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THE NUTRIENT CONTENT OF TWO FOLIA MORPHOTYPES OF *CENTELLA ASIATICA* (L) GROWN IN MADAGASCAR

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ABSTRACT

Centella asiatica is a herbal plant generally used for its curative properties. Two foliar morphotypes were recently identified in Madagascar: a reniform tetraploid (2n = 4x = 36) in the Center and the East of the island and a round diploid (2n = 2x = 18) in the West. The objective of this study was to evaluate the nutritional properties of these two morphotypes and to compare them with other green leafy vegetables. Reniform leaves were collected in Antananarivo and Moramanga and round leaves were collected in Tsiroanomandidy and Analavory. Macronutrient content was determined by standard methods, mineral contents were analysed by inductively coupled plasma atomic emission spectroscopy. Ascorbic acid and carotenoids were quantified by HPLC. Food composition showed significant differences between the morphotypes. Reniform leaves have higher protein content (19–22 % dry weight (DW)) than round leaves (17–21 % DW). Lipid content are from 2.5 to 6.0 % DW. Reniform leaves have higher iron content than round leaves. Iron content of *C. asiatica*'s leaves range from 3.8 to 12.5 mg/100 g fresh weight (FW). Reniform leaves from Moramanga have the highest protein, lipid and iron content. Round leaves from Analavory have the highest calcium and magnesium content, which can cover 41 % and 17 % of the Recommended Dietary Allowances (RDA), respectively. Leaves of *C. asiatica* have low vitamin C content (1.3 to 7.7 mg/100 g FW). Consumption of 100 g of *C. asiatica* leaves could cover 10 to 21 % of vitamin A daily requirements of women for reniform leaves and 21 to 37 % of vitamin A requirement of women for round leaves. Round leaves from Analavory have the highest β-carotene content. It is hoped that from these findings, the people of Madagascar will be encouraged to include *C. asiatica* in their diets for its nutritional benefits.

Key words: *Centella asiatica*, reniform leaves, round leaves, protein, lipid, vitamin A, minerals, amino acids

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INTRODUCTION

Food Security is a major challenge worldwide; in Madagascar 36% of the households were affected in 2014 [1]. This has led to an increase in malnutrition. Infant mortality stood at 54 per 1,000 live births while the BMI for most women in reproductive age (27 %) was less than 18.5. Vitamin A deficiency was reported to be at 42% among the under-fives having low serum concentrations [2].

Madagascar is known for the variety of its natural wealth, which can be potentially beneficial for health against malnutrition. Recently, several studies were conducted in Madagascar about Green Leafy Vegetables (GLV) [3, 4]. Green leafy vegetables are rich in protein, vitamins and minerals [3]. They are also a good source of provitamin A carotenoids [5]. Promotion of GLV consumption can contribute to the reduction of vitamin A deficiency and food insecurity because they are affordable for Malagasy people and easy to prepare [4].

Centella asiatica (L.) is a herbal medicinal plant used in various applications. It has long been used by traditional healers in Java, Indonesian Islands and Madagascar [3]. Research has proven that this plant has different functional properties: memory enhancing, skin protective activities, and is also used to treat leprosy due to its triterpenes [6, 7]. *C. asiatica* is also reported for its antioxidant, antiulcer, anti-inflammatory and cytotoxic activities [8]. It is commonly used for making herbal tea. However, in Asian countries, it is consumed in salads, soups and in juices [9, 10].

The quality of *C. asiatica* leaves is evaluated by its level of triterpenes (asiatic acid, madecassic acid, asiaticoside, madecassoside) which have wound-healing properties. This level can be affected by the location and diverse environmental conditions [3, 9].

In Madagascar, leaves of *C.asiatica* are collected and exported as a medicinal plant for transformation. Malagasy *C.asiatica* leaves from Madagascar contain 3 to 7 times more asiaticoside than leaves from India [7]. Recently, two foliar morphotypes of *C.asiatica* were identified in Madagascar, a morphotype tetraploids (2n = 4x = 36) with small reniform leaves in the East and the Center and a morphotype diploids (2n = 2x = 18) with large round leaves in the West of the Island [11]. The reniform leaf morphotype is two times richer in asiaticoside than the round leaf morphotype and it is the only morphotype collected for medicinal industrial processing purposes [12].

The main objective of this study was to compare two morphologic varieties of *C.asiatica* by analysing their macro- and micronutrients levels.

MATERIALS AND METHODS

Raw material and sample preparation

Leaves of *C. asiatica* were collected in 4 localities (Figure 1). In each locality, about 5 kg of *C. asiatica* leaves were collected and transported into the laboratory. Young leaves (YL) were separated from mature leaves (ML) according to their color and size. Young leaves are light green and ML are dark green. Young leaves are the little sized leaves

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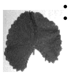
near the root of the plant. The damaged leaves and the stems were discarded. Both YL and ML were washed three times in distilled water, weighed, named and stored at -80°C before analysis. Nutritional composition of the YL and ML in the 4 areas were determined (8 groups of leaves). Analyses were conducted with freeze-dried leaves for minerals, proteins, β-carotene, and amino acids; with fresh leaves for vitamin C; and dried leaves for lipid content.

Figure 1: Localities of the collection of *C. asiatica* leaves

 : locality of collection of the reniform leaves

 : locality of collection of the round leaves

<u>Chemicals</u>

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Hexane, methanol, dichloromethane, methyl-tert-butyl-ether (MTBE), metaphosphoric acid, sulfuric acid, 1-ascorbic acid, tris(2-carboxyethyl) phosphine (TCEP), norleucine, acid methane sulfonic and sodium hydroxide were obtained from Sigma-Aldrich (St. Louis, USA). β-carotene standard (≥ 98 %) was purchased from Extrasynthese (Genay, France). Sodium citrate was obtained from Biochrom (Biochrom, Cambridge, UK).

Proximate composition
Moisture contents of the 8 groups of samples were determined as described in the Official Methods of Analysis [13]. Samples were dried in an oven (24h/105°C).

Crude fat was extracted with n-hexane [14]. Protein contents of the samples were estimated by multiplying the nitrogen content of each sample by the factor 6.25. Total nitrogen content was determined by Dumas method (JAOAC 51, 766).

Total carbohydrate was determined by subtracting the dry weight of the sample protein, lipid and ash content [15].

Energy value was calculated by using Atwater index: 1 g of carbohydrate and 1 g of protein provide 4 kcal; 1 g of crude fat provides 9 kcal.

Mineral composition
Mineralization (500°C) of the sample in an ash furnace (Thermo Scientific™ 141 Thermolyne™ 142 6000 series 408) was performed prior to the analysis of Ca and Mg, and similarly for trace elements (Fe and Zn) until the ashes were cleared. The sample was digested with perchloric acid until the destruction of organic matter, as previously described. Mineral contents were performed by inductively coupled plasma atomic emission spectroscopy (ICP - AES) and quantified against standard solutions of known concentration [16].

Amino acid composition
Shyh-Horng *et al.* [17] method was used with minor modifications. About 15 mg of the sample was put in hydrolysis tube with Norleucine 25 µmol.mL^{-1} and acid methane sulfonic under vacuum. The solutions were then placed in an hydrolysis ReactiTherm Heating Module (Pierce, Rockford, USA) for 120 min at 150°C. 4N NaOH was used to stop the reaction after cooling. The solution was adjusted with buffer solution of sodium citrate (pH 2.2), filtered and put in a Biochrom 30+ analyzer (Biochrom, Cambridge, UK) for the determination of total amino acids.

Amino acid indexes were calculated. The quality of a protein is determined by the lowest amino acid score. Protein references were taken according to FAO/OMS/UNU [18].

$$\text{amino acid index} = \frac{\text{amino acid rate in the protein of the sample}}{\text{amino acid rate in the reference protein}} \times 100$$

Ascorbic acid quantification
The methods described by Behrens *et al.* [19] and Mertz *et al.*[20] were used.

Extraction
About 500 mg of fresh sample crushed with liquid nitrogen was mixed two times in 5 mL of 4 % metaphosphoric acid, put under strong agitation for 10 min then centrifuged (10 min; 2397 g; 4°C). The analysis on HPLC was performed on the supernatant. To determine total ascorbic acid, dehydroascorbic acid of the supernatant was reduced with 10 mM TCEP. The quantification was done after 2 hours of incubation.

HPLC analysis
Ascorbic acid was analyzed by reversed phase HPLC using an Agilent 1200 system (Agilent, Santa Clara, USA). They were separated using an Uptisphere C18 column 4.6 x 250 mm x 5 µm (Interchim, Montluçon, France) and a mobile phase made of sulfuric acid 0.01 %. The isocratic flow rate was set at 1 $mL.min^{-1}$, and the injection volume was 20 µL. Quantification of ascorbic acid was performed at 245 nm using a calibration curve of ascorbic acid standard from 0 to 202.5 $mg.L^{-1}$ (r^2 >0.99).

Carotenoids quantification

Preparation of carotenoid extract
Carotenoid extraction was performed with the method described by Dhuique-Mayer *et al.* [21] with some modifications. About 10 mg of freeze-dried sample was extracted two times in ethanol/hexane 4/3 (0.1 % BHT) in a shaker and extractor system Fast Prep ®24 (MpBio, California, USA); then, re-extracted with hexane. The hexanic phases were collected and evaporated to dryness under-vacuum Genevac EZ-2 Plus Centrifuge-Evaporator (Sp Scientific, Warminster, USA). The residue was dissolved in 500 µL MTBE/ MeOH 80/20: v/v and 500 µL CH_2Cl_2 before HPLC injection.

HPLC analysis
Carotenoids were analyzed by reversed phase HPLC using an Agilent 1100 system (Agilent, Santa Clara, USA). They were separated using a YMC C30 column (4.6 x 250 mm; 5 µm) and the mobile phase was water as eluent A, methanol as eluent B, methyl tertiary butylether as eluent C. The flow rate was set at 1 $mL.min^{-1}$, the column was maintained at 25°C, and the injection volume was 20 µL. A solvent gradient was programmed as follows: initial conditions 40 % A-60 % B; 0-7 min, 20% A-80 % B, 7-10 min, 4 % A-81 % B- 15 % C; 10-60 min, 4%A-11 % B-85 % C; 60-71 min, 100 % B; held for 71-72 min, and returned to initial conditions for equilibration. Quantification of β-carotene used the standard calibration curve (r^2 = 0.99).

Statistical analysis
Results were analyzed statistically with Statgraphics plus 5.1, December 2001 (Statpoint Technologies Inc., Virginia, USA). Analysis of variance (ANOVA) was used to show statistical differences between the sample composition; comparison of paired samples, then hypothesis test followed by t-test were used to show statistical differences between the YL and ML of each geographical origin.

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RESULTS AND DISCUSSION

This study gives more information on nutritional properties of *C. asiatica* as a potential GLV.

Nutritional composition of the two foliar morphotypes of *C. asiatica* leaves

Proximate composition

Protein and lipid contents of the samples are presented in Table 1. Reniform leaves of Moramanga had the highest protein content 22 % DW (2.7 % FW) and the highest lipid content: 6 % DW (0.7 % FW).

Mean comparison of the protein content of reniform and round leaves revealed a significant difference ($p < 0.05$). Reniform leaves have higher protein content than round leaves. Higher values of protein were reported by some authors: lower value was reported in *C. asiatica* grown in Florida 19.4 % DW [22] and in *Brassica juncea* 20.5 % DW [23]. As a GLV, *C. asiatica* has the same amount of protein as other GLV but it is less rich in protein than green seed vegetables which are also consumed by Malagasy people in alternation of GLV. The West African food composition table [24] reported higher protein content in white beans boiled (8.0 % FW), lentils (9.3 % FW). Nevertheless, promotion of the consumption of *C. asiatica* as GLV may contribute to the decrease of protein-energy malnutrition of Malagasy people as they are more available and affordable than green seed vegetables.

No significant difference was observed between the mean lipid content of reniform and round leaves. Although, reniform leaves from Moramanga had the highest lipid content among them. Lipid content of *C. asiatica* from Antananarivo was similar as *C. asiatica* from Florida 2.9 % DW. However, lipid content of *Moringa oleifera* (7.1 % DW) was higher than those found in our samples [22]. Green leafy vegetables are not a good source of fat, but addition of oil or oleaginous seeds during the cooking will enhance the nutritional properties of this GLV.

The ash contents were from 13.5 to 16.4 % DW (1.8-2.7 % FW). The round leaves from Analavory had the highest ash content. Lower ash content in *C.asiatica* and *Brassica juncea* were reported, respectively 12.0 % DW, 10.7 % DW [22, 23]. High ash content reflects high mineral content.

Mineral contents of the leaves are presented in Table 2. Mature leaves from Analavory had the highest calcium and magnesium content 0.55 and 0.09 g/100 g FW, respectively. According to the Food and Nutrition Board [24], the consumption of 75 g of leaves from Analavory can cover 41 % of Recommended Dietary Allowance (RDA) of calcium and 17 % of the RDA for magnesium.

Magnesium is beneficial against circulatory diseases and calcium is necessary for bone formations [25].

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Iron and zinc in *C. asiatica* leaves ranged from 3.8 to 12.5 mg/100 g FW and 1.2 to 3.2 mg/100 g FW, respectively. Reniform leaves had higher iron content than round leaves. Consumption of 75 g of reniform leaves can provide more than 70 % of RDA for minerals and consumption of 75 g of round leaves can provide more than 35 % of RDA for minerals. Zinc content of 75 g *C. asiatica* covers less than 24 % of the RDA. Malagasy GLV have less iron and zinc content [3]. For these Malagasy GLV, iron content ranged from 1.8 to 2.1 mg/100 g FW and zinc content from 0.2 to 0.4 mg/100 g FW. Lack of iron is the origin of anemia, it can cause late psychomotor development, it threatens the health of pregnant mother and unborn children. In Madagascar, anemia affects 35 % of women [26].

Amino acid content and protein quality

Seven essential amino acids were present in all the samples (threonine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine), presented in Table 3. Tryptophan was not determined because it needs basic hydrolysis, which was not performed.

Amino acid contents of the leaves were compared to essential amino acid daily requirements for adult by FAO/WHO/UNU [27]. The values were higher than the amino acids required for adults except for requirements in sulfur amino acids.

Essential amino acids represented 40-42 % of total amino acids in *C. asiatica*. Amino acid indexes were calculated in comparison with the standard amino acid pattern (age: more than 2 years old) of FAO/OMS/UNU [27] to evaluate the quality of *C. asiatica'*s protein. Results are shown in Table 4. The leaves can be considered a good source of protein if the amino acid indexes were more than 100 %. The limiting amino acids have the lower amino acid indexes. For leaves, they are the sulfur amino acids [28]. In our study, it was the lysine for the reniform leaves from Antananarivo and the round leaves from Tsiroanomandidy, methionine and cystein for the reniform leaves from Moramanga and the round leaves from Analavory.

Vitamin contents

Vitamin C contents

Vitamin C contents of *C. asiatica* leaves were from 1.3 to 7.7 mg/100 g FW; the reniform leaves from Moramanga had the highest vitamin C content. Significant differences were observed between the vitamin C content of YL and ML from all sites. These contents are lower than those found in other GLV: 42.11 mg/100g FW in *C. asiatica* [29]; 87.2 mg/100 g FW in *Brassica juncea* [23]. Differences may be due to the matrix itself or the method of analysis. *C. asiatica* from Madagascar cannot be considered a good source of vitamin C.

Provitamin A contents

B-Carotene results are presented in Table 5. The round leaves from Analavory had the highest β-carotene content (18.9mg/100 g DW). Mature leaves had higher β-carotene content than YL except for reniform leaves from Antananarivo.

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Reniform leaves of *C. asiatica* had lower β-carotene content than found in other Malagasy GLV. But β-carotene contents of the round leaves (12.1 - 18.6 mg/100 g DW) can be compared to some GLV consumed in Madagascar [3]: *Brassica pekinensis* (15.8 mg/100 g DW); *Manihot esculenta* leaves (18.5 mg/100 g DW), and *Brassica sinensis* (19.3 mg/100 g DW). Higher values were found in watercress leaves (26.1 mg/100 g DW) and in *Brassica campestris* (20 mg/100 g DW).

Similar values of β-carotene in *C. asiatica* leaves were reported [29]: 8.9mg/100 g DW.

Retinol activity equivalent (RAE) was calculated (Table 6) in order to compare vitamin A daily requirement to vitamin A content of the leaves: 1 RAE = 12 µg β-carotene [30].

Daily requirement in vitamin A is 400 RAE for children under 6 years old and 700 RAE for women [31]. Consumption of 100 g of *C. asiatica* leaves could cover 10 to 21 % of vitamin A daily requirements of women for reniform leaves and 21 to 37 % of vitamin A requirement of women for round leaves.

The total vitamin A activity in 100 g FW of *C. asiatica* ranged from 67 to 145 µg RAE for the reniform and from 150 to 259 µg RAE for the round leaves (Table 6). Round leaves are better source of vitamin A for consumption than reniform leaves.

Higher values were found in *C. asiatica* from Sri Lanka: 354 to 643 µg RAE/100g FW [29].

Vitamin A promotes visual health [32]. It is also associated to child mortality as vitamin A is known to enhance the immune system [33]. Daily consumption of foods rich in provitamin A carotenoids is one of the strategies to decrease vitamin A deficiency in Madagascar [34]. *C. asiatica* could be introduced in Malagasy diet among GLV especially the round leaves from Analavory, which have the same amount of provitamin A carotenoids as other GLV consumed in Antananarivo (Madagascar).

CONCLUSION

Results from this study showed that the nutritional composition of *C. asiatica* leaves could be compared to other GLV, especially as a source of protein and provitamin A carotenoids. Protein contents were more than 20 % DW for the reniform leaves from Moramanga; consumption of 100 g of *C. asiatica* round leaves from Analavory can cover more than 30 % of daily requirement of vitamin A in women. They can replace other GLV as rice accompaniment for families because they can be foraged in the rice paddy fields instead of buying other GLV. From the results of this study, the researchers recommend that *C. asiatica* plant be included in the diets of the people of Madagascar so they can benefit from its nutritional properties. The round leaves from Analavory and reniform leaves from Moramanga need further investigation. They were the most interesting samples among the 4 areas of collection regarding their nutritional composition.

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Table 1: Proximate composition of the young and mature leaves of two foliar morphotypes of *C. asiatica* collected in Madagascar (mean of triplicate ± standard deviation; values in the same line with the same letter are not significantly different at p ≤ 0.05)

Leaf morphotype	Reniform				Round			
Collection locality	Antananarivo		Moramanga		Tsiroanomandidy		Analavory	
Stage of maturity of the leaves	Young	Mature	Young	Mature	Young	Mature	Young	Mature
Moisture content (%)	83.1 (0.5) [a]	82.9 (0.5) [a]	88.0 (0.7) [b]	86.2 (0.5) [b]	87.4 (1.2) [b]	87.4 (0.7) [b]	84.0 (0.8) [a]	83.5 (1.0) [a]
N x 6.25 Protein content (% DW)	20.7	18.6	21.9	20.1	20.2	21.3	17.1	16.7
Lipid content (% DW)	2.5 (0.3) [a]	-	6.0 (1.4) [c]	-	3.8 (0.5) [b]	-	4.0 (0.3) [b]	-
Total carbohydrates (% DW)	43.6	-	42.9	-	48.3	-	52.0	-
Energy value (kcal/100 g DW)	280.3	-	307.4	-	308.3	-	311.7	-

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Table 2: Mineral contents of the leaves of the two foliar morphotypes of *C. asiatica* collected in Madagascar

Leaf morphotype	Reniform				Round			
Collection locality	Antananarivo		Moramanga		Tsiroanomandidy		Analavory	
Stage of maturity of the leaves	Young	Mature	Young	Mature	Young	Mature	Young	Mature
Ash content (% FW)	2.41	2.30	1.77	2.18	1.86	1.91	2.40	2.70
Ca (% FW)	0.41	0.40	0.26	0.35	0.28	0.33	0.45	0.55
Mg (% FW)	0.08	0.07	0.05	0.06	0.06	0.07	0.08	0.09
Fe (mg/100 g FW)	7.49	9.78	8.85	12.55	3.79	4.17	5.06	6.09
Zn (mg/100 g FW)	2.26	1.85	2.57	3.07	1.23	2.20	2.74	3.20

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Table 3: Amino acid content (mg/g DW of protein) of the two foliar morphotypes of *C. asiatica* collected in Madagascar and the daily requirement of amino acids according to FAO

Leaf morphotype	**Reniform**				**Round**				**Adult essential amino acid requirements** (mg/g protein per day)*
Collection locality	**Antananarivo**		**Moramanga**		**Tsiroanomandidy**		**Analavory**		
Stage of maturity of the leaves	**Young**	**Mature**	**Young**	**Mature**	**Young**	**Mature**	**Young**	**Mature**	
Histidine	21.1	25.1	23.6	23.6	23.6	23.6	24.0	24.2	15
Isoleucine	38.9	45.9	44.0	43.9	42.1	44.5	43.8	45.9	30
Leucine	69.3	83.0	75.5	76.7	77.0	80.6	81.1	83.6	59
Lysine	45.8	54.6	51.5	51.6	52.5	53.9	55.1	56.0	45
Valine	52.0	59.6	56.9	57.5	56.8	59.0	58.2	60.0	39
Methionine	11.3	13.7	12.9	10.1	7.8	12.3	14.2	16.4	MET + CYS = 22
Phenylalanine	40.6	50.2	47.3	48.2	46.5	49.2	48.6	50.7	PHE + TYR (Tyrosine) = 38
Threonine	36.4	42.7	39.4	40.6	40.0	40.1	41.4	42.8	23
Cysteine	8.5	13.6	8.8	9.0	9.0	9.6	8.7	8.6	
Aspacticacid + Arginine	115.1	143.4	138.0	60.0	151.2	132.5	135.8	142.6	
Proline	55.8	59.7	55.5	58.1	64.3	60.6	52.0	56.8	

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Serine	32.7	38.4	36.1	36.9	36.2	36.8	37.3	38.7	
Glutamicacid	107.4	123.8	121.9	130.5	117.3	118.6	114.8	118.7	
Glutamine	45.6	54.4	50.7	51.8	50.0	52.0	52.6	54.0	
Glycine	49.6	57.2	54.7	55.9	53.7	56.5	56.7	58.9	
Alanine	33.2	48.7	34.3	39.9	52.3	34.6	40.0	45.1	
Arginine	19.8	27.4	21.7	19.1	16.8	21.9	22.9	25.0	

*: *FAO/WHO/UNU. 2007. Protein and amino acid requirements in human nutrition. Report of a joint; FAO/WHO/UNU Expert Consultation. World Health Organ. Tech. Rep. Geneva. Report No.: 724*

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Table 4: Essential amino acid score of the protein of the leaves of *C. asiatica* collected in Madagascar (%)

Leaf morphotype	Reniform				Round			
Collection locality	Antananarivo		Moramanga		Tsiroanomandidy		Analavory	
Stage of maturity of the leaves	Young	Mature	Young	Mature	Young	Mature	Young	Mature
Histidine	111.3	132.1	124.1	124.1	124.1	124.2	126.6	127.2
Isoleucine	139.0	163.8	157.2	156.8	150.2	158.9	156.6	163.9
Leucine	105.0	125.7	114.4	116.2	116.6	122.1	122.8	126.7
Lysine	79.0	94.2	88.8	89.1	90.5	93.0	95.1	96.6
Valine	148.7	170.4	162.7	164.3	162.3	168.6	166.3	171.6
Threonine	107.2	125.7	115.8	119.6	117.6	118.0	121.8	125.9
Methionine + Cysteine	79.0	109.1	86.9	76.5	67.3	87.6	91.4	99.9
Phenylalanine + Tyrosine	117.3	147.0	133.7	136.8	132.3	139.5	138.4	145.5

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Table 5: β-carotene content of *C. asiatica* leaves (mg/100 g DW)

Leaf morphotype	Reniform				Round			
Collection locality	Antananarivo		Moramanga		Tsiroanomandidy		Analavory	
Stage of maturity of the leaves	Young	Mature	Young	Mature	Young	Mature	Young	Mature
β-carotene (mg/100 g DW)	10.32 (0.98) ^{c}	7.37 (0.55) ^{3}	6.76 (0.24) ^{d}	8.54 (0.20) ^{3}	14.36 (0.53) ^{a}	21.33 (0.18) ^{1}	12.11 (0.56) ^{b}	18.86 (1.17) ^{2}

Mean (standard deviation); mean in the same line with the different exponent are significantly different

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Table 6: Retinol Activity Equivalent (RAE) and % of coverage of the daily requirement in vitamin A of *C. asiatica* leaves from Madagascar

Leaf morphotype	**Reniform**				**Round**			
Collection locality	**Antananarivo**		**Moramanga**		**Tsiroanomandidy**		**Analavory**	
Stage of maturity of the leaves	**Young**	**Mature**	**Young**	**Mature**	**Young**	**Mature**	**Young**	**Mature**
µg RAE/100 g FW	145.2 (13.7)	107.9 (9.0)	67.5 (2.4)	97.2 (2.4)	150.3 (5.6)	225.2 (3.5)	160.9 (7.5)	259.0 (16.1)
% coverage of the daily requirement of vitamin A for children*/100 g leaves	36.3 (3.4)	27.0 (2.2)	16.9 (0.6)	24.3 (0.6)	37.6 (1.4)	56.3 (0.9)	40.2 (1.9)	64.7 (4.0)
% coverage of the daily requirement of vitamin A for women*/100 g leaves	20.7 (2.0)	15.4 (1.3)	9.6 (0.3)	13.9 (0.3)	21.4 (0.8)	32.17 (0.5)	23.0 (1.1)	37.0 (2.3)

** FAO/WHO (2002*

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REFERENCES

1. **Badjeck B, Ndiaye CI and M Rakotonirainy** Mission FAO/PAM d'évaluation de la sécurité alimentaire à Madagascar. *Rapport spécial, 23 octobre 2014* [Online] Accessed at: http://www.fao.org/giews/ and http://www.wfp.org/foodsecurity/reports/CFSAM on16th March 2016.

2. **INSTAT (Institut National de la Statistique) et ICF Macro** *Enquête Démographique et de Santé de Madagascar 2008-2009*. Antananarivo, Madagascar : INSTAT et ICF Macro, 2010.

3. **Seevaratnam V, Banumathi P, Premalatha MR, Sundaram SP and T Arumugam** Functional properties of *Centella asiatica* (L.): A REVIEW. *Int J Pharm Sci* 2012; **4**(**5**):8-14.

4. **Randrianatoandro VA, Avallone S, Picq C, Ralison C and S Trèche** Recipes and nutritional value of dishes prepared from green-leafy vegetables in an urban district of Antananarivo (Madagascar). *Int J Food Sc Nutr.*2010;**61**(**4**):404-416.

5. **Rakotonirainy N, Razafindratovo V, Sarter S, Andrianarisoa B, Dabat MH, Ralison C and S Trèche** Fréquences et modalités de consommation des légumes feuilles dans la Commune Urbaine d'Antananarivo (CUA). Communication, Forum national de la recherche, Antsiranana, Madagascar, 3-5 décembre 2008.

6. **Gohil KJ, Patel JA and AK Gajjar** Pharmacological Review on *Centella asiatica*: A Potential Herbal Cure-all. *Indian J PharmSci* 2010; **72**(**5**): 546-556.

7. **Rouillard-Guellec F, Robin J, Rakoto Ratsimamanga A, Ratsimamanga S, and P Rasaoanaivo** Étude comparative de *Centella asiatica* d'origine malgache et d'origine indienne. *Acta bot. Gallica* 1997; **144** (**4**):489-493.

8. **Vohra K, Pal G, Gupta VK, Singh S and Y Bansal** An insight on *Centella asiatica* linn.: A review on recent research. *Pharmacologyoline* 2011; **2**: 440-462.

9. **Hashim P, Sidek H, Helan MHM, Sabery A, Palanisamy UD and M Ilham** Triterpene Composition and Bioactivities of *Centella asiatica. Molecules* 2011; **16**:1310-1322.

10. **Gupta S, Gowri BS, Jyothi Lakshmi A and J Prakash** Retention of nutrients in green leafy vegetables on dehydration. *J Food Sci Technol* 2013; **50(5)**: 918–925.

11. **Rakotondralambo RSO, Rodier Goud M, Rivallan R, Lussert A, Danthu P, Frédéric de Lamotte, Ralambofetra E, Ramavovololona P, Noyer JL and FC Baurens** Insight into biology, genetics and evolution of the *Centella asiatica* polyploid complex in Madagascar. *Industrial Crops and Products* 2013; **47**:118-125.

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 19 No. 3 June 2019 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

12. **Rahajanirina V, Rakotondralambo Raoseta SO, Roger E, Razafindrazaka H, Pirotais S, Boucher M and P Danthu** The Influence of Certain Taxo-nomic and Environmental Parameters on Biomass Production and Triterpenoid Content in the Leaves of *Centella asiatica* (L.) Urb. from Madagascar. *Chemistry & Biodiversity* 2012; **9**:298-308.

13. **AOAC.** Official methods of analysis. 16th ed. Washington, DC: Association of Official Analytical Chemists. 1995.

14. **AOAC.** Crude fat in feeds, cereal grains and forages (hexane extraction). Association of Official Analytical Chemists. AOAC 2003.05. 18th ed. Washington, DC: AOAC International. 2003.

15. **Adrian J, Potus and R Frangne** La science alimentaire de A à Z. Technique et documentation Lavoisier. Paris, France, 1995.

16. **Pinta M** Méthode de référence pour la détermination des éléments Ca, Mg, Fe, Mn, Zn et Cu par Absorption atomique. *Oléagineux* 1973; **28**(**2**):87-92.

17. **Shyh-Horng C and W Kung-Tsung** Simplified protein hydrolysis with methanesulfonic acid at elevated temperature for the complete amino acid analysis of proteins. *J Chromatogr* A 1998; **448**: 404-410.

18. **FAO/OMS/UNU.** Besoins énergétiques et besoins en protéines. Genève, Suisse: Série Rapports Techniques 1986: 724, OMS.

19. **Behrens WA and M Rene** A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic and dehydroascorbic acid in tissues, biological fluids, and foods. *Anal Biochem* 1987; **165**:102-107.

20. **Mertz C, Brat P, Caris-Veyrat C and Z Gunata** Characterization and thermal lability of carotenoids and vitamin C of tamarillo fruit (*Solanumbetaceum Cav.*). *Food Chem* 2010; **119**: 653–659.

21. **Zhang M, Hettiarachchy NS, Horax R, Kannan A, Praisoody AMD, Muhundan A and RC Mallangi** Phytochemicals, antioxidant and antimicrobial anctivity of *Hibiscus sabdariffa*, *Centella asiatica, Moringa oleifera* and *Murraya koenigii* leaves. Jour Med Pl Res 2011: **5(30)**: 6672-6680.

22. **Ng XN, Chye FY and MA Ismail** Nutritional profile and antioxidative properties of selected tropical wild vegetables. *Int Food Res Jour* 2012; **19(4)**: 1487-1496.

23. **FAO.** West African Food Composition Table 2012. ISBN 978-92-5-007207-4.

24. **Jenifer King L, Rita Miller J, James Blue Jr P, William O'Brien Jr D and W John Erdman Jr** Inadequate dietary magnesium intake increases atherosclerotic plaque development in rabbits. *Nutr Res* 2009; **29(5)**: 343-349.

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 19 No. 3
June 2019
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

25. **PNAN.** Plan National d'Action pour la Nutrition III 2017-2021.Antananarivo, Madagascar: Repoblikan'i Madagasikara, 2017.

26. **FAO/WHO/UNU.** Protein and amino acid requirements in human nutrition. Report of a joint FAO/WHO/UNU Expert Consultation. Geneva, Switzerland: World Health Organ. Tech. Rep. Geneva. Report No.: 724. 2007.

27. **Lisiewska Z, Kmiecik W and A Korus** The amino acid composition of kale (*Brassica oleracea* L. var. acephala), fresh and after culinary and technological processing. *Food Chem* 2008; **108(2)**: 642-648.

28. **Akhtar S, Karak C, Biswas P, Chattopadhyay A and P Hazra** Indigenous Leafy Vegetables: A Potential Source of β-Carotene and Ascorbic Acid. *International Journal of Vegetable Science* 2012; **18(4)**: 370-375.

29. **Chandrika UG, Salim N, Wijepala GDDJ, Perera KSU and AKE Goonetilleke** Carotenoid and mineral content of different morphotypes of *Centella asiatica* L. (Gotukola). *Int Journ Food Sc Nutr* 2011; Early Online: 1-6.

30. **FAO/WHO.** Human vitamin and mineral requirements. Bangkok, Thailand: Report of a joint FAO/WHO expert consultation, 2002.

31. **Kao JF, Chui SY, Tsou MJ and WD Chiang** Effects of Chinese domestic cooking methods on the carotenoid composition of vegetables in Taiwan. *Food Sc Tech* 2012; **46**: 485-492.

32. **Semba RD** Vitamin A, Immunity, and Infection. *Clin Infectious Diseases*1994; **119**:489-499.

33. **PNAN.** Plan National d'Action pour la Nutrition 2012-2015.Antananarivo, Madagascar: Repoblikan'i Madagasikara, 2012.