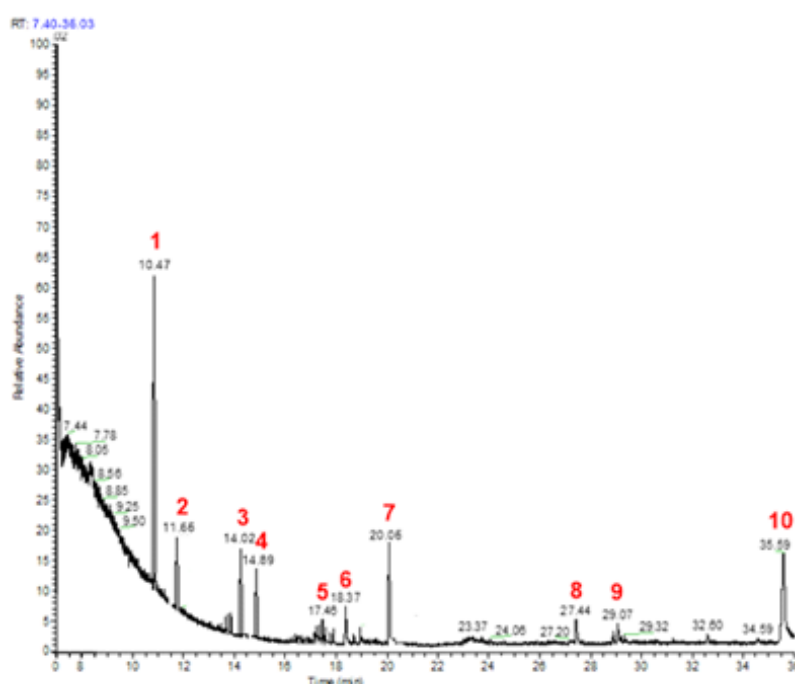

Figure 2: GC-MS of C. olitorius chloroform crude extract

Table 5. Identification of Chloroform extract of C. olitorius leaves.

Peak No	Retention time	Molecular weight	Identification	Type of compound
1	10.47	296	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol
2	11.65	172	4,8 dimethyl, 1-Nonanol	alcohol
3	14.02	214	2-hexyl, 1-octanol	alcohol
4	14.89	490	12-O-acetylingol 8-tiglate	Terpene alcohol
5	17.46	220	Butylated hydroxyl toluene	Aromatic alcohol
6	18.37	298	Eicosanol	alcohol
7	20.06	388	7,12 dioxo, 5α cholan-24-oic acid	diterpene
8	27.44	224	2,3,3 trimethyl-2-(4-methyl-pentanoyl)-cyclopentanone	ketone
9	29.07	410	squalene	triterpene
10	35.59	430	Vitamin E	vitamin
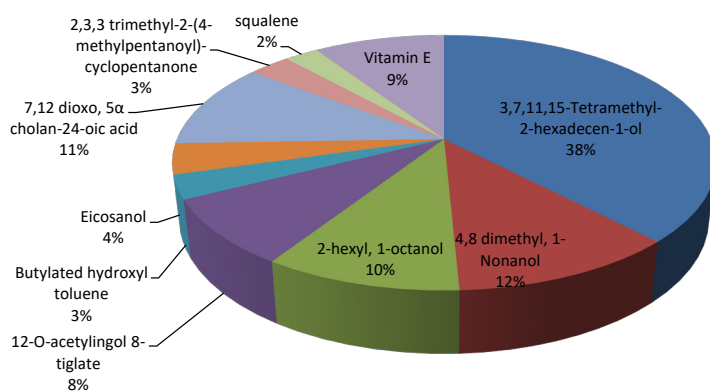
Figure 3. Quantification of identify of C. olitorius chloroform extract

The yield of *C. olitorius* for organic fraction was of 0.250 g, using the extraction process explained above in the Material and Methods section. The individual identified components, with their relative percentages, are reported in Table 5. The analysis revealed 10 compounds mainly consisting in hydrocarbon: terpene, alcohol ketone. The major fraction compounds were found to be 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (38%), the other ones were found in lower percentages: 4,8, dimethyl, 1-Nonanol (12%), 7,12 dioxo,5α cholan-24-oic acid (11%), 2-hexyl, 1-octanol (10%), vitamin E (9%), 12-O-acetylingol 8-tiglate (8%), Eicosanol (4%), 2,3,3 trimethyl-2-(4methylpentanoyl)-cyclopentanone (3%), Butylated hydroxyl toluene (3%), squalene (2%).

6.4 DISCUSSION

Corchorus olitorius is used in traditional medicine for treating many different diseases, as previous literature has reported (Wang et al., 2011). Detecting, chemical composition and quantification are required to link a putative compound to ethnical uses. Terpenes and phenolic acids were the main constituents of C. olitorius antioxidant properties. Chlorogenic acid and its derivatives were found abundance, more so than in other common leafy vegetables like lettuce.

Considering antioxidant properties, flavonoids and anthocyanins inside leaves have to be considered as active compounds which are able to influence human metabolism and its probiotic and oxidant scavengers in the human gut, promoting human health. It is interesting to see that inside the second fraction, flavones have been the main chemical constituents; only one anthocyanin has been detected. Flavones like quercetin and kaempferol are generally higher in *C. olitorius* than European leafy vegetables product commonly used in "ready-to-eat salad". Kaempherol, for example, are present in lettuce at an average of 1-28 mg kg^{-1} Fw according to varieties like red radish 4 mg and kale, 30 mg kg^{-1} Fw (Bilyk, and Sapers, 1985).

Moreover, coumarin has been detected in abundance in leaves of *C. olitorius*. Coumarin is regarded to be the drug used in treatments for vascular affection due to their anticoagulant activity (Oboh *et al.*, 2012). However, coumarin could be considered as an anti-nutritional factor according to the interaction with vitamin K absorption (Udall 1965). The interaction between coumarin and the human body is important considering the consumption per die and the possible amplification of some drug actions, like those of Coumadin (Izzo *et al.*, 2005).

Active terpenes has been detected in leaves, and the main constituent of this fraction has a particularly important biological function as antimicrobial compounds. This aspect can link chemical composition with all the bibliography written in which the extract of *C. olitorius* leaves demonstrated antimicrobial activity, as in Zakaria *et al.*, 2006. In particular, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and 4,8 dimethyl, 1-Nonanol are the main constituents of a terpene extract of *Corchorus* with 40%. These compounds are the main candidates which can justify the antimicrobial properties of *C. olitorius* extract and the use of this vegetable as food for treating microbial gut diseases as diarrhoea. In fact, these two compounds in vitro had the properties to control developing bacteria. In addition, butylated hydroxyl toluene, which is only 3% of the fraction, also has an antimicrobial in vitro, underlining the putative attitude regarding the use of *C. olitorius* terpene fraction as a natural microbial agent.

From a dietary point of view, our study confirmed the presence of the important antioxidant compound vitamin E, which is an important antioxidant compound. However, our results showed higher content than reported in literature, depending on the procedure used 1.134 g on 100 g DW. Further, *C. olitorius* has higher 310 mg kg^{-1} Dw of quercetin compared to other vegetables like broccoli 60 mg kg^{-1} Dw or red spinach 29.5 mg kg^{-1} Dw. It is much like other African leafy vegetables, like *Moringa oleifera* (232.5 mg kg^{-1} Dw), with regard to its importance for human nutrition. Also, the *Brassica* family has less quercetin, such as black cabbage 0.4 mg kg^{-1} or red cabbage 4 mg kg^{-1} Fw (Bilyk, and Sapers, 1985). The main constituent of phenolic acid inside *C. olitorius* leaves is chlorogenic acid. It is considered an important antioxidant which is common in the plant. *C. olitorius* has 1.72 g kg^{-1} of chlorogenic acid in dried leaves. Compared to tomato (300 mg kg^{-1} Dw), *C. olitorius* has 4 time more chlorogenic acid

inside the edible part. However, is important to note that green coffee, for example, has sixty time more content: 65 g kg^{-1} Dw. This trend is confirmed by reaching 3.84 g kg-1 of dried leaves, if we considered all fractions formed by caffeic acid derivatives. Anthocyanin in *C. olitorius* leaves are present at an interesting level (33 mg kg^{-1} Dw) compared to other leafy vegetables like lettuce 24.9 mg kg^{-1} Fw (Cartea *et al.*, 2010).

6.5 CONCLUSION

Quantification of phenol compounds and terpenes confirmed the presence of a high content of antioxidant and active compounds. All these compounds help human health by stimulating and promoting gut microbial and reducing oxidant toxicity in cells. *C. olitorius* has a potential future as a nutraceutical plant for human nutrition.

CHAPTER VII: CONCLUSION

Jute is an important commercial crop. It is the second source of natural fibre after cotton. It is 100% biodegradable and recyclable. Moreover, its leaves have an important use as leafy vegetables in Africa and Asia. It is also present in ethnic medicine which aims to treat several diseases such as inflammation, fever and vascular diseases. For all these reasons, the interest around this plant is currently increasing. Western and developing countries have the same interest to develop a strategy to increase production and improve knowledge about the pharmacological use of jute leaves that could be collected from the waste of the fibre market. Moreover, improving cultivation methods and the selection of varieties can also influence food varieties. This last aspect could prove essential for improving the research on ethnic vegetables such as *C. olitorius* in Africa in order to improve food security in many communities. *C. olitorius*, in fact, is also used as a natural food supplier for woman during pregnancy and child-rearing. This research worked to explore this issue in order to improve cultivation methods and post-harvest treatments, as well as cover knowledge about the leaves' chemical composition. Understanding the chemical composition of leaves and plant physiology, as shown in chapters 5-6, could be an interesting step forward in order to link the ethno-pharmacological use of leaves to the real active compounds inside them, in addition to understanding how we can activate them using physiological stress.

The complexity of these goals requires more studies over the next few years, although an important path has been mapped by previous literature. It is important to consider that only a small percentage of plant compounds have been investigated. The majority of biodiversity is found in developing countries. Such is the case of *C. olitorius*, which has the highest biodiversity in West Africa. This aspect creates a great opportunity to improve the collaboration between different areas of the world in the name of respect of natural resources, biodiversity and common health. The responsible use ethnobotanical knowledge could characterize the new, ethical business of the future.

CHAPTER VIII: REFERENCES

ACHIGAN-DAKO, E. G., FUCHS, J., AHANCHEDE, A., & BLATTNER, F. R. (2008). FLOW CYTOMETRIC ANALYSIS IN LAGENARIA SICERARIA (CUCURBITACEAE) INDICATES CORRELATION OF GENOME SIZE WITH USAGE TYPES AND GROWING ELEVATION. PLANT SYSTEMATICS AND EVOLUTION, 276(1-2), 9-19.

ACHO, F. C., ZOUE, L. T., & NIAMKE, S. L. (2015). EFFECT OF REFRIGERATION STORAGE ON NUTRITIVE AND ANTIOXIDANT PROPERTIES OF FIVE LEAFY VEGETABLES CONSUMED IN SOUTHERN CÔTE D'IVOIRE. PAKISTAN JOURNAL OF NUTRITION, 14(7), 401.

AGBEMAFLE, R., OBODAI, E. A., GENEVIVE, E. A., & AMPRAKO, D. N. (2012). Effects of boiling time on the concentrations of vitamin c and beta-carotene in five selected green vegetables consumed in Ghana. Applied Science Research, 3, 2815-2820.

AKINWUMI, K. A., OSIFESO, O. O., JUBRIL, A. J., ADEDOJA, A. W., OGUNBIYI, E. T., ADEBO, F. M., and ODUNOLA, O. A. (2016). Potassium Dichromate Toxicities: Protective Effect of Methanol Extract of Corchorus olitorius in Albino Rats. Journal of medicinal food, 19(5), 457-465.

ALI, M. A., NAHER, Z., RHAMAN, M., HAQUE, A. and ALIM, A. (2002). Selection prediction for yield of fibre in jute (Corchorus capsularis and C. olitorius). OnLine Journal of Biological Sciences, 2, 295-297.

Azuma, K., Nakayama, M., Koshioka, M., Ippoushi, K., Yamaguchi, Y., Kohata, K., and Higashio, H. (1999). Phenolic antioxidants from the leaves of Corchorus olitorius L. Journal of agricultural and food chemistry, 47(10), 3963-3966.

BALDI, L. AND CASATI, D. (2009). UN DISTRETTO DELLA IV GAMMA? IL COMPARTO CHE VENDE TEMPO LIBERO. AGRIREGIONIEUROPA, 5(16), 50-52.

BANCHEVA, S., AND GREILHUBER, J. (2006). GENOME SIZE IN BULGARIAN CENTAUREA SL (ASTERACEAE). PLANT SYSTEMATICS AND EVOLUTION, 257(1-2), 95-117.

BASU, T., SATYA, P., SARKAR, D., KAR, C. S., MITRA, J. and KARMAKAR, P. G. (2016). Organelle genetic diversity in a global collection of Jute (Corchorus capsularis and C. olitorius, Malvaceae). South African Journal of Botany, 103, 54-60.

BENOR, S., FUCHS, J., and BLATTNER, F. R., (2011). Genome size variation in Corchorus olitorius (Malvaceae sl) and its correlation with elevation and phenotypic traits. Genome, 54(7), 575-585.

BERGQUIST, S. Å., GERTSSON, U. E., KNUTHSEN, P. and OLSSON, M. E. (2005). Flavonoids in baby spinach (Spinacia oleracea): changes during plant growth and storage. Journal of agricultural and food chemistry, 53, 9459-9464.

BILYK, A., & SAPERS, G. M. (1985). DISTRIBUTION OF QUERCETIN AND KAEMPFEROL IN LETTUCE, KALE, CHIVE, GARLIC CHIVE, LEEK, HORSERADISH, RED RADISH, AND RED CABBAGE TISSUES. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, 33(2), 226-228.

BLOM-ZANDSTRA, M. (1989). Nitrate accumulation in vegetables and its relationship to quality. Annals of Applied Biology, 115(3), 553-561.

BOUAYED, J., HOFFMANN, L. and BOHN, T. (2011). Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bio-accessibility and potential uptake. Food Chemistry, 128, 14-21.

BRUNING-FANN, C. S. and KANEENE, J. B. (1993). The effects of nitrate, nitrite and N-nitroso compounds on human health: a review. Veterinary and human toxicology, 35, 521-538.

BRYAN, N. S. (2006). NITRITE IN NITRIC OXIDE BIOLOGY: CAUSE OR CONSEQUENCE?: A SYSTEMS-BASED REVIEW. FREE RADICAL BIOLOGY AND MEDICINE, 41(5), 691-701.

Cao, S., Zhenfeng Y., Yonghua Z., 2013. Sugar metabolism in relation to chilling tolerance of loquat fruit. Food Chem. 136, 139-143.

CARTEA, M. E., FRANCISCO, M., SOENGAS, P., & VELASCO, P. (2010). PHENOLIC COMPOUNDS IN BRASSICA VEGETABLES. MOLECULES, 16(1), 251-280.

CATALDO, D. A., MAROON, M., SCHRADER, L. E. and YOUNGS, V. L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Communications in Soil Science and Plant Analysis, 6, 71-80.

Cawley, J., Sweeney, M. J., Sobal, J., Just, D. R., Kaiser, H. M., Schulze, W. D., Wansink, B., 2015. The impact of a supermarket nutrition rating system on purchases of nutritious and less nutritious foods. Public health nutr. 18, 8-14.

CHAPIN, F.S. (1980). THE MINERAL NUTRITION OF WILD PLANTS.ANNUAL REVIEW OF ECOLOGY AND SYSTEMATICS, 11, 233–260.

CHAUHAN, B. S., AND JOHNSON, D. E. (2008). Seed germination and seedling emergence of nalta jute (Corchorus olitorius) and redweed (Melochia concatenata): important broadleaf weeds of the tropics. Weed science, 56(6), 814-819.

CHEN, B. M., WANG, Z. H., LI, S. X., WANG, G. X., SONG, H. X. and WANG, X. N. (2004). Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. Plant Science, 167, 635-643.

COCETTA, G., BALDASSARRE, V., SPINARDI, A., & FERRANTE, A. (2014). Effect of cutting on ascorbic acid oxidation and recycling in fresh-cut baby spinach (Spinacia oleracea L.) leaves. Postharvest Biology and Technology, 88, 8-16.

COLELLI, G., AND ELIA, A. (2009). I prodotti ortofrutticoli di IV gamma: aspetti fisiologici e tecnologici. Italus Hortus, 16(1), 55-78.

COLLINS, E. D., & CHANDRASEKARAN, K. (2012). A wolf in sheep's clothing? An analysis of the 'sustainable intensification'of agriculture. Friends of the Earth International, Amsterdam.

Cram, W. J., 1976. Negative feedback regulation of transport in cells. The maintenance of turgor, volume and nutrient supply. In: Pirson, A., Zimmermann H.M, Encyclopaedia of Plant Physiology. Springer, Berlin Heidelberg, pp 284-316.

DAS, A. K., BAG, S., SAHU, R., DUA, T. K., SINHA, M. K., GANGOPADHYAY, M. AND DEWANJEE, S. (2010). Protective effect of Corchorus olitorius leaves on sodium arsenite-induced toxicity in experimental rats. Food and chemical toxicology, 48, 326-335.

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES. PRODUCTION GUIDELINES JEW'S MALLOW (CORCHORUS OLITORIUS L.). ISSUED ON FEBRUARY 2012, AGRICULTURE, FORESTRY AND FISHERIES, REPUBLIC OF SOUTH AFRICA. AVAILABLE: TTP://WWW.DAFF.GOV.ZA/DOCS/BROCHURES/JEWMALLOWPG.PDF.

DEATON, A. AND PAXSON, C. (1998). Economies of scale, household size, and the demand for food. Journal of political economy, 106(5), 897-930.

DER AGOPIAN, R. G., PERONI-OKITA, F. H. G., SOARES, C. A., MAINARDI, J. A., DO NASCIMENTO, J. R. O., CORDENUNSI, B. R., PURGATTO, E., (2011). Low temperature induced changes in activity and protein levels of the enzymes associated to conversion of starch to sucrose in banana fruit. Postharvest Biol. Technol. 62 133-140.

DEWANJEE, S., SAHU, R., KARMAKAR, S. and GANGOPADHYAY, M. (2013). Toxic effects of lead exposure in Wistar rats: involvement of oxidative stress and the beneficial role of edible jute (Corchorus olitorius) leaves. Food and chemical toxicology, 55, 78-91.

DREHER, D., and JUNOD, A. F. (1996). Role of oxygen free radicals in cancer development. European Journal of cancer, 32(1), 30-38.

EDMONDS, J.M. 1990. Herbarium survey of African Corchorus L. species. Systematic and ecogeographic studies on crop genepools4. International Board for Plant Genetic Resources, Rome, Italy.

EMONGOR, V. F., MATHOWA T. and KABELO S. (2004). Acid and Ethephon on the Germination of Corchorus (Corchorus Widens) Seed. Journal of Agronomy, 3, 196-200.

FALLOVO, C., ROUPHAEL, Y., REA, E., BATTISTELLI, A. and COLLA, G. (2009). Nutrient solution concentration and growing season affect yield and quality of Lactuca sativa L. var. acephala in floating raft culture. Journal of the Science of Food and Agriculture, 89(10), 1682-1689.

FERRANTE, A. and MAGGIORE, T. (2007). Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables. Postharvest Biology and Technology, 45, 73-80.

FLORINDO, A. A., BROWNSON, R. C., MIELKE, G. I., GOMES, G. A., PARRA, D. C., SIQUEIRA, F. V., AND HALLAL, P. C. (2015). Association of knowledge, preventive counseling and personal health behaviors on physical activity and consumption of fruits or vegetables in community health workers. BMC public health, 15(1), 1.

FORCE, L., CRITCHLEY, C., & VAN RENSEN, J. J. (2003). New fluorescence parameters for monitoring photosynthesis in plants. Photosynthesis Research, 78(1), 17-33.

FRANCINI, A., ROMEO, S., CIFELLI, M., GORI, D., DOMENICI, V., SEBASTIANI, L. 2016. 1 H NMR and PCA-based analysis revealed variety dependent changes in phenolic contents of apple fruit after drying. Food Chemistry (in press).

FREIBERGER, C. E., VANDERJAGT, D. J., PASTUSZYN, A., GLEW, R. S., MOUNKAILA, G., MILLSON, M. AND GLEW, R. H. (1998). Nutrient content of the edible leaves of seven wild plants from Niger. Plant foods for Human nutrition, 53(1), 57-69.

FRITZ, C., PALACIOS-ROJAS, N., FEIL, R. and STITT, M. (2006). Regulation of secondary metabolism by the carbon–nitrogen status in tobacco: nitrate inhibits large sectors of phenylpropanoid metabolism. The Plant Journal, 46, 533-548.

FURUMOTO, T., WANG, R., OKAZAKI, K., HASAN, A. F., ALI, M. I., KONDO, A., and FUKUI, H. (2002). Antitumor Promoters in Leaves of Jute (Corchorus capsularis and Corchorus olitorius). Food Science and Technology Research,8(3), 239-243.

Giro A., Ferrante A., 2016. Yield and quality of Corchorus olitorius baby leaf grown in a floating system. J. Hortic. Sci. Biotech. 1-8.

GLEICK, P. H. (1994). Water, war & peace in the Middle East. Environment: science and policy for sustainable development, 36(3), 6-42.

Goh, Y. J., Klaenhammer, T. R., 2015. Genetic mechanisms of prebiotic oligosaccharide metabolism in probiotic microbes. Annu. Rev. Food Sci. Technol. 6, 137-156.

ILHAN, S., SAVAROĞLU, F. and ÇOLAK, F. (2007). Antibacterial and antifungal activity of Corchorus olitorius L.(molokhia) extracts. International Journal of Natural and Engineering Sciences, 1(3), 59-61.

ISLAM, M. M. (2013). Biochemistry, medicinal and food values of Jute (Corchorus capsularis L and C. olitorius L) leaf: a review. International Journal of Enhanced Research in Science Technology and Engineering, 2(11), 35-44.

IZZO, A. A., DI CARLO, G., BORRELLI, F., & ERNST, E. (2005). Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction. International journal of cardiology, 98(1), 1-14.
JOSHI, A., DAS, S. K., SAMANTA, P., PARIA, P., SEN, S. K. and BASU, A. (2014). Chromosome-specific physical localisation of expressed sequence tag loci in Corchorus olitorius L. Plant Biology, 16(6), 1133-1139.

KADER, A. A. "QUALITY PARAMETERS OF FRESH-CUT FRUIT AND VEGETABLE PRODUCTS." FRESH-CUT FRUITS AND VEGETABLES (2002): 11-20.

KAISER, G. AND HEBER, U. (1984). SUCROSE TRANSPORT INTO VACUOLES ISOLATED FROM BARLEY MESOPHYLL PROTOPLASTS. PLANTA, 161(6), 562-568.

KALAPOS, TIBOR, RIKI VAN DEN BOOGAARD, AND HANS LAMBERS. "EFFECT OF SOIL DRYING ON GROWTH, BIOMASS ALLOCATION AND LEAF GAS EXCHANGE OF TWO ANNUAL GRASS SPECIES." PLANT AND SOIL 185.1 (1996): 137-149.

KAMGA, R.T., KOUAMÉ, C., ATANGANA, A.R., CHAGOMOKA, T., & NDANGO, R. (2013). NUTRITIONAL EVALUATION OF FIVE AFRICAN INDIGENOUS VEGETABLES. JOURNAL OF HORTICULTURAL RESEARCH, 21, 99–106.

KAMINSKI, KACPER PIOTR, ET AL. "CONTRASTING WATER-USE EFFICIENCY (WUE) RESPONSES OF A POTATO MAPPING POPULATION AND CAPABILITY OF MODIFIED BALL-BERRY MODEL TO PREDICT STOMATAL CONDUCTANCE AND WUE MEASURED AT DIFFERENT ENVIRONMENTAL CONDITIONS." JOURNAL OF AGRONOMY AND CROP SCIENCE 201.2 (2015): 81-94.

KHAN, M. S. Y., BANO, S., JAVED, K., & MUEED, M. A. (2006). A comprehensive review on the chemistry and pharmacology of Corchorus species-A source of cardiac glycosides, triterpenoids, ionones, flavonoids, coumarins, steroids and some other compounds. Journal of scientific and industrial research, 65(4), 283.

KANG, H.M. and SALTVEIT, M.E. (2003). Wound-induced increases in phenolic content of fresh-cut lettuce is reduced by a short immersion in aqueous hypertonic solutions. Postharvest Biology and Technology, 29, 271-277.

KASIM, M. U., KASIM R. (2012). Colour changes of fresh-cut Swiss chard leaves stored at different light intensity. Am. J. Food Tech. 7, 13-21.

KAULMANN, A., JONVILLE, M.C., SCHNEIDER, Y.J., HOFFMANN, L., & BOHN, T. (2014). Carotenoids, polyphenols and micronutrient profiles of Brassica oleraceae and plum varieties and their contribution to measures of total antioxidant capacity. Food Chemistry, 155, 240–250.

KIM, J. H., LEE, G., CHO, Y. L., KIM, C. K., HAN, S., LEE, H. and KIM, Y. M. (2009).

Desmethylanhydroicaritin inhibits NF-κB-regulated inflammatory gene expression by modulating the redox-sensitive PI3K/PTEN/Akt pathway. European journal of pharmacology, 602, 422-431.

KLEIN, A.O., & HAGEN, C.W. JR (1961). ANTHOCYANIN PRODUCTION IN DETACHED PETALS OF IMPATIENS BALSAMINA L. PLANT PHYSIOLOGY, 36, 1–9.

KOVÁČIK, J., & BAČKOR, M. (2007). Changes of phenolic metabolism and oxidative status in nitrogen-deficient Matricaria chamomilla plants. Plant and Soil, 297,

KUNDU, B. C., 1951: Origin of jute. Indian J. Genet. Plant Breed. 11, 95—99.;

LARSSON S., WIREN A., ERICSSON T. and LUNDGREN L. (1986). Effects of light and nutrient stress on defensive chemistry and susceptibility to Galerucella lineola (Coleoptera, Chrysomelidae) in two Salix species. Oikos, 47, 205-210.

LESTER, GENE E., DONALD J. MAKUS, and D. MARK HODGES. "Relationship between fresh-packaged spinach leaves exposed to continuous light or dark and bioactive contents: effects of cultivar, leaf size, and storage duration." Journal of agricultural and food chemistry 58.5 (2010): 2980-2987.

LEVY, M. A., THORKELSON, C., VÖRÖSMARTY, C., DOUGLAS, E., & HUMPHREYS, M. (2005, JUNE). Freshwater availability anomalies and outbreak of internal war: results from a global spatial time series analysis. In Human Security and Climate Change: An International Workshop (pp. 21-23).

LI, Q. AND KUBOTA C. (2009). Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environmental and Experimental Botany, 67, 59-64.

LICHTENTHALER, H.K. (1987). Chlorophyll and carotenoids: pigments of photosynthetic membranes. Methods in Enzymology, 148, 350–382.

LOUMEREM, M., & ALERCIA, A. (2016). DESCRIPTORS FOR JUTE (CORCHORUS OLITORIUS L.). GENETIC RESOURCES AND CROP EVOLUTION, 63(7), 1103-1111.

LUNDBERG, J. O., & GOVONI, M. (2004). INORGANIC NITRATE IS A POSSIBLE SOURCE FOR SYSTEMIC GENERATION OF NITRIC OXIDE. FREE RADICAL BIOLOGY AND MEDICINE, 37(3), 395-400.

MALLICK, S., PAL, B. C., KUMAR, D., CHATTERJEE, N., DAS, S., & SAHA, K. D. (2013). EFFECT OF CORCHORUSIN-D, A SAIKOSAPONIN LIKE COMPOUND, ON B16F10 MELANOMA CELLS (IN VITRO AND IN VIVO). JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH, 15(11), 1197-1203.

MARIN, A., FERRERES, F., BARBERÁ, G. G. and GIL, M. I. (2015). Weather Variability Influences Color and Phenolic Content of Pigmented Baby Leaf Lettuces throughout the Season. Journal of agricultural and food chemistry, 63, 1673-1681.

MARTIN, C., ZHANG, Y., TONELLI, C., PETRONI, K., (2013). Plants, diet, and health. Annu. Rev. Plant Biol. 64, 19-46.

MATUSHKE I., 2009 - Rapid urbanization and food security: Using food density maps to identify future food security hotspots. –FAO Contributed paper for International Association of Agriculture Economist Conference, Beijing, China, August 16-22, pp. 15.

MCKNIGHT, G. M., DUNCAN, C. W., LEIFERT, C., & GOLDEN, M. H. (1999). Dietary nitrate in man: friend or foe?. British Journal of Nutrition, 81(05), 349-358.

MEDINA, M. S., TUDELA, J. A., MARÍN, A., ALLENDE, A., GIL, M. I., 2012. Short postharvest storage under low relative humidity improves quality and shelf life of minimally processed baby spinach (Spinacia oleracea L.). Postharvest Biol. Technol. 67, 1-9.

MGUIS, K., ALBOUCHI, A., & BEN BRAHIM, N. (2014). Germination responses of Corchorus olitorius L. to salinity and temperature. African Journal of Agricultural Research, 9(1), 65-73.
MILLER, G.L. (1959). USE OF DINITROSALICYLIC ACID REAGENT FOR DETERMINATION OF REDUCING SUGAR. ANALYTICAL CHEMISTRY., 31, 426–428.

MORSY, N. E., RAYAN, A. M., & YOUSSEF, K. M. (2015). Physico Chemical Properties, Antioxidant Activity, Phytochemicals and Sensory Evaluation of Rice-Based Extrudates Containing Dried Corchorus olitorius l. Leaves. Journal of Food Processing & Technology, 6(1), 1.

MOSIHUZZAMAN, M., QUDDUS, A., NAHAR, N. and THEANDER, O. (1989). Comparative study of carbohydrates in the two major species of jute (Corchorus capsularis and Corchorus olitorius. Journal of the Science of Food and Agriculture, 48, 305-310.

MUSA, A., EZENWA, M.I., OLADIRAN, J.A., AKANYA, H.O., AND OGBADOYI, E.O. (2010). Effect of soil nitrogen levels onsome micronutrients, antinutrients and toxic substances in Corchorus olitorius grown in Minna, Nigeria. African Journal Agricolture Research, 5, 3075–3081.

NAKATANI, N., KAYANO, S. I., KIKUZAKI, H., SUMINO, K., KATAGIRI, K. AND MITANI, T. (2000). Identification, Quantitative Determination, and Antioxidative Activities of Chlorogenic Acid Isomers in Prune (Prunus d omestica L.). Journal of Agricultural and Food Chemistry, 48(11), 5512-5516.

Nature 522, 146–148 (11 June 2015) doi10.1038/522146a.

NDEHEDEHE, C. E., AGUTU, N. O., OKWUASHI, O., and FERREIRA, V. G. (2016). Spatio-Temporal Variability of Droughts and Terrestrial Water Storage over Lake Chad Basin using Independent Component Analysis. Journal of Hydrology.

NDLOVU, J., AND AFOLAYAN, A.J. (2008). Nutritional analysis of the South African wild vegetable Corchorus olitorius L. Asian Journal of Plant Sciences, 7, 615–618.

NEILL, S.O. and GOULD, K. S. (2003). Anthocyanins in leaves: light attenuators or antioxidants?. Functional Plant Biology, 30, 865-873.

NISHIUMI, S., YABUSHITA, Y., FUKUDA, I., MUKAI, R., YOSHIDA, K. I. and ASHIDA, H. (2006). Molokhia (Corchorus olitorius L.) extract suppresses transformation of the aryl hydrocarbon receptor induced by dioxins. Food and chemical toxicology, 44, 250-260.

NKOMO, M., & KAMBIZI, L. (2009). Effects of pre-chilling and temperature on seed germination of Corchorus olitorius L.(Tiliaceae)(Jew's Mallow), a wild leafy vegetable. African Journal of Biotechnology, 8(6), 1078.

NWAKAEZE, A. E., IROHA, I. R., EJIKEUGWU, P. C., AFIUKWA, F. N., UDU-IBIAM, O. E., OJI, A. E., and EZE, E. A. (2014). Evaluation of antibacterial activities of some Nigerian medicinal plants against some resistant bacteria pathogens.African Journal of Microbiology Research, 8(18), 1832-1836.

OBOH, G., ADEMILUYI, A. O., AKINYEMI, A. J., HENLE, T., SALIU, J. A. and SCHWARZENBOLZ, U. (2012). Inhibitory effect of polyphenol-rich extracts of jute leaf (Corchorus olitorius) on key enzyme linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting) in vitro. Journal of Functional Foods, 4(2), 450-458.

OGUNLESI, M., OKIEI, W., AZEEZ, L., OBAKACHI, V., OSUNSANMI, M., and NKENCHOR, G. (2010). Vitamin C contents of tropical vegetables and foods determined by voltammetric and titrimetric methods and their relevance to the medicinal uses of the plants. Int J Electrochem Sci, 5, 105-115.

OGUNLESI, T. A. (2010). Maternal socio-demographic factors influencing the initiation and exclusivity of breastfeeding in a Nigerian semi-urban setting. Maternal and child health journal, 14(3), 459-465.

OLANIYI, J. O., & AJIBOLA, A. T. (2008). Growth and yield performance of Corchorus olitorius varieties as affected by nitrogen and phosphorus fertilizers application. American-Eurasian Journal of Sustainable Agriculture, 2(3), 234-241.

OHTANI, K., OKAI, K., YAMASHITA, U., YUASA, I. and MISAKI, A. (1995). Characterization of an acidic polysaccharide isolated from the leaves of Corchorus olitorius L.(Moroheiya). Bioscience, biotechnology, and biochemistry, 59, 378-381.

OKE, O. L. (1964). Chemical Studies on Corchorus. Indian Journal of Medical Research, 52(12), 1266-72.

OKE, O. L. (1968). Chemical changes in some Nigerian vegetables during growth. Experimental Agriculture, 4(04), 345-349.

ORECH, F.O., CHRISTENSEN, D.L., LARSEN, T., FRIIS, H., AAGAARD-HANSEN, J., AND ESTAMBALE, B.A. (2007). Mineral content of traditional leafy vegetables from western Kenya. International Journal of Food Sciences and Nutrition, 58, 595–602.

OWOYELE, B. V., ABDULMAJEED, W., ADISA, B. M., OWOLABI, O. O. and OYELEKE, S. A. (2015). Anti-Ulcerogenic and Gastric Antisecretory Effects of Corchorus olitorius Extract in Male Albino Rats. Journal of Herbs, Spices & Medicinal Plants, 21(1), 1-8.

PARIDA, A., DAS, A. B. AND DAS, P. (2002). NaCl stress causes changes in photosynthetic pigments, proteins, and other metabolic components in the leaves of a true mangrove, Bruguiera parviflora, in hydroponic cultures. Journal of Plant Biology, 45(1), 28-36.

PARVIN, S., MARZAN, M., RAHMAN, S., DAS, A. K., HAQUE, S. and RAHMATULLAH, M. (2015). Preliminary phytochemical screening, antihyperglycemic, analgesic and toxicity studies on methanolic extract of aerial parts of Corchorus olitorius L.

PHUWAPRAISIRISAN, P., PUKSASOOK, T., KOKPOL, U. and SUWANBORIRUX, K. (2009). Corchorusides A and B, new flavonol glycosides as α-glucosidase inhibitors from the leaves of Corchorus olitorius. Tetrahedron Letters, 50(42), 5864-5867.

RAVEN, J. A., & SMITH, F. A. (1976). Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytologist, 76(3), 415-431.

RIBEIRO, R. M., RUIZ, J. M., GARCIA, P. C., LOPEZ-LEFEBRE, L. R., SÁNCHEZ, E. and ROMERO, L. (2001). Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. Plant Science, 160, 315-321.

ROSE, P., CHOON, N.O. and WHITEMAN M. (2005). Protective effects of Asian green vegetables against oxidant induced cytotoxicity. World Journal of Gastroenterology, 11, 7607.

Roura, S. I., Davidovich, L. A., Valle, C. D., 2000. Postharvest changes in fresh Swiss chard (Beta vulgaris, type cycla) under different storage conditions. J. Food Qual. 23, 137-147.

SAMUOLIENĖ, G., SIRTAUTAS, R., BRAZAITYTĖ, A., DUCH OVSKIS, P., 2012. LED lighting and seasonality effects antioxidant properties of baby leaf lettuce. Food chem. 134(3), 1490-1499.
Sanchez-Mata, M. C., M. Camara, Dıez-Marques, C., 2003. Extending shelf-life and nutritive value of green beans (Phaseolus vulgaris L.), by controlled atmosphere storage: macronutrients. Food chem. 80.3, 309-315.

SANTAMARIA, P. (2006). Nitrate in vegetables: toxicity, content, intake and EC regulation. Journal of the Science of Food and Agriculture, 86, 10-17.

SCUDERI, D., RESTUCCIA, C., CHISARI, M., BARBAGALLO, R. N., CAGGIA, C., AND GIUFFRIDA, F. (2011). Salinity of nutrient solution influences the shelf-life of fresh-cut lettuce grown in floating system. Postharvest biology and technology, 59(2), 132-137.

SHACKLETON, S. E., DZEREFOS, C. M., SHACKLETON, C. M. and MATHABELA, F. R. (1998). Use and trading of wild edible herbs in the central lowveld savanna region, South Africa. Economic Botany, 52(3), 251-259.

SHUKLA, S., MEHTA, A., BAJPAI, V. K., and SHUKLA, S. (2009). In vitro antioxidant activity and total phenolic content of ethanolic leaf extract of Stevia rebaudiana Bert. Food and Chemical Toxicology, 47, 2338-2343.

Sindhu K., Kantharaj U., 1995. Rocket, An Ancient Underutilized Vegetable Crop and Its Potential. Rocket Genetic Resources Network, in: Padulosi, S., (Ed), Report of the First Meeting, Lisbon, Portugal, 13–15 November 1994. International Plant Genetic Resources Institute. Rome, Italy, 1995; pp. 46-47.

ŠMARDA, P., & BUREŠ, P. (2006). Intraspecific DNA content variability in Festuca pallens on different geographical scales and ploidy levels. Annals of Botany, 98(3), 665-678.

SOLOMONSON L.P. and BARBER M.J. (1990). Assimilatory nitrate reductase: functional properties and regulation. Annual Review of Plant Biology, 41, 225–253.

SPINARDI A., FERRANTE A. 2012. Effect of storage temperature on quality changes of minimally processed baby lettuce. Journal of Food, Agriculture & Environment, 10(1), 38-42.

TAIWO, B. J., TAIWO, G. O., OLUBIYI, O. O., & FATOKUN, A. A. (2016). Polyphenolic compounds with anti-tumour potential from Corchorus olitorius (L.) Tiliaceae, a Nigerian leaf vegetable. Bioorganic & Medicinal Chemistry Letters, 26(15), 3404-34

TALHOUK, R. S., KARAM, C., FOSTOK, S., EL-JOUNI, W., & BARBOUR, E. K. (2007). Anti-inflammatory bioactivities in plant extracts. Journal of medicinal food, 10(1), 1-10.

TOMASI, N., PINTON, R., DALLA COSTA, L., CORTELLA, G., TERZANO, R., MIMMO, T., AND CESCO, S. (2015). New 'solutions' for floating cultivation system of ready-to-eat salad: A review. Trends in Food Science & Technology, 46(2), 267-276.

TSORMPATSIDIS, E., HENBEST, R. G. C., DAVIS, F. J., BATTEY, N. H., HADLEY, P. and WAGSTAFFE, A. (2008). UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution'grown under polyethylene films. Environmental and Experimental Botany, 63, 232-239.

TUDELA, J. A., MARÍN, A., GARRIDO, Y., CANTWELL, M.,MEDINA-MARTÍNEZ, M. S., & GIL, M. I. (2013). Off-odour development in modified atmosphere packaged baby spinach is an unresolved problem. Postharvest biology and technology, 75, 75-85.

UDALL, J. A. (1965). HUMAN SOURCES AND ABSORPTION OF VITAMIN K IN RELATION TO ANTICOAGULATION STABILITY. JAMA, 194(2), 127-129.

VAN JAARSVELD, P., FABER, M., VAN HEERDEN, I., WENHOLD, F., VAN RENSBURG, W.J., & VAN AVERBEKE, W. (2014). NUTRIENT CONTENT OF EIGHT AFRICAN LEAFY VEGETABLES AND THEIR POTENTIAL CONTRIBUTION TO DIETARY REFERENCE INTAKES. JOURNAL OF FOOD COMPOSITION AND ANALYSIS, 33, 77–84.

WANG, L., YAMASAKI, M., KATSUBE, T., SUN, X., YAMASAKI, Y. and SHIWAKU, K. (2011). Anti-obesity effect of polyphenolic compounds from molokheiya (Corchorus olitorius L.) leaves in LDL receptor-deficient mice. European journal of nutrition, 50, 127-133.

WARD, J. M., KÜHN, C., TEGEDER, M. AND FROMMER, W. B. (1997). SUCROSE TRANSPORT IN HIGHER PLANTS. INTERNATIONAL REVIEW OF CYTOLOGY, 178, 41-71.

WRIGHT, R. P., LENTZ, D. L., BEAUBIEN, H. F. and KIMBROUGH, C. K. (2012). New evidence for jute (Corchorus capsularis L.) in the Indus civilization. Archaeological and Anthropological Sciences, 4(2), 137-143.

WU, M. and KUBOTA, C. (2008). Effects of high electrical conductivity of nutrient solution and its application timing on lycopene, chlorophyll and sugar concentrations of hydroponic tomatoes during ripening. Scientia Horticulturae, 116, 122-129.

YAN, Y., WANG, Y. W., CHEN, S. L., ZHUANG, S. R. and WANG, C. K. (2013). Anti-inflammatory effects of phenolic crude extracts from five fractions of Corchorus olitorius L. Food chemistry, 138, 1008-1014.

YEMM, E.W. and WILLIS, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal, 57, 508–514.

YOSHIKAWA, M., MURAKAMI, T., SHIMADA, H., YOSHIZUMI, S., SAKA, M., YAMAHARA, J., & MATSUDA, H. (1998). Medicinal foodstuffs. XIV. On the bioactive constituents of moroheiya. (2): New fatty acids, corchorifatty acids A, B, C, D, E, and F, from the leaves of Corchorus olitorius L.(Tiliaceae): structures and inhibitory effect on NO production in mouse peritoneal macrophages. Chemical and pharmaceutical bulletin, 46(6), 1008-1014.

ZAKARIA, Z. A., SAFARUL, M., VALSALA, R., SULAIMAN, M. R., FATIMAH, C. A., SOMCHIT, M. N., & JAIS, A. M. (2005). The influences of temperature and naloxone on the antinociceptive activity of Corchorus olitorius L. in mice. Naunyn-Schmiedeberg's archives of pharmacology, 372(1), 55-62.

ZAKARIA Z. A., FARAH, W., NAZARATULMAWARINA, R., and FATIMAH, C. (2006). The in vitro antibacterial activity of Corchorus olitorius extracts. International Journal of Pharmacology, 2(2), 213-215.

ZAKARIA, Z. A., SULAIMAN, M. R., GOPALAN, H. K., ABDUL GHANI, Z. D. F., RADEN MOHD. NOR, R. N. S., MAT JAIS, A. M., and ABDULLAH, F. C. (2007). Antinociceptive and anti-inflammatory properties of Corchorus capsularis leaves chloroform extract in experimental animal models. Yakugaku Zasshi,127(2), 359-365.

ZEGHICHI S, KALLITHKARA S, SIMOPOULUS AP (2003) NUTRITIONAL COMPOSITION OF MOLOKHIA (*CORCHORUS OLITORIUS*) AND STAMNAGATHI (CICHORIUM SPINOSUM). IN: SIMOPOULUS AP, GOPALAN C (EDS) PLANTS IN HUMAN HEALTH AND NUTRITION POLICY. KARGER, BASEL, PP 1–21.

ZEID, A. H. S. A. (2002). Stress metabolites from *Corchorus olitorius* L. leaves in response to certain stress agents. Food Chemistry, 76(2), 187-195.

ACKNOWLEDGMENT