PARTICULAR BENEFITS CAN BE ATTRIBUTED TO *Moringa oleifera* LAM LEAVES BASED ON ORIGIN AND STAGE OF MATURITY

Julien Armel ASSIENE AGAMOU¹, Edith N. FOMBANG^{*, 1} and Carl M. F. MBOFUNG^{1,2}

¹Department of Food Science and Nutrition (ENSAI), University of Ngaoundere, BP 455, Ngaoundere, Adamawa Region, Cameroon

²College of Technology, University of Bamenda, P.O. Box 39, Bamili, North West Region, Cameroon

Received – October 08, 2015; Revision – November 01, 2015; Accepted – December 15, 2015

Available Online – December 17, 2015

DOI: [http://dx.doi.org/10.18006/2015.3\(6\).541.555](http://dx.doi.org/10.18006/2015.3(6).541.555)

KEYWORDS

Moringa oleifera leaves

Maturity

Locality

Nutrient composition

Bioactive compounds

Utilisation

ABSTRACT

Chemical compositions of plants are influenced by the environmental conditions under which they are grown. In the present study, *Moringa oleifera* leaves were collected from eight localities of Cameroon, at two stages of maturity (young and mature). Chemical composition and nutritional benefits of collected plant leaves were studied. Macronutrients and mineral composition of leaves were significantly ($p=0.0001$) influenced by the locality from which they were harvested and the stage of maturity, with significant interactions between them. Principal component analysis was used to group leaves from different localities and at different stages of maturity. Results of the study revealed that mature leaves are richer in proteins, fiber, divalent cations (Ca, Mg, Fe, Mn, Cu) and bioactive compounds, and thus can be used to combat protein and mineral deficiencies, as well as stress related diseases; while the young leaves are more concentrated in available carbohydrates, total carotenoids, K, P, and Zn; and can be used to combat vitamin A and zinc deficiency, as well as for producing energetic foods. Levels of these components vary with the origin of leaves. Calcium, in particular, was three times higher in mature leaves (1.58%) compared to young leaves (0.59%), and mature leaves had one and a half times (20.7%) more iron than young leaves (12.5%). Leaves at different stages of maturity and from different localities are grouped according to their unique nutritional and antioxidant benefits based on their nutrients and bioactive compounds contents.

* Corresponding author

E-mail: edfombang@yahoo.fr (Edith N. FOMBANG)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher
(<http://publisher.jebas.org/index.html>).
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1 Introduction

Moringa oleifera L. is a tree native to Asia and widely cultivated in sub-Saharan Africa (Armelle et al., 2010; Ogbe & Affiku, 2011; Agoyi et al., 2014). Leaves of this tree are well appreciated for their nutritional value and their bioactive compounds contents (polyphenols, phytates) (Fahey, 2005; Moyo et al., 2011), which help in the fight against various chronic diseases (Kumar et al., 2010; Biswas et al., 2012; Agoyi et al., 2014). There are evidences that suggested that *M. oleifera* leaf powder could be successfully employed to combat protein and micro-nutrient malnutrition especially in infants and nursing mothers (Oduro et al., 2008; Armelle et al., 2010; Agoyi et al., 2014). It is also useful as a hypotensive and in the prevention of cancer (Fahey, 2005; Oduro et al., 2008; Kumar et al., 2010; Biswas et al., 2012; Agoyi et al., 2014). The virtues of *M. Oleifera* leaves are closely linked to their chemical compositions which in turn are influenced by environmental factors such as soil type and climatic conditions (Jenab et al., 2001; Sarni-Manchado & Cheynier 2006; Ogbe & Affiku, 2011). In addition, leaf constituents equally show variation with stage of maturity (Yang et al., 2006; Jongrungruangchok et al., 2010; Bamishaiye et al., 2011) and season of harvest (Yang et al., 2006; Shih et al., 2011). These factors could account for the variability in nutrient composition of *M.oleifera* leaves observed in the literature. For instance, Moyo et al. (2011), in South Africa, reported protein contents of 30.29g per 100 g DM (Dry Matter) in *M. oleifera* leaf powders while Ogbe & Affiku (2011), in Nigeria, reported protein contents of 17.01 g per 100 g DM. Variations in nutrient contents of leaves could affect the efficiency of their use.

Cameroon, with a high biodiversity and diversity of foods (Kengué et al., 2008) has experienced an increased growth in the cultivation and consumption of *M. oleifera* leaves throughout the country in the past decade. The leaves are

consumed fresh as a vegetable or dried and reduced into powder. The most utilised form is the powder which is added to foods. However, for the production of *M. oleifera* leaf powder, the leaves are harvested at random with no attention being paid to the stage of maturity or locality of harvest, which could influence the composition of the leaves and consequently their nutritional and therapeutic values. The present study therefore aims at investigating the variation in nutrient composition of *M. oleifera* leaves harvested from eight different localities in Cameroon, at two stages of maturity and attributing particular benefits to them based on their composition.

2 Materials and methods

2.1 Description of localities

Leaves of *M. oleifera* were harvested from eight different localities representing 7 of the 10 Regions of Cameroon (Figure 1). The others 3 Regions are areas of low *M. oleifera* cultivation. The climatic conditions and soil types of these localities are presented in table 1.

2.2 Sampling

Young (bright green coloured leaves at the apex of the branch) and mature (dark green coloured leaves at the base of the branch) leaves of *M. oleifera* Lam were harvested in August 2012 from all the eight localities in Table 1. These colour differences were selected based on the fact that farmers use them to distinguish between young and mature leaves as it facilitates their comprehension and use as opposed to duration after pruning. Two farms (each at least 1000 m²) in each locality, well-kept and without fertilizer application were selected for sampling. Leaves of each maturity stage were collected through a simple random sampling method from both farms early in the morning and pooled together to represent the locality.

Table 1 Climatic conditions and soil type of different localities in Cameroon in August 2012.

Locality	Region	Climatic conditions in August 2012		Soil type
		Mean precipitation (mm)	Mean Temperature (°C) (Minimal – Maximal)	
Bafia (Gouiffé)	Centre	220.7	23.1 (21.0 - 26.6)	Ferralitic
Bertoua (Bonis)	East	181.2	23.1 (20.7 – 27.0)	Ferralitic
Doumé	East	333.1	23.0 (20.6 - 26.7)	Ferralitic
Mbouda (Babété)	West	250.8	18.3 (15.7 – 23.1)	Ferralitic
Edéa	Littoral	220.0	26.2 (24.2 - 29.5)	Yellow soil
Garoua	North	137.1	28.6 (25.0 – 34.0)	Vertisols
Maroua	Far North	197.3	29.9 (25.8 – 36.3)	halomorphic
Méiganga	Adamawa	247.1	21.8 (18.9 – 26.2)	Vertisols

Source: CMIP 5, MPI-ESM-LR RCP

Figure 1 Map of Cameroon showing sampling areas

2.3 Preparation of powders

After harvesting, leaflets were detached from the leaf, washed with clean water and dried at 55°C in an electric dryer (Riviera & Bar QD105A, Paris, France) for 5 hours. The dried leaves were ground (Zaiba ZB-2225, China) and sieved through a 1mm mesh sieve to obtain powder. The powders were then stored in airtight bottles at 4°C prior to analysis.

2.4 Determination of Moisture content

Moisture content of leaf powders was determined according to AOAC (1990) method.

2.5 Evaluation of Nutrient composition

2.5.1 Total protein, ash and carotenoids content estimation

Total protein content ($N \times 6.25$) was determined using the Kjeldahl method (AOAC, 1990). Similarly, ash content of the dried leaf powder was also determined according to the guide line given by AOAC (1990). Total carotenoids content was estimated according to AOAC (1975) method.

2.5.2 Available carbohydrates

Available carbohydrates were determined by the DNS (3,5 Dinitrosalicylic acid) method of Fischer & Stein (1961) following digestion of the samples with H_2SO_4 (1.5N) to breakdown carbohydrates to simple sugars.

2.5.3 Total lipids content

Total lipids content was determined using the soxhlet method as described by Bourely (1982).

2.5.4 Minerals contents

Different minerals like Ca, Mg, K, Na, Cu, Zn, Mn and Fe were analysed after extraction by atomic absorption spectrophotometry (Jones & Vernon, 1990). Phosphorus was analysed using Murphy & Riley (1962) reagent and read colorimetrically.

2.5.5 Vitamin C

Amount of vitamin C was determined by titrimetric method with some modifications (Tomohiro, 1990). One gram of powder was mixed with 10 ml of acetic acid (90%). To this was added 5 ml of distilled water and the whole mixed with a magnetic stirrer. Ascorbic acid was evaluated by measuring dehydroascorbic acid obtained after oxidation of ascorbic acid by 2,6-dichlorophenol-indophenol. During titration, acetic acid (10%) was used as colour indicator and the end of titration was reached when the colour of the solution changed from purplish pink to colourless. Ascorbic acid (10%) was used as standard and results were expressed as mg ascorbic acid per 100 g of dry matter.

2.5.6 Total fiber content

Total fiber was determined as described by the method given by Wolff (1968) with little modifications.

2.6 Determination of Bioactive components

2.6.1 Total polyphenols contents

Total polyphenols were determined according to Makkar et al. (1993) with some modification. Ten (10 ml) of ethanol (70%) was added to 0.5 g of powder and the mixture stirred for 2 hours (using a Prolabo 54 433 agitator, Paris, France) at 220 rpm, to extract total polyphenols. After centrifuging (DL 6000 mark, rotor 15 cm, Japan) at 3000g for 20 min at 4°C, 0.02 ml of the supernatant was mixed with 0.2 ml of Folin-Ciocalteu reagent diluted (1/16), 0.4 ml of sodium carbonate (20%) and 1.38 ml distilled water. The mixture was vortexed and incubated in a water bath at 40°C for 20 minutes in darkness. Gallic acid (0.2g/l) was used as standard and absorbance was read at 725 nm.

2.6.2 Total tannins contents

Total tannins were determined according to Makkar et al. (1993). Polyphenol extract (1 ml) and 1ml of distilled water were precipitated with 0.1 g of PVPP (polyvinyl polypyrrolidone) to remove total tannins (100 mg PVPP is sufficient to bind 2 mg of tannins). Total polyphenols in the supernatant was determined as previously described in section 2.6.1 and the total tannins content obtained by difference with the total polyphenols before precipitation.

2.6.3 Phytates contents

Phytates were determined as described by Vaintraub & Lapteva (1988) with some modifications. Phytates were extracted from 0.5 g of powder suspended in 10 ml of 3.5% HCl for 2 hours using a prolabo 54 433 agitator Paris, France. The extract was centrifuged (DL 6000, rotor 15 cm, Japan) at 1000 g for 20 minutes at 10°C. The supernatant was treated with 1 g sodium chloride and kept at 4°C for 24 hours. This was later centrifuged at 1000 g for 10 minutes at 10°C. A 3 ml aliquot of the supernatant was added to 1 ml of modified Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ + 0.3% sulfo-salicylic acid) and centrifuged at 1000 g for 10 min at 10 °C. The absorbance was read at 500 nm. Sodium phytate solution (40 µg per ml) was used as standard (100 g of sodium phytate equals 59.9 g of phytic acid).

2.7 Statistical analysis

Analyses were carried out in triplicates. Microsoft Excel 2010 software was used for calculation of means and standard deviations; Stat-graphic centurion 15.2 software (StatPoint Technologies, Inc, Warrenton, Virginia, USA) for analysis of variance and means separated using the Duncan multiple range test at $P < 0.05$.

Table 2 Influence of stage of maturity and locality on macronutrients contents of young and mature leaves of *M. oleifera* (g/100 g DM).

Maturity of leaves	Locality	Moisture content	Total proteins	Available carbohydrate	Total lipids	Total ash	Total Fibers
Young Leaves	Bafia	11.5 ± 0.5 ^a	20.36 ± 0.48 ^a	34.86 ± 0.1 ^a	7.64 ± 1.14 ^{abc}	9.94 ± 0.45 ^{abc}	23.61 ± 1.01 ^a
	Bertoua	10.5 ± 0.5 ^a	21.51 ± 0.37 ^a	36.65 ± 1.74 ^{ab}	9.10 ± 0.10 ^{ac}	7.18 ± 0.55 ^{ab}	22.65 ± 0.55 ^{ac}
	Doumé	11.5 ± 0.2 ^a	21.88 ± 0.05 ^{ac}	37.15 ± 1.17 ^a	8.83 ± 0.05 ^{ac}	8.89 ± 0.05 ^{abc}	20.44 ± 0.55 ^{ab}
	Edéa	9.5 ± 0.5 ^a	22.70 ± 0.54 ^{abc}	33.14 ± 1.03 ^a	9.00 ± 0.44 ^{ac}	8.06 ± 0.53 ^{abc}	23.92 ± 1.34 ^{ac}
	Mbouda	13.5 ± 0.5 ^b	25.89 ± 0.09 ^a	33.97 ± 0.22 ^{ab}	8.53 ± 0.65 ^{abc}	8.02 ± 0.93 ^{ab}	21.14 ± 1.71 ^{abc}
	Garoua	8 ± 0.6 ^b	22.41 ± 0.13 ^{abc}	34.93 ± 0.35 ^a	9.64 ± 1.00 ^{ab}	9.00 ± 0.21 ^{ab}	20.05 ± 0.16 ^a
	Maroua	9 ± 0.2 ^{ac}	24.03 ± 0.48 ^{ab}	34.55 ± 0.82 ^{ab}	8.16 ± 0.62 ^{abc}	9.49 ± 0.55 ^{abc}	21.12 ± 0.12 ^{abc}
	Méiganga	9 ± 0.5 ^{ac}	22.12 ± 0.01 ^{ac}	35.41 ± 0.38 ^b	6.75 ± 0.53 ^a	9.89 ± 0.05 ^{abc}	22.40 ± 1.63 ^{abc}
	Mean	10.31 ± 0.44	22.61 ± 0.27	35.08 ± 0.73	8.46 ± 0.57	8.90 ± 0.45	21.92 ± 0.88
Matures Leaves	Bafia	11.5 ± 0.5 ^a	23.74 ± 0.02 ^b	26.90 ± 0.72 ^a	10.78 ± 0.51 ^a	9.15 ± 0.11 ^{ab}	26.21 ± 2.71 ^b
	Bertoua	9.5 ± 0.5 ^a	23.98 ± 0.89 ^{ab}	30.70 ± 0.07 ^a	8.15 ± 0.53 ^{abc}	6.81 ± 0.11 ^a	27.03 ± 0.44 ^b
	Doumé	9.5 ± 0.5 ^a	23.35 ± 1.18 ^{abc}	28.46 ± 0.18 ^a	10.67 ± 0.74 ^a	8.95 ± 0.08 ^{ac}	25.64 ± 1.24 ^{ab}
	Edéa	7 ± 0.3 ^a	24.46 ± 0.95 ^{ab}	27.91 ± 0.17 ^{ab}	6.82 ± 0.54 ^a	8.95 ± 0.11 ^{ac}	27.95 ± 1.65 ^b
	Mbouda	12.5 ± 0.5 ^b	23.55 ± 0.18 ^{ab}	28.27 ± 1.33 ^{ab}	8.02 ± 0.04 ^{abc}	10.46 ± 0.05 ^a	26.70 ± 1.04 ^b
	Garoua	9 ± 0.3 ^{ac}	22.16 ± 0.91 ^{ac}	29.24 ± 0.62 ^{abc}	8.64 ± 1.23 ^{abc}	10.32 ± 0.54 ^{ab}	26.90 ± 0.27 ^b
	Maroua	10.5 ± 0.5 ^a	22.72 ± 0.81 ^{abc}	29.95 ± 1.55 ^{ac}	8.67 ± 1.21 ^{abc}	7.7 ± 1.09 ^{ab}	27.58 ± 0.76 ^b
	Méiganga	8.5 ± 0.5 ^{ac}	24.95 ± 0.33 ^{ab}	28.64 ± 1.06 ^{ac}	7.36 ± 0.04 ^{ab}	8.84 ± 0.05 ^{abc}	26.92 ± 0.54 ^b
	Mean	9.75 ± 0.45	23.61 ± 0.66	28.76 ± 0.71	8.64 ± 0.60	8.97 ± 0.50	26.87 ± 1.08
Probability	Maturity (M)	0.0365	0.0000	0.0000	0.3795	0.5876	0.0000
	Locality (L)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0071
	Interaction (ML)	0.0061	0.0000	0.0053	0.0000	0.0000	0.0917

Values are means ± standard deviation (n = 3). Means in the same column with different superscripts are significantly different from each other (P < 0.05). Values in bold within each stage of maturity represent maximum and minimum contents

Principal component analysis (PCA) was used to group leaves according to their chemical composition. XL-stat 2007 software was used for that purpose.

3 Results

3.1 Moisture contents

Mean moisture content of young and mature leaf powders of *M. Oleifera* were 10.31 and 9.75 g per 100g of DM respectively (Table 2). Moisture content ranged from 8 to 13.50% in young leaf powders while it ranged from 7 to 12.50% in mature leaf powders.

3.2 Macronutrients composition

3.2.1 Total protein content

Mean protein contents were significantly (p<0.05) higher in mature leaves (23.61g/100g DM) compared to young leaves (22.61g/100g DM), with the exception of Mbouda and Maroua localities (Table 2). Protein content of leaves was also significantly (P=0.0001) influenced by locality and stage of

maturity, but among these two locality had a greater influence on protein contents, 31.57% than maturity 11.98%, with their interaction having a cumulative effect 45.04%. Mbouda and Meiganga localities had the highest total protein contents for young (25.89% DM) and mature (24.95% DM) leaves respectively; whereas Bafia (20.36% DM) and Garoua (22.16% DM) had respectively the lowest.

3.2.2 Available carbohydrates contents

Unlike proteins, available carbohydrate contents were significantly (p<0.05) higher in young leaves (35.08 ± 0.73 g per 100g DM) as compared to mature leaves (28.76 ± 0.71 g per 100g DM) (Table 2) for all localities. This is in agreement with the observed significant (p=0.0001) effect of locality and stage of maturity on total available carbohydrates content, with maturity having a greater influence 83.86%. Available carbohydrate content ranged from 33.14% to 37.15% DM in young leaves while it ranged from 27.91% to 30.70% DM in mature leaves. *M. oleifera* leaves collected from Doume and Bertoua had the highest available carbohydrate contents whereas those from Edéa and Bafia had the lowest available contents for young and mature leaves respectively.

Table 3 Influence of stage of maturity and locality on mineral contents of young and mature leaves of *M. Oleifera*.

Maturity of leaves	Locality	Macro-minerals (g/100g DM)					Micro-minerals (mg/100g DM)			
		Ca	Mg	K	Na	P	Fe	Zn	Mn	Cu
Young Leaves	Bafia	0.51 ± 0.03 ^{bc}	0.33 ± 0.02 ^{def}	2.71 ± 0.27 ^{hi}	0.03 ± 0.01 ^a	0.63 ± 0.03 ^{sh}	14.84 ± 1.02 ^{cd}	2.63 ± 0.44 ^{fb}	4.42 ± 0.75 ^{cdcf}	0.40 ± 0.02 ^{fb}
	Bartoua	0.37 ± 0.02 ^{ab}	0.27 ± 0.02 ^{abc}	1.69 ± 0.17 ^{ac}	0.08 ± 0.02 ^{abc}	0.39 ± 0.02 ^a	12.30 ± 0.84 ^a	1.77 ± 0.30 ^{abc}	3.36 ± 0.57 ^{accc}	0.32 ± 0.02 ^{ab}
	Doumé	0.33 ± 0.02 ^a	0.23 ± 0.01 ^a	2.05 ± 0.2 ^{ccc}	0.07 ± 0.02 ^{abc}	0.45 ± 0.02 ^a	10.29 ± 0.71 ^a	2.21 ± 0.37 ^{acd}	2.44 ± 0.41 ^a	0.30 ± 0.01 ^a
	Edéa	0.37 ± 0.02 ^{ab}	0.29 ± 0.02 ^{abc}	2.30 ± 0.23 ^{cb}	0.07 ± 0.02 ^{abc}	0.58 ± 0.03 ^{acd}	12.45 ± 0.85 ^a	2.87 ± 0.48 ^{sh}	2.66 ± 0.45 ^{ab}	0.34 ± 0.02 ^{ab}
	Mbouda	0.68 ± 0.05 ^{de}	0.35 ± 0.02 ^{cd}	2.95 ± 0.3 ⁱ	0.43 ± 0.02 ⁱ	0.65 ± 0.03 ^b	13.83 ± 0.95 ^{bc}	3.23 ± 0.54 ^b	3.34 ± 0.56 ^{accc}	0.40 ± 0.02 ^{fb}
	Garoua	0.83 ± 0.06 ^e	0.29 ± 0.03 ^{abc}	2.57 ± 0.26 ^{sh}	0.24 ± 0.07 ^c	0.55 ± 0.03 ^a	12.26 ± 0.84 ^{ab}	2.40 ± 0.40 ^{fb}	2.57 ± 0.43 ^{ab}	0.30 ± 0.01 ^a
	Maroua	1.03 ± 0.07 ^e	0.35 ± 0.02 ^{cd}	2.45 ± 0.24 ^{sh}	0.18 ± 0.05 ^{de}	0.60 ± 0.03 ^{cb}	12.51 ± 0.86 ^a	2.61 ± 0.44 ^{fb}	4.74 ± 0.80 ^{cdcf}	0.33 ± 0.02 ^{ab}
	Méiganga	0.59 ± 0.04 ^{cd}	0.28 ± 0.03 ^{abc}	2.55 ± 0.25 ^{sh}	0.10 ± 0.03 ^{bc}	0.56 ± 0.03 ^{ac}	12.24 ± 0.84 ^{ab}	1.98 ± 0.33 ^{abc}	2.70 ± 0.45 ^{ab}	0.30 ± 0.01 ^a
	Mean	0.59 ± 0.04	0.30 ± 0.02	2.41 ± 0.24	0.15 ± 0.03	0.55 ± 0.03	12.59 ± 0.86	2.46 ± 0.41	3.28 ± 0.55	0.34 ± 0.02
Mature Leaves	Bafia	1.63 ± 0.12 ⁱ	0.52 ± 0.03 ^g	2.11 ± 0.21 ^{def}	0.07 ± 0.02 ^{abc}	0.54 ± 0.03 ^d	19.41 ± 1.33 ^{ef}	1.91 ± 0.32 ^{bcd}	11.62 ± 1.97 ⁱ	0.45 ± 0.03 ^h
	Bartoua	1.36 ± 0.10 ^h	0.33 ± 0.02 ^{acd}	1.18 ± 0.11 ^a	0.04 ± 0.01 ^{ab}	0.27 ± 0.01 ^a	19.12 ± 1.31 ^e	0.93 ± 0.15 ^a	5.95 ± 1.00 ^{sh}	0.36 ± 0.02 ^{ccc}
	Doumé	1.83 ± 0.14 ^j	0.34 ± 0.02 ^{cd}	1.64 ± 0.16 ^b	0.23 ± 0.07 ^c	0.40 ± 0.02 ^a	19.36 ± 1.33 ^{ef}	1.38 ± 0.23 ^{ab}	6.46 ± 1.09 ^h	0.38 ± 0.02 ^{acd}
	Edéa	1.19 ± 0.09 ^g	0.49 ± 0.03 ^f	1.57 ± 0.15 ^a	0.09 ± 0.02 ^{abc}	0.61 ± 0.03 ^{gh}	22.57 ± 1.55 ^h	2.07 ± 0.35 ^{acd}	4.79 ± 0.81 ^{sh}	0.39 ± 0.02 ^{cd}
	Mbouda	1.84 ± 0.14 ^j	0.57 ± 0.04 ^h	2.29 ± 0.23 ^{cb}	0.19 ± 0.05 ^{de}	0.42 ± 0.02 ^{ac}	22.54 ± 1.55 ^h	1.86 ± 0.31 ^{abcd}	5.14 ± 0.87 ^{gh}	0.41 ± 0.02 ^{gh}
	Garoua	1.18 ± 0.09 ^g	0.31 ± 0.02 ^{ccc}	2.18 ± 0.22 ^{acd}	0.20 ± 0.06 ^c	0.45 ± 0.02 ^a	16.42 ± 1.13 ^d	1.73 ± 0.29 ^{abc}	3.04 ± 0.51 ^{ccc}	0.25 ± 0.01 ^a
	Maroua	1.84 ± 0.14 ^j	0.37 ± 0.02 ⁱ	1.90 ± 0.19 ^{ccc}	0.13 ± 0.03 ^{cd}	0.43 ± 0.02 ^{ac}	25.09 ± 1.73 ⁱ	1.53 ± 0.25 ^{abc}	5.86 ± 0.99 ^{sh}	0.43 ± 0.03 ^{sh}
	Méiganga	1.76 ± 0.13 ^j	0.26 ± 0.01 ^{ab}	2.17 ± 0.22 ^{acd}	0.09 ± 0.02 ^{abc}	0.41 ± 0.02 ^{ac}	21.26 ± 1.46 ^{gh}	1.55 ± 0.26 ^{ac}	3.99 ± 0.67 ^{cccd}	0.34 ± 0.02 ^{cd}
	Mean	1.58 ± 0.12	0.40 ± 0.03	1.88 ± 0.19	0.13 ± 0.03	0.44 ± 0.02	20.72 ± 1.43	1.62 ± 0.27	5.86 ± 0.99	0.38 ± 0.02
Probability	Maturity (M)	0.0000	0.0000	0.0000	0.1910	0.0000	0.0000	0.0000	0.0000	0.0000
	Locality (L)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Interaction (ML)	0.0000	0.0000	0.8236	0.0000	0.0000	0.0000	0.5029	0.0000	0.0003
Values are means ± standard deviation (n = 3). Means in the same column with different superscripts are significantly different from each other (P < 0.05) Values in bold within each stage of maturity represent maximum contents										

Table 4 Influence of stage of maturity and locality on total carotenoids and vitamin C contents in young and mature leaves of *M. oleifera* (mg/100g DM).

Maturity of leaves	Locality	Total carotenoids	Vitamin C
Young Leaves	Bafia	88.20 ± 1.72 ^d	56.43 ± 1.32 ⁱ
	Bertoua	83.01 ± 1.55 ^c	58.27 ± 1.13 ⁱ
	Doumé	116.41 ± 1.12 ^j	44.15 ± 1.29 ^{bc}
	Edéa	129.19 ± 0.54 ^k	39.33 ± 1.42 ^a
	Mbouda	153.06 ± 1.74 ^l	50.53 ± 1.51 ^{fg}
	Garoua	115.07 ± 1.54 ^{ij}	58.27 ± 0.80 ⁱ
	Maroua	106.45 ± 1.28 ^f	46.61 ± 1.31 ^{cde}
	Méiganga	108.31 ± 1.53 ^{fg}	48.16 ± 1.28 ^{ef}
	Mean	112.46 ± 1.38	50.22 ± 1.26
Mature Leaves	Bafia	109.24 ± 1.15 ^{gh}	53.28 ± 1.49 ^h
	Bertoua	66.98 ± 0.85 ^a	58.43 ± 1.96 ⁱ
	Doumé	94.83 ± 1.44 ^c	43.49 ± 1.65 ^b
	Edéa	86.19 ± 1.11 ^d	45.45 ± 1.62 ^{bcd}
	Mbouda	113 ± 1.91 ⁱ	51.62 ± 2.37 ^{gh}
	Garoua	111.46 ± 1.94 ^b	61.31 ± 0.95 ^j
	Maroua	79.33 ± 0.28 ^b	45.52 ± 1.61 ^{bcd}
	Méiganga	80.17 ± 0.84 ^b	47.13 ± 1.29 ^{de}
	Mean	92.77 ± 1.19	50.78 ± 1.62
Probability	<i>Maturity (M)</i>	0,0000	0,2020
	<i>Locality (L)</i>	0,0000	0,0000
	<i>Interaction (ML)</i>	0,0000	0,0003

Values are means ± standard deviation (n = 3). Means in the same column with different superscripts are significantly different from each other (P < 0.05); Values in bold within each stage of maturity represent maximum contents.

3.2.3 Total lipids contents

Young (8.46 ± 0.57g per 100g DM) and mature leaves (8.64 ± 0.60 g per 100g DM) did not show any significant differences in lipid contents and accordingly maturity had no significant (p=0.37) influence on lipids contents (table 2). Locality however, significantly (p=0.0001) influenced lipid content as there exists significant differences between localities for the same maturity stage.

3.2.4 Total ash

Young and mature leaves had mean total ash contents of 8.90 ± 0.45 and 8.97 ± 0.50 g per 100 g DM respectively (table 2) with no significant (p>0.05) difference between them. However from one locality to another there was significant difference (p=0.0001) in ash content.

3.2.5 Total fiber contents

Fiber are the non-digestible polysaccharide residues of plants, and mean total fiber was significantly (p<0.05) higher in mature leaves (26.87 ± 1.08 g per 100 g DM) compared to

young leaves (21.92 ± 0.88 g per 100 g DM). Locality and stage of maturity had significant (p = 0.0001) effects on fiber contents (table 5), with maturity it contributing more (74.49%) to the effects than locality. However, the locality of Edea had the highest total fiber for both stages maturity.

3.3 Mineral and Vitamin composition

3.3.1 Macro-minerals (Ca, Mg, K, Na, P)

The mineral composition of *M. oleifera* leaf powders from different localities are presented on Table 3. Mean contents of macro-minerals (g per 100g DM) in young and mature leaves were respectively 0.59 and 1.58 for Ca; 0.30 and 0.40 for Mg; 2.41 and 1.88 for K; 0.15 and 0.13 for Na; 0.55 and 0.44 for P. Magnesium was significantly (p<0.05) higher in mature leaves whereas potassium and phosphorus presented significantly (p<0.05) higher contents in young leaves. Sodium content remained unchanged with maturity, whereas Calcium content tripled in mature leaves compared to young leaves. Leaf macro-mineral composition was significantly (p=0.0001) influenced by locality and stage of maturity, with the exception of sodium where only locality had a significant influence.

However when we consider the sum of squares of these two factors we realise that locality has a greater effect on magnesium (46.21%), potassium (55%), sodium (60.41%) and phosphorus (56.39%) contents, whereas maturity has a greater influence on calcium content (77.91%) which is evident in the tripling of calcium content with maturity. Considering the young leaves, the locality of Mbouda has the highest concentration of the different macro-minerals, with the exception of calcium.

3.3.2 Micro-minerals (Fe, Zn, Mn, Cu)

Micro-minerals (Fe, Zn, Mn, Cu) content was also significantly ($p=0.0001$) influenced by locality and stage of maturity. Maturity had a greater influence on iron (77.18%) and zincs (42.40%) contents, whereas locality had a greater influence on manganese (41.39%) and copper (62.51%) contents. Iron, manganese and copper are more concentrated in mature leaves compared to young leaves which are more concentrated in zinc. Young and mature leaves of *M. oleifera* have respectively mean contents (mg per 100g DM) of 12.59 and 20.72 for Fe; 2.46 and 1.62 for Zn; 3.28 and 5.86 for Mn; 0.34 and 0.38 for Cu. Mbouda and Maroua localities had highest iron contents in young and mature leaves respectively. Mbouda had equally highest concentration of micro-minerals for young leaves, except in the case of manganese.

3.3.3 Total carotenoids contents

Except in Bafia, carotenoids were significantly ($p<0.05$) more concentrated in young leaves compared to mature leaves. Locality and maturity both had significant ($p=0.0001$) influences on carotenoids contents, with locality accounting for (57.20%) of this effect. Mean total carotenoids contents in young and mature leaves of *M. oleifera* were 112.46 ± 1.38 and 92.77 ± 1.19 mg per 100 g DM respectively (table 4). Mbouda locality had the highest total carotenoids contents at both maturity stages.

3.3.4 Vitamin C contents

The stage of maturity of the leaves had no significant ($p=0.20$) effect on their vitamin C content, but this was significantly ($p=0.0001$) influenced by the origin (locality) of the leaves. Young and mature leaves had vitamin C contents of 50.22 ± 1.26 and 50.78 ± 1.62 mg per 100g DM respectively (table 4).

3.4 Bioactive compounds (Total polyphenols, Tannins, Phytates)

Table 5 shows the bioactive compound contents of *M. oleifera* leaf powders collected from the eight different localities. Neither the origin of the leaves, nor the stage of maturity had any significant ($p>0.05$) influence on polyphenols and tannin contents with essentially the same values between localities and between maturities. Mean total polyphenols contents in young and mature leaves were 1.12 ± 0.04 and 1.17 ± 0.04 g

per 100g DM respectively. Among the young leaves, the locality of Bafia had the highest concentration of bioactive compounds.

Tannins contents of young and mature leaves were 0.42 ± 0.04 and 0.47 ± 0.05 g per 100g DM respectively (table 5) and no significant ($p>0.05$) difference was reported between these two. Tannins were positively correlated with total polyphenol ($r=0.867$; $p<0.05$, Table 7).

On the contrary, the individual effect of locality and maturity as well as the interaction between them significantly ($p=0.0001$) influenced phytates content obtained in this study; but the sum of squares of each factor indicated that locality had a greater influence (59%). Mean phytate contents were significantly ($p<0.05$) higher in mature leaves (340.04) compared to young leaves (312.77) mg per 100 g DM (table 5). The locality of Bafia had the highest phytate content for both young and mature leaves. Phytates in this study, show a significant negative correlation with zinc ($r = -0.574$, $p<0.05$, Table 7).

4 Discussions

4.1 Chemical composition of *M. oleifera* as influenced by locality and maturity

4.1.1 Macronutrients

The locality from which leaves were harvested and their maturity stage had a significant ($p=0.001$) influence on their macronutrients composition (proteins, available carbohydrates, fiber), with the exception of ash and lipid contents which were only significantly ($p=0.001$) affected by locality (table 2). Protein contents of leaves are usually influenced by soil nitrogen content, and soil nitrogen varies from one locality to another and with soil type thereby affecting plant nitrogen concentration. Nitrogen is used for the synthesis of amino acids and proteins which are stored in leaves and other parts of the plant (Folly, 2000).

Plants use these reserves for their growing needs. Young leaves, being at a more active stage of growth, will use much of these reserves for their growth (Zufferey, 2000), reason for the significant ($p<0.05$) lower protein contents observed in young leaves (22.61%) compared to mature leaves (23.61%). Values of protein recorded in this study are similar to those of Shih et al. (2011) in Taiwan but lower than the 28.34 and 31.08% DM reported earlier by Yang et al. (2006) respectively for young and mature leaves in Taiwan. However, the fact that mature leaves had higher protein contents compared to young leaves is consistent with present study observations. *M. oleifera* proteins are said to be of good quality as Sánchez-Machado et al. (2010) in Mexico had reported that they are rich in the essential amino acids with the exception of methionine which is generally deficient in green leaves.

Table 5 Influence of stage of maturity and locality on bioactive compounds in *M. oleifera* leaf powder (g/100gDM).

Maturity of leaves	Locality	Total polyphenols	Total tannins	Phytates*
Young Leaves	Bafia	1.67 ± 0.04 ^c	0.72 ± 0.03 ^c	339.61 ± 2.87 ^h
	Bertoua	1.03 ± 0.01 ^{abcd}	0.36 ± 0.04 ^{abc}	326.58 ± 2.81 ^{ef}
	Doumé	1.12 ± 0.07 ^{abcd}	0.41 ± 0.08 ^{abc}	314.30 ± 2.12 ^c
	Edéa	0.90 ± 0.03 ^a	0.61 ± 0.01 ^{abc}	277.77 ± 1.79 ^a
	Mbouda	1.03 ± 0.08 ^{abc}	0.23 ± 0.07 ^a	313.65 ± 3.96 ^c
	Garoua	1.01 ± 0.03 ^{abc}	0.29 ± 0.04 ^{abc}	281.44 ± 1.05 ^a
	Maroua	0.95 ± 0.03 ^{ab}	0.26 ± 0.05 ^{ab}	323.40 ± 3.87 ^{de}
	Méiganga	1.23 ± 0.08 ^{bcd}	0.51 ± 0.07 ^{abc}	325.44 ± 3.79 ^{def}
	Mean	1.12 ± 0.04	0.42 ± 0.04	312.77 ± 2.78
Mature Leaves	Bafia	1.16 ± 0.06 ^{abcd}	0.55 ± 0.09 ^{abc}	366.29 ± 2.82 ⁱ
	Bertoua	1.33 ± 0.03 ^d	0.51 ± 0.06 ^{abc}	364.92 ± 1.19 ⁱ
	Doumé	1.18 ± 0.01 ^{abcd}	0.50 ± 0.08 ^{abc}	362.83 ± 3.92 ⁱ
	Edéa	1.28 ± 0.08 ^{cd}	0.70 ± 0.08 ^{bc}	304.17 ± 1.40 ^b
	Mbouda	1.08 ± 0.05 ^{abcd}	0.23 ± 0.02 ^a	321.77 ± 1.92 ^d
	Garoua	1.03 ± 0.01 ^{abc}	0.33 ± 0.03 ^{abc}	331.52 ± 1.81 ^g
	Maroua	1.08 ± 0.02 ^{abcd}	0.42 ± 0.01 ^{abc}	340.65 ± 1.83 ^h
	Méiganga	1.23 ± 0.08 ^{bcd}	0.55 ± 0.03 ^{abc}	328.14 ± 1.90 ^{fg}
	Mean	1.17 ± 0.04	0.47 ± 0.05	340.04 ± 2.10
Probability	<i>Maturity (M)</i>	0.4897	0.1767	0.0000
	<i>Locality (L)</i>	0.1849	0.2588	0.0000
	<i>Interaction (ML)</i>	0.2106	0.5461	0.0000

Values are means ± standard deviation (n = 3). Means in the same column with different superscripts are significantly different from each other (P < 0.05); *mg/100 g DM; Values in bold within each stage of maturity represent maximum contents.

Plant leaves are the site for photosynthetic activity which produces carbohydrates for energy and other needs of the plant. According to Zufferey (2000) this photosynthetic activity is more intense in young leaves in active stage of growth and needing more nutrients for growth, compared to mature ageing leaves. This explains why available carbohydrates were systematically significantly (p < 0.05) higher in young leaves (35.08%) compared to mature leaves (28.76%) of *M. oleifera* for each locality (table 2), and why maturity had a greater influence 83.86% on carbohydrate content than locality. Available carbohydrate contents of young leaves in this study are closer to the range of 35.7 to 43.3 g per 100 g DM reported by Yameogo et al. (2011) for *M. oleifera* leaves in Burkina Faso. Similar observations of higher carbohydrates content in young leaves compared to mature leaves of *M. oleifera* have been made by Yang et al. (2006) in Taiwan; and by Bamishaiye et al. (2011) in Nigeria.

Lipid contents for young and mature leaves were high (8.46 ± 0.57 and 8.64 ± 0.60 g per 100g DM) with no significant differences between maturities. These values are within the range of 4 to 9 % DM reported by Sánchez-Machado et al. (2010) in leaves of *M. oleifera* in Mexico. *M. oleifera* leaf lipids appear to be of good nutritional quality as they have been reported to be rich in unsaturated fatty acids (70%) (Sánchez-Machado et al., 2010).

Fibers are more elaborated in mature plant organs (Foulk et al., 2011) given that mature leaves have in addition to a primary

cell wall (made up of fiber - pectin, cellulose, hemi cellulose), a rigid secondary cell wall which is also constituted of several fibers - lignin, and cellulose (Cheftel et al., 1984). Therefore, even though locality and maturity had significant (p = 0.0001) effects on fiber contents (table 5), maturity contributed more (74.49%) of the effects than locality. Fiber contents in this study ranged from 20.05 to 27.95g per 100 g DM and are in agreement with the range of 19.2 to 30% obtained by Oduro et al. (2008) and Sánchez-Machado et al. (2010) in *M. oleifera* leaf powder from Ghana and Mexico respectively.

Plants obtain their minerals from the soil, and climatic conditions and minerals contents of soils differ from one locality to another and with soil type (Kengué et al., 2008) thereby influencing the mineral contents of plants. This is supported by the significant (p=0.0001) effect of locality on ash content. Ash contents of *M. oleifera* leaves in this study are high (>8%), indicating a high concentration of minerals (table 2) with no significant (p>0.05) difference between maturities. Similar values of ash were reported by Jongrungruangchok et al. (2010) and Shih et al. (2011) with *M. oleifera* leaves in Thailand and Taiwan respectively.

4.1.2 Macro minerals (Ca, Mg, K, Na, P)

Macro minerals composition was significantly (p=0.0001) influenced by locality and maturity, with the exception of sodium which was only significantly affected by locality.

The absorption of minerals by plants from the soil changes with maturity of plants and in accordance with their role in leaves. Consequently, higher concentration of magnesium in mature leaves could be attributed to chlorophyll which contains magnesium in its structure, and accumulates in leaves as they mature (Folly, 2000). Higher calcium content in mature leaves (1.58 g per 100 g DM) may be due to its role in the calcium- fiber (pectin) network which contributes in ensuring the rigidity of the secondary cell wall (Cheftel et al., 1984; Kirkby & Pilbeam, 1984). A positive correlation ($r = 0.740$; $p < 0.05$, table 7) obtained between calcium and fiber supports this assertion. Values of calcium are similar to (0.54 g per 100 g DM), but lower than (2.04 g per 100 g DM) that reported by Yang et al. (2006) respectively in young and mature leaves of *M. oleifera* in Taiwan.

4.1.3 Micro minerals (Fe, Zn, Mn, Cu)

Maturity had a greater influence on iron (77.18%) and zinc (42.40%) contents, whereas locality had a greater influence on manganese (41.39%) and copper (62.51%) contents. Iron, manganese and copper are more concentrated in mature leaves whereas young leaves are more concentrated in zinc (table 3). Minerals contribute to several reactions in plants which influences their composition. Iron was the most abundant micro-mineral in *M. oleifera* leaf powders. Iron participates in photosynthesis and is a constituent of many enzymes while zinc and copper are cofactors in many reactions (Koolman & Roehm, 2005). Iron contents of young and mature leaves are respectively lower than those reported by Yang et al. (2006), 18.54 and 30.18 mg per 100 g DM, for young and mature leaves of *M. oleifera* in Taiwan. The mean content of zinc is in the range of 1.5 to 3 mg per 100g DM reported by Armelle et al. (2010) whereas copper and manganese have values below the range reported by these authors.

4.1.4 Vitamins

Carotenoids (precursor of vitamin A) are responsible for most of the yellow, orange and red colour of fruits and vegetables (Folly, 2000). They also occur along with chlorophyll in chloroplasts. They are the underlying pigments in green leaves which become visible when chlorophyll is destroyed (Belitz et al., 2009). Carotenoids content was higher in young leaves (112.46 ± 1.38 mg per 100g DM), and could be related to its photo-protective role in photosynthesis which would be most intense in young leaves (Zufferey, 2000; Koolman & Roehm, 2005). Mean total carotenoids contents of young (112.46) and mature leaves (92.77) of *M. oleifera* in this study, compare to values (101) mg per 100 g DM recorded by Djuikwo et al. (2011), for *M. oleifera* leaves from Bini-Dang (Ngaoundere).

Vitamin C is a heat labile vitamin and thus its contents could have been affected by the drying process. However, mean vitamin C contents of young and mature leaves in this study are in the range of 15-100 mg per 100 g DM obtained by Armelle et al. (2010) in *M. oleifera* leaf powders from Ghana.

4.1.5 Bioactive compounds

Polyphenols and tannins were not significantly affected by either maturity of leaves or locality. Tannins were significantly positively correlated with polyphenol ($r = 0.867$; $p < 0.05$, Table 7), which is expected as tannins are a component of polyphenol. Polyphenols have been lauded for their beneficial effects in the fight against chronic diseases like atherosclerosis, diabetes (Fahey, 2005; Biswas et al., 2012). Mean total polyphenol contents obtained in this study are within the range of 0.67 to 2.02 g per 100 g DM obtained by Tchiégang & Kitikil (2004) and Moyo et al. (2011) with *M. oleifera* leaf powder.

Table 6 Groupings of *M. oleifera* leaf powders from different localities and maturities according to chemical composition and their potential applications.

<i>M. oleifera</i> leaf powder Groups	Composition of <i>M. oleifera</i> leaf powder Groups	Major components	Potential uses
Group 1	Mature leaves of <i>M. oleifera</i> from Mbouda (MLMB), Bafia (MLBA), Edea (MLED), Maroua (MLMA), Doume (MLDO) and Meiganga (MLME)	Total proteins Mg, Cu, Mn, Ca, Fe Total fibers	Minerals and protein deficiencies.
Group 2	Mature leaves of <i>M. oleifera</i> from Bertoua (MLBE)	Phytates Total tannins Total polyphenols	Antioxidants
Group 3	Young leaves of <i>M. oleifera</i> from Bertoua (YLBE), Bafia (YLBA), Meiganga (YLME), Doume (YLDO)	Available carbohydrates	Energetic foods and Beverages
Group 4	Young leaves of <i>M. oleifera</i> from Mbouda (YLMB), Maroua (YLMA), Edea (YLED) and Garoua (YLGA)	Total carotenoids Na, Zn, K, P	Antioxidants and Vitamin A deficiency
Group 5	Mature leave of <i>M. oleifera</i> of Garoua (MLGA)	Total ash Total lipids Vitamin C	Antioxidants and Nutritional

Figure 2a Correlation circle of similarities between nutrients and bioactive compounds of *M. oleifera* leaf powders [Temperature (T °C) and Precipitation (p mm) are supplementary variables].

(MLBA/YLBA: Mature Leaves/Young Leaves from Bafia ; MLBE/YLBE : Mature Leaves/Young Leaves from Bertoua ; MLDO/YLDO :Mature Leaves/Young Leaves from Doumé; MLMB/YLMB :Mature Leaves/Young Leaves from Mbouda ; MLED/YLED : Mature Leaves/Young Leaves from Edéa ; MLGA/YLGA : Mature Leaves/Young Leaves from Garoua ; MLMA/YLMA: Mature Leaves/Young Leaves from Maroua ; MLME/YLME :Mature Leaves/Young Leaves from Méiganga)

Figure 2b Grouping of *M. oleifera* leaves powders according to their chemical composition [temperature (T °C) and precipitation (p mm) are supplementary variables].

Table 7 Pearson's Correlation Matrix.

Variables	Total Ash	Total polyphenol	Total Tannins	Phytates	Available Carbohydrate	Total Lipid	Total proteins	Total fiber	Ca	Mg	K	Na	P	Zn	Cu	Mn	Fe	Total carotenoids	Vitamin C
Total Ash																			
Total polyphenol	0.145																		
Total Tannins	0.077	0.867																	
Phytates	-0.168	0.487	0.485																
Available Carbohydrate	-0.155	-0.089	-0.268	-0.403															
Total lipids	-0.189	-0.357	-0.264	0.210	-0.129														
Total proteins	-0.203	-0.257	-0.136	-0.119	-0.475	-0.067													
Total fibers	-0.012	0.265	0.401	0.518	-0.878	-0.113	0.231												
Ca	0.109	0.043	0.183	0.330	-0.871	0.162	0.458	0.740											
Mg	0.293	0.057	0.105	0.129	-0.667	0.065	0.337	0.507	0.563										
K	0.495	-0.103	-0.328	-0.385	0.390	-0.126	-0.055	-0.575	-0.366	-0.099									
Na	0.115	-0.450	-0.526	-0.202	-0.003	0.206	0.480	-0.299	0.096	0.076	0.469								
P	0.362	0.024	-0.002	-0.484	0.271	-0.199	0.035	-0.431	-0.448	0.101	0.726	0.257							
Zn	0.189	-0.218	-0.376	-0.574	0.496	-0.069	0.022	-0.651	-0.623	-0.078	0.791	0.387	0.873						
Cu	-0.155	0.304	0.280	0.332	-0.475	0.143	0.334	0.409	0.480	0.691	-0.066	0.014	0.105	-0.009					
Mn	-0.032	0.204	0.364	0.391	-0.646	0.458	0.254	0.494	0.627	0.659	-0.321	-0.177	-0.151	-0.360	0.701				
Fe	-0.017	0.229	0.360	0.436	-0.868	-0.081	0.384	0.893	0.880	0.626	-0.448	-0.085	-0.353	-0.570	0.626	0.549			
Total carotenoids	0.274	-0.517	-0.638	-0.328	0.261	0.207	0.188	-0.545	-0.376	0.024	0.707	0.650	0.567	0.765	-0.112	-0.262	-0.490		
Vitamin C	0.050	0.212	0.072	0.265	0.061	0.061	-0.269	0.001	-0.053	0.010	0.008	0.030	-0.224	-0.209	-0.192	0.038	-0.088	-0.177	
Correlation coefficients in bold are significant at $p > 0.05$																			

In opposite, to polyphenols and tannins, the individual effect of locality and maturity as well as the interaction between them significantly ($p=0.0001$) influenced phytate content with locality showing a greater influence (59%). Phytates are the main form of preservation of phosphorus in leaves. Phosphorus is an important mineral for the plants metabolism. According to Lestienne (2004), phytates have a tendency to accumulate in plant organs as they mature, which is in accordance with results obtained in this study. Mean values of phytate contents obtained in this study are 312.77 mg per 100 g for young leaves and 340.04 mg per 100 g DM for mature leaves; and are much lower than those reported (2.59 g per 100 g DM) by Ogbé & Affiku (2011) for *M. oleifera* leaf powder in Lafia Nasarawa State (Nigeria). Phytates limit the bioavailability of minerals (iron, zinc, calcium) by chelating them (Connie & Srimathi, 2002). Phytates in this study, show a significant negative correlation with zinc ($r = -0.574$, $p < 0.05$, Table 7). This could indicate a chelating activity of Phytates on zinc. On the other hand, phytates possess antioxidant properties, and could play an important role in cell protection (John & Feng, 2002).

4.2. Grouping of *M. oleifera* leaf powders according to their chemical composition

Much variation was observed in the chemical composition of young and mature leaves of *M. oleifera* from the different localities studied. Principal component analysis was thus carried out to group *M. oleifera* leaves according to similarities in their chemical composition. The first principal component (F1) represents 36.35% of variations between variables while the second principal component (F2) represents 18.28%. Correlation circle (figure 2a) shows grouping of variables. Five groups can be observed from this figure. The first group is composed mainly of the minerals Mg, Cu, Mn, Ca, Fe; proteins and fiber. The second group consists of bioactive compounds (Phytates, total tannins, and total polyphenols) and the third group is made up solely of available carbohydrate. The fourth group is composed of Na, K, P, Zn and carotenoids. The fifth group is not well represented on both the F1 and F2 axis and is made up of ash, lipids and vitamin C. Temperature and precipitation have no significant effects on these different groups. When we represent the factors (*M. oleifera* leaves at different maturity and from different localities) on these groups, we can separate them according to their major constituents (Figure 2b and Table 6). Consequently, group 1 is characterized by mature leaves of *M. oleifera* from Mbouda, Bafia, Edea, Maroua, Doume and Meiganga which are rich in divalent cations, proteins and fiber contents. Thus mature leaves from these localities could contribute to solving nutritional deficiencies linked to proteins, Fe, and Ca. With respect to their high fiber content, they could help in facilitating digestion, and in the prevention of atherosclerosis and colon cancer (Gropper et al., 2009). The second group constituted of mature leaves of *M. Oleifera* from Bertoua is more concentrated in bioactive molecules and could be a good antioxidant source helping in the fight against stress related diseases (cardiovascular diseases, diabetes, cancer etc). The

third group consists of young leaves from Meiganga, Doume, Bafia, Bertoua which are more concentrated in available carbohydrates contents, suggesting that they could be useful in the development of energetic foods. The fourth group, made up of young leaves of *M. oleifera* from Mbouda, Maroua, Edea and Garoua are good sources of sodium, potassium, phosphorus, total carotenoids (Vitamin A precursor) and zinc. Young leaves from these localities are thus important in alleviating vitamin A and zinc deficiencies. Their high potassium content suggests their use in the prevention and management of hypertension (Whitney & Rolfes, 2011). The fifth group not well represented on F1-F2 axis is constituted by mature leaves of *M. oleifera* from Garoua and is characterized by good ash, lipids and vitamin C contents. This last group in addition to its nutritional role could be an important source of antioxidant. Table 6 give a summary of these different groups of *M. oleifera* leaf powders and their predominant constituents as well as their particular benefits with respect to health and nutrition.

Conclusion

The results of this study suggest that particular uses can be attributed to *M. oleifera* leaf powders from different localities and maturity, as the composition of these leaves vary significantly with locality and maturity. The grouping of *M. oleifera* leaves according to their chemical composition reveals that mature leaves are in general a rich source of protein, fiber, divalent cations, and bioactive compounds, and thus can help in the fight against protein, mineral deficiencies and stress related diseases. Young leaves are more concentrated in available carbohydrate, total carotenoids and monovalent cations. *M. oleifera* leaves of different stages of maturity and of different origins could thus be exploited for their unique nutritional and antioxidant benefits.

Acknowledgement

The authors would like to thank Mr TAGNE Théophile, Ibrahim SKIVO, Willy NGON and AWOUALA Balado of Moringa Cameroon network (MoCam) and Sister Monique of “Monastère St Benoît de Babeté” for their assistance with the collection of the samples.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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