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Physico-chemical composition and antioxidant activity of three local cultivars of *Hibiscus sabdariffa* (Malvaceae) consumed in Congo

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Abstract

Roselle (Hibiscus sabdariffa) is cultivated for its leaves, fibers, fruits and seeds. Its leaves are consumed fresh and cooked proved to be rich in micro-constituent exogenous antioxidants. This study was conducted to identify and to quantify the nourishing elements contents in leaves of Moussa 1, Moussa 2 and Poutou Poutou, local cultivars of Hibiscus sabdariffa. For dry powder of leaves, the aqueous content, total acidity and total sugars content was determined. Polyphenol and flavonoids were extracted from the aqueous, ethanolic and aqueous ethanolic solution and read on a spectrophotometer. The antioxidant and peroxidasic activity were measured with fresh leaves. The most significant total sugar contents is obtained at the cultivar Moussa 2 follow-up of Moussa 1 and Poutou Poutou. The local cultivars, Poutou Poutou are rich in polyphenols and flavonoids. The cultivar Poutou Poutou presented high percentages of polyphenols (10.30, 5.05 and 3.07 EAG/g.MS) and flavonoid (2.54, 1.4 and 0.84) respectively of the ethanolic, aqueous ethanolic and aqueous extracts compared to the cultivars Moussa 1 and Moussa 2. For three extraction solution, the ethanolic extract presents polyphenolic and flavonoids contents as well as antioxidant activity more significant compared with aqueous ethanolic extract. The cultivar Moussa 1 presents an antioxidant capacity very significant follow-up of Poutou Poutou and Moussa 2. The strong peroxidasic activity as well as acidity is recorded at the cultivar Moussa 1 compared to cultivars Poutou Poutou and Moussa 2.

Keywords: Polyphenol, Flavonoids, Physico-chemical, *Hibiscus sabdariffa*, République du Congo.

Introduction

Roselle (*Hibiscus sabdariffa*) belongs to the Malvaceae family and is native to tropical and subtropical regions. This herbaceous plant is cultivated for its leaves, fibers and fruits. On the whole of tropical zone, *Hibiscus sabdariffa* covers close to 21 million hectares and her world production estimated to 25 million of tons. Asia provides around 71% of this production and Africa, 19% of the world production. *Hibiscus sabdariffa* is suitable to numerous foods, medicinal and cosmetic uses[1,2]. They are used to prepare sauces. Her leaves proved to be rich in micro-constituent exogenous antioxidants. The calyx is rich in phenolic compounds notably flavonoids, anthocyans and was especially exploited to produce juices, jams or the sweet[3]. Her seed enters in food spices manufacture and cosmetic products[1,4,5]. The regular consumption of the extract or products basis of *Hibiscus sabdariffa* reduces nutritional deficiency as well as the risks of the inflammation, cancer, cardiovascular pathologies and would decrease the lipids and cholesterol rate, and the arterial tension[6].

In Brazzaville markets, the variability of the *Hibiscus sabdariffa* is very important. The leaves and calyx were widely used as food and appreciated by consumers. This diversity offers the existence of interesting local cultivars for consumption current of this vegetable-leaf. However, the nutritional contribution in quantity as well as different local cultivars of *Hibiscus sabdariffa* is badly known. In the optics to satisfy the socioeconomic requirements relative to nutritional quality in order to assure the food security of the consumer, the knowledge of the chemical composition of *Hibiscus sabdariffa* constitutes was indispensable scientific support to the mastery of the quality of this vegetable. This work was initiated to identify and to quantify the nourishing elements brought by the local cultivar of *Hibiscus sabdariffa.*

Material and methods

Preparation of leave's extracts: The plant material was constituted of leaves of three local varieties of *Hibiscus sabdariffa* (Poutou poutou, Moussa 1 and Moussa 2) and bought to consumer maturity in Brazzaville market.

Three local varieties of *Hibiscus sabdariffa* were: i. Variety known as *Poutou- poutou* present green leaves and generally three (3) lobes partially cut out. The green stems carries the

hairs, ii. Two other varieties were called *Moussa*, their leaves generally present three (3) at five (5) lobes, completely or partially cut out. The stem was deprived of hairs. For *Moussa 1* leaves were sometimes green clear yellow clear for young leaves to dark orange stem. For *Moussa 2* leaves were green dark sometimes come to the purple to dark purple stem.

The leaves of three local varieties of *Hibiscus sabdariffa* were divided into three (3) lots: i. The first lot was composed of leaves were dried at 50°C during 5 days in order to evaluate polyphenol content, flavonoids content and antioxidant activity, ii. The second lot was constituted of leaves were dried at 103°C during 24 hours and kept for study of physicochemical properties (soluble sugars, free acidity), iii. The third lot was composed of leaves were stored at 4°C for peroxidasic activity study.

The leaves were dried to darkness and grounded in order to obtain a powder, which was useful for preparation of the leave's extracts. The powder leave was subjected to maceration in aqueous, ethanolic and aqueous ethanolic solution. For aqueous and ethanolic extracts, the powder leave (10g) was extracted with 100ml of aqueous and ethanol respectively for 24 hours at room temperature. For aqueous ethanolic extract, the powder leave (10g) put in 100ml of cold aqueous ethanol solution (50%) during 72 hours, under agitation. The extracts were then filtered. The extract was evaporated to dryness at 50°C, 103°C, 70°C for ethanolic, aqueous and aqueous ethanolic extracts respectively. For extraction solution, the residues were obtained and preserved for later studies.

Evaluation of physico-chemical composition of *Hibiscus sabdariffa* extracts: For aqueous content, the fresh leaves (5g) of three local varieties (Poutou poutou, Moussa 1 and Moussa 2) were dried at 103°C in an oven during 3 days until obtaining a constant weight. The percent of water was calculated following relation:

$$\% \, W = \frac{Wf - Wd}{Wf} \times 100$$

Were: wf: weight of fresh leaves (g) and wd: weight of dry leaves (g).

For total acidity contents, it was determined by titration following Ilkay and Aziz method[7]. The filtrate of leaves (10ml) was mixed with distilled water (10ml). To this mixture, some drops of phenolphthalein was added and titled with NaOH (0.1N). Total acidity was calculed following relation:

Total acidity = 0.49 x V.
Were: V: volume of added NaOH

For total sugars content, it was determined following phenol method reported by Goodon[8] using glucose as standard. The dry powder (1g) was extract twice with 10 ml of cold aqueous ethanolic solution (20/80). These extract (0.1ml) was successively added distilled water (0.4ml), phenol (0.3ml) and H_2SO_4 (1.8ml). After 30 minutes at room temperature, absorbance at 490nm was read on a Spectrophotometer against a blank that contained phenol instead to sample. All measurements were conducted in triplicate and total sugars compounds were expressed in terms of mg/ml per dry extract (g)[9].

Dosage of total polyphenolic compounds: The levels of total polyphenolic compounds were determined in three plants extracts following Folin-Ciocalteu method[10], using gallic acid as standard. The dosage of phenolic compounds have been conducted as follows: to an ethanolic, aqueous-ethanolic or aqueous extract (0.1ml), distilled water (0.9ml) was added to make 1ml (Eppendorff tube, 2ml), followed by 0.9 ml of Folin-Ciocalteu reagent (1N). After shaking, it was added 0.2 ml of sodium carbonate (20%). After 40 minutes at room temperature, absorbance at 725 nm was read on a Spectrophotometer against a blank contained aqueous, ethanolic and aqueous ethanolic solution instead to extract. All measurements were conducted in triplicate and total phenolic compounds were expressed in terms of equivalent amounts of gallic acid (GAE) per g dry extract (g).

Evaluation of total flavonoid contents: Flavonoids were evaluated following colorimetric assay described by Mompon[11]. Standard solution of catechin was used. At zero time, 0.075ml of $NaNO_2$ (5%) was added to the tube containing 0.25ml of the Standard solution of catechin and 1 ml of distillate water. After 5 minutes, 0.075ml of $AlCl_3$ (10%) was added. At 6 minutes, 0.5 ml of NaOH (1N) and 2.5ml of distillate water were added. Absorbance was measured at 510nm. Total flavonoid contents in plants were expressed as mg catechin equivalent (CE) per g dry extract, based on the calibration curve of catechin. Samples were analyzed in three replications a colorimetric assay.

Determination of antioxidant activity: The method applied to measure the antioxidant activity was the free radical scavenging by using DPPH reported by Chun[12]. This activity was determined by measure of inhibitory activity with ultraviolet spectrophotometer at 517nm against the blank. Evaluation of antioxidant activity was carried using 1ml of the 100 µM of DPPH solution in ethanol and mixed with 0.1 ml of differents concentrations extract (10, 5, 2.5, 1.25 and 0.625mg/ml in ethanol). The reaction mixture was incubated in the dark for 30 minutes and thereafter the optical density was read at 517 nm. Samples were analyzed in three replications. The antioxidant activity was determined as percentage of inhibition (% IP) of DPPH radical by following formula:

$$\% \text{ inhibition} = [(Ab - As) / (Ab)] \times 100$$

Were: Ab: absorbance of the sample test after zero minute and As: absorbance of the control after 30 minutes.

From a plot of concentration against % IP, a linear regression analysis was performed to determine the IC50 value for each extract. The DPPH radical scavenging activity of phenolic compounds was expressed as IC50 value in micrograms per ml

of fresh weight. A low IC50 value represents a high antioxidant activity.

Extraction and determination of peroxidase activity: Peroxidases were extracted following Attibayeba and Paulet[13]. Fresh leaves (10g) of *Hibiscus sabdariffa* were grounded finely and homogenized at 4°C in HCl (20mM, pH 7.0)/KCl (50mM)/ $MgCl_2$ (5mM) (10ml) and ascorbic acid buffer (0.16g). The homogenate was stirred for 15 minutes and centrifuged at 11.000 rpm for 20 minutes. The culot was discarded and the supernatant was transferred in small sample bottles and stored at 4°C in the dark until determination of peroxidase activity. For peroxidase activity, absorbance at 470 nm was read on a spectrophotometer with gaïacol as substrate. Then, the substrate was prepared by dissolving 20 ml of gaïacol (0.62%), 1ml of H_2O_2 (10v), 2ml of KH_2PO_4 (1M, pH 6.0) and distilled water (77ml) was added. The reaction was started by adding peroxidasic extract (0.1ml) with 2ml of substrate. One unit of enzymatic activity was defined as amount of oxidized enzyme 1 µmol / min of hydrogen under assay conditions.

Statistical analyses: The version 7.5.3 of Xlstat software was used for the statistical analyses as a whole. In order to identify the best extraction yields and local varieties of *Hibiscus sabdariffa,* the comparison of means was applied. This comparison of means was performed according to Student-Newmann and Keuls test at 5% level.

Results and discussion

Aqueous contents of three local cultivars of *Hibiscus sabdariffa*: Aqueous content was evaluated on the leaves extract of three *Hibiscus sabdariffa*. The results of analysis are showed in Figure-1. These result indicated that the leaves of Poutou Poutou, Moussa 2 and Moussa 1 was high aqueous contents of 86, 85.6 and 85.2% respectively. For cultivars Moussa 2 and Moussa 1, the aqueous contents does not vary significantly. The high aqueous contents on the leaves of three cultivars were similar to those found by Morton 1987. These authors was showed that the aqueous contents on *Hibiscus sabdariffa* leaves from Philippine and Guatemala origin were varied from 85.6 to 86.2%.

Total acidity contents of three local cultivars of *Hibiscus sabdariffa:* Quantitative comparison of total acidity contents was determined following phenol method. Total acidity of cultivar Moussa 1 (2.6 eq.H_2SO_4/l) was higher compared of others cultivars Moussa 2 (M2) and Poutou Poutou (1.5 eq.H_2SO_4/l) (Figure-2). These results showed that the total acidity on leaves varied significantly according to cultivar. This result revealed the existence of three groups homogeneous (a, b and c). The higher total acidity was observed on cultivar Moussa 1 leaves (a) group and lower total acidity on cultivar Poutou Poutou (group c). The total acidity with Moussa 1 (M1) is twice higher than that of Poutou Poutou. Results obtained on cultivar Moussa 1 and cultivar Poutou Poutou are in accord with those described by Cissé[14] who reported that the cultivar M1 called Thai showed increase total acidity than the cultivar M2 called Vimto. This variability of the total acid content of three local cultivars would be related to the genetic difference between the cultivars. However, the data found in this study on leaves indicate that the acid content on cultivars Moussa 1 and Moussa 2 was lower than those found in calyx of cultivar Tai and Vimto as related by Cissé[14]. This difference can be explained by the studied organ and the expression mode. The decrease of total acidity contents showed that the leaves of three cultivars were less acid than those of calyx cultivar Tai and Vimto of *Hibiscus sabdariffa*.

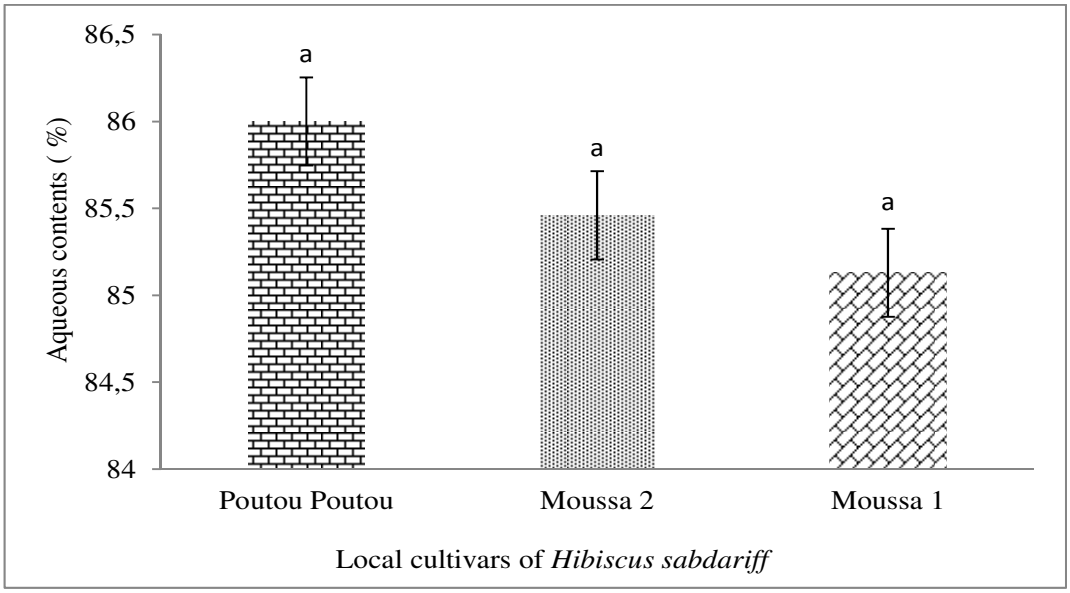

Figure-1: Aqueous contents of three local cultivars of *Hibiscus sabdariff*

Total sugar contents: Figure-3 showed a high total sugar content on cultivar Moussa 2 (0.78 mg/ml.g-1MS). By contrast, total sugar content of cultivars Poutou Poutou (0.05 mg/ml.g-1MS) and Moussa 1 (0.03 mg/ml.g-1MS) was lower and did not reveal any significant difference group (b). The highest total sugar contents with cultivar Moussa 2 could be explained by good synthesis of carbohydrates resulting to photosynthesis. Indeed, cultivar Moussa 2 have some dark green leaves rich in chlorophyll pigments returning thus an efficient photosynthesis.

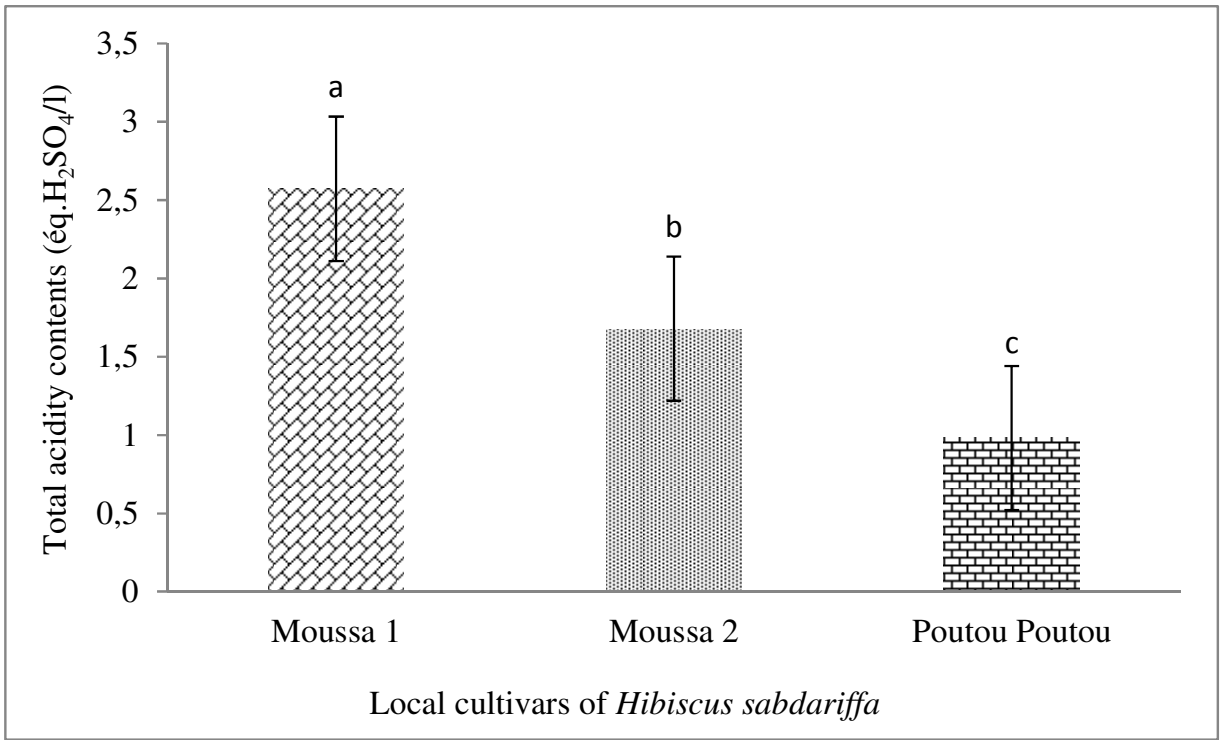

Figure-2: Total acidity contents of three local cultivars of *Hibiscus sabdariffa.*

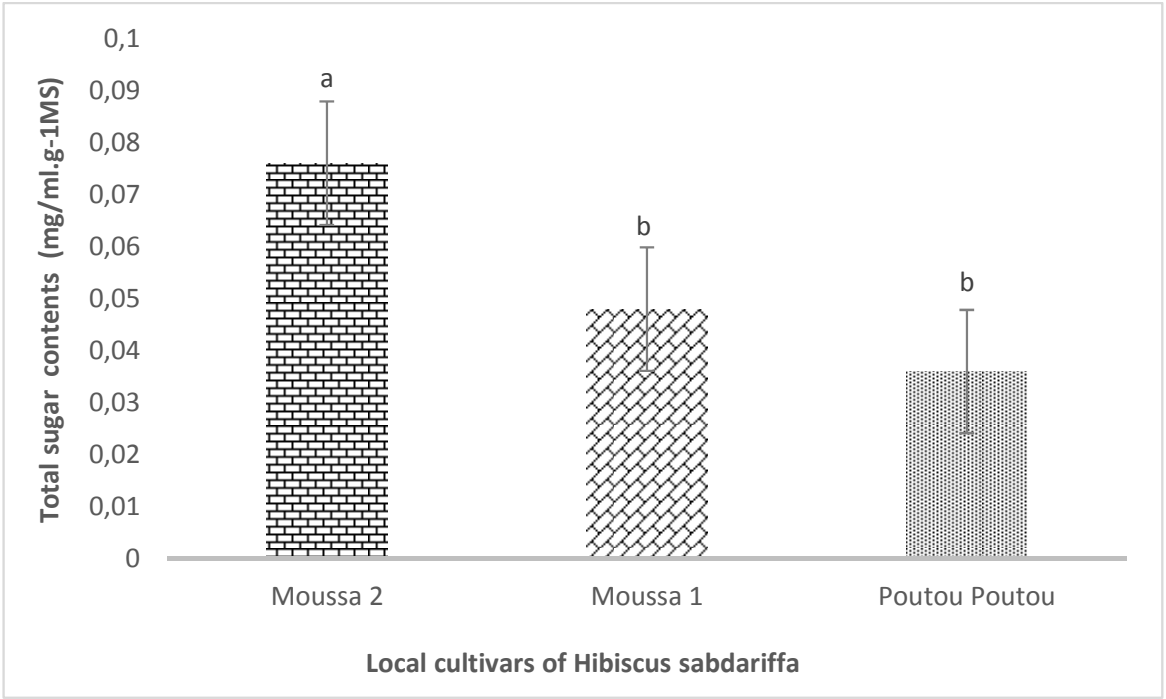

Figure-3: Total sugar contents of three local cultivars of *Hibiscus sabdariffa.*

Total polyphenolic compounds: Total polyphenolic compounds of three cultivars of *Hibiscus sabdariffa* was determined using UV-spectrophotometer. It was observed that highest total polyphenolic contents was found in ethanolic extract on cultivars Poutou Poutou (10.3mg. EAG/g MS), Moussa 1(10.17mg. EAG/g MS) and Moussa 2 (9.99 mg. EAG/g MS) compared to those of aqueous-ethanolic and aqueous extract (Figure-4). Quantitative comparison the total polyphenolic compounds between cultivars Poutou Poutou, Moussa 1 and Moussa 2 was revealed a high quantity with cultivar Poutou Poutou. Aqueous-ethanolic extract, cultivars Poutou Poutou was higher total polyphenolic content (5.05 mg. EAG/g MS) compared to those of cultivars Moussa 1(3.20 mg. EAG/g MS) and Moussa 2 (2.76 mg. EAG/g MS) respectively. For aqueous extracts, total polyphenolic compounds on cultivars Poutou Poutou (3.07 mg. EAG/g MS), Moussa 1 (2.27 mg. EAG/g MS) and Moussa 2 (3.07 mg. EAG/g MS) was weak compared to similar values of total polyphenolic content with cultivars Poutou Poutou and Moussa 2 (Table-1).The data showed clearly that total polyphenol compounds varied significantly according to extract and cultivar. For ethanolic and aqueous ethanolic extract, the levels of total polyphenolic compounds were significantly increased on cultivar Poutou Poutou. These results are contrary to those obtained by Cissé[14] on the calyx of cultivar Vimto and Thai. This difference would be explained by organ nature and extraction method used. In regard of the levels of total polyphenolic compounds, *Hibiscus sabdariffa* calyx would contain more than the leaves.

Table-1: Total polyphenolic compounds of three local cultivars of *Hibiscus sabdariffa.*

Cultivars	Extraction solvent		
	Ethanolic	Aqueous ethanolic	Aqueous
Poutou- Poutou	10.30a	5.05a	3.07a
Moussa1	9.99a	3.20b	2.27a
Moussa 2	10.17a	2.76b	3.07a

Total flavonoids compounds: Total flavonoid compounds were quantified using an UV-Vis Spectrophometric apparatus. The results of analysis are showed in Figure-5. Total flavonoid compounds on cultivars Poutou Poutou (2.54 mg E.Ct/gMs), Moussa1 (2.46 mg E.Ct/gMs) and Moussa 2 (2.2 mg E.Ct/gMs) were higher with ethanolic extract compared to those aqueous ethanolic and aqueous extract. For aqueous ethanolic extract, the cultivars Moussa 1 (1.75 mg E.Ct/gMs) was higher total flavonoid compounds than cultivars Poutou Poutou (1.45 mg E.Ct/gMs) and Moussa 2 (1.31 mg E.Ct/gMs) respectively. Three local cultivar of *Hibiscus sabdariffa*, the results revealed that total polyphenolic and total flavonoid compounds were higher with ethanolic extract (Table-2).

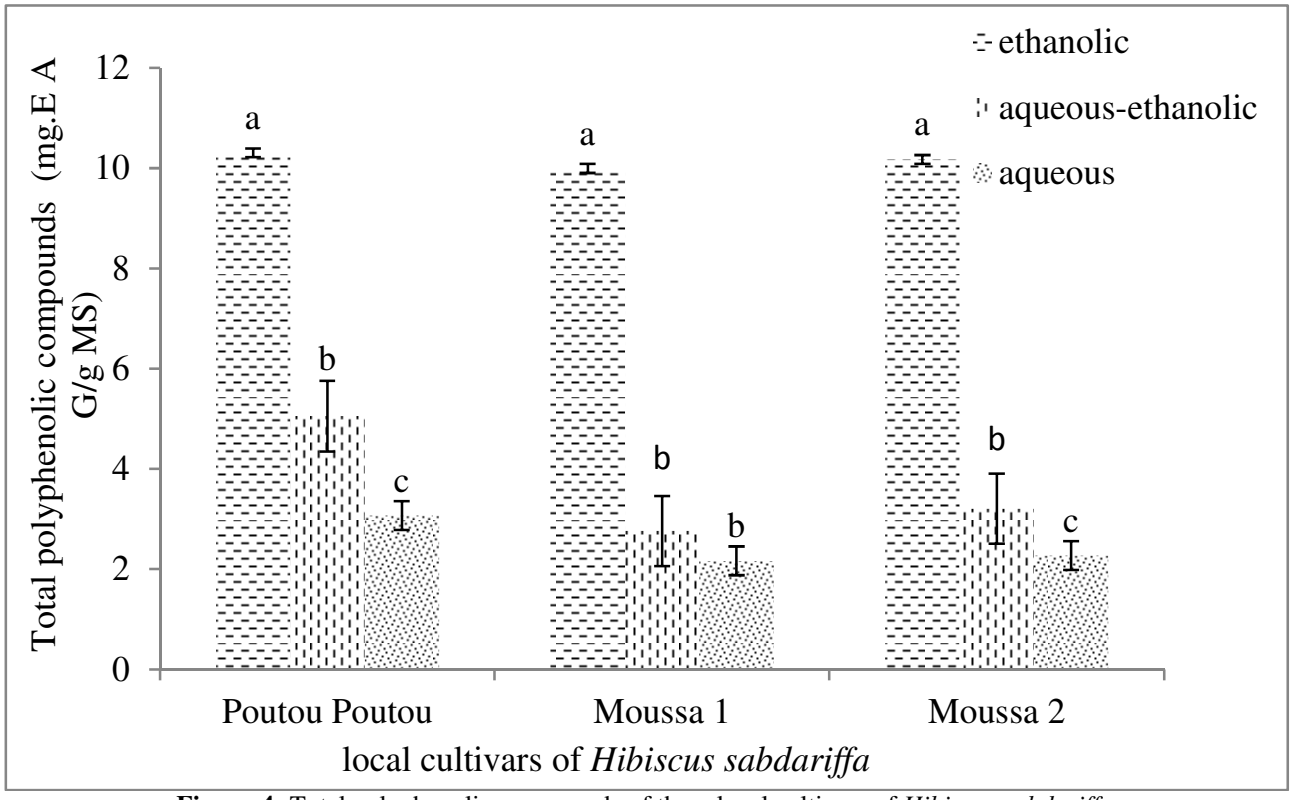

Figure-4: Total polyphenolic compounds of three local cultivars of *Hibiscus sabdariffa*

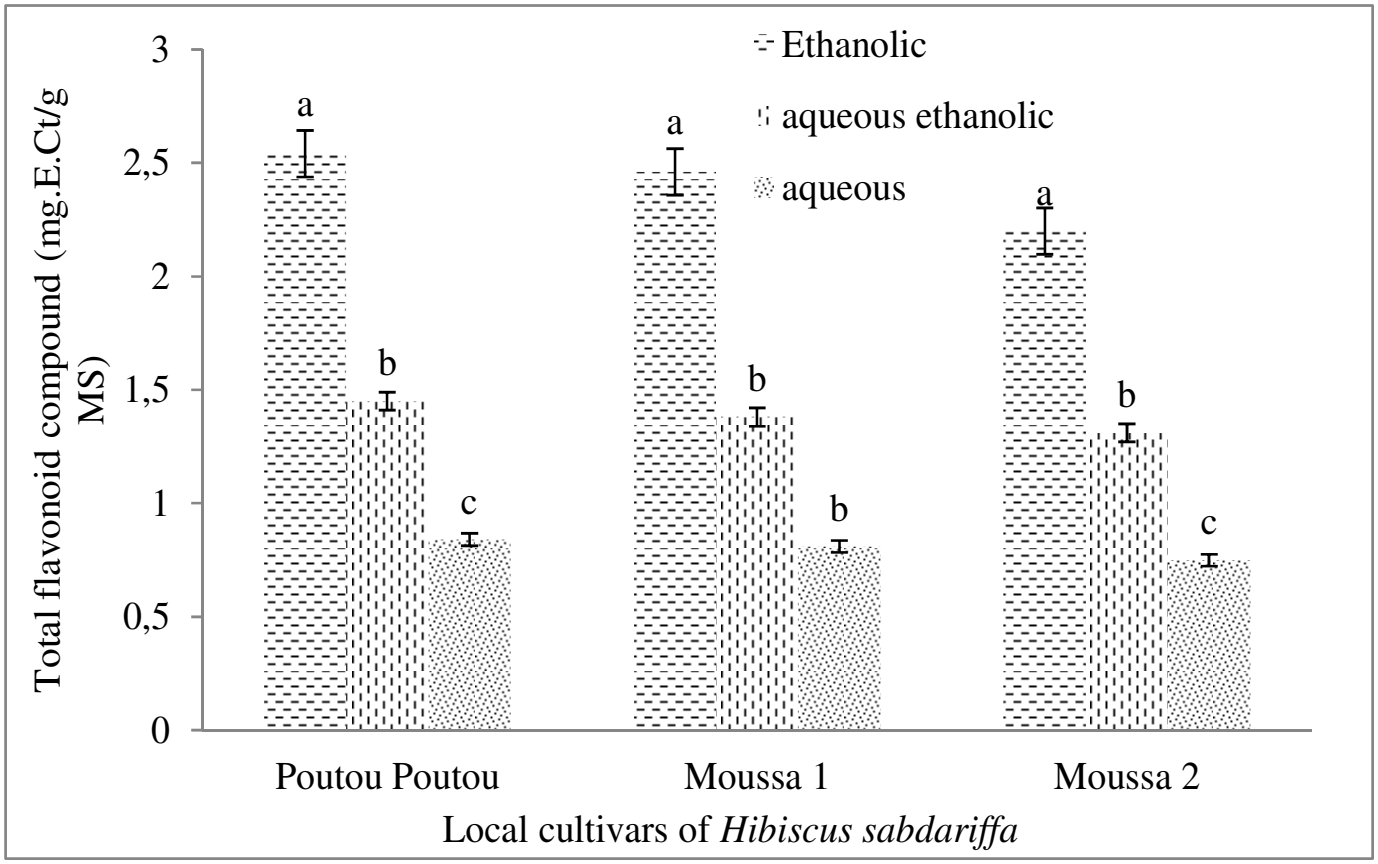

Figure-5: Total flavonoid compounds of three local cultivars of *Hibiscus sabdariffa*

Table-2: Total flavonoid compounds of three local cultivars of *Hibiscus sabdariffa.*

Cultivars	Extraction solvent		
	Ethanolic	Aqueous ethanolic	Aqueous
Poutou- Poutou	2.54a	1.45a	0.84a
Moussa1	2.46a	1.75a	0.81a
Moussa 2	2.20a	1.31a	0.75a

The levels of phenolic compounds were lower with aqueous ethanolic and aqueous extract. Similar results were obtained by Talbi[15] on *Nigelle sativa*, Jokic[16] on soya beans and Mahmoudi[17] on *Cynara scolymus* L. In addition, these results are contrary with those described by Nsemi[18] on *Daniella oliveri* and *Desmodium adscendens.* This author showed that aqueous ethanolic extract presented higher levels of phenol compounds than that of the aqueous extract. This prevalence of phenolic compounds on aqueous ethanolic extract would be explained by their strong solubility with organic solvent. Moreover, it is noted the alkalinity and ionization of these phenolic compounds with solvent containing alcohol[19]. For ethanolic extract, quantity of total flavonoid compounds was compared between cultivars Poutou Poutou, Moussa 1 and Moussa 2. The results revealed a high quantity of total flavonoids contents on cultivar Poutou Poutou. These results are contrary to those obtained by Cissé[14] on Vimto and Thai calyx due by nature of the organ used.

Evaluation of antioxidant activity: On the three local cultivar of *Hibiscus sabdariffa*, ethanolic extracts revealed good antioxidant activities (Figure-6, Table-3). For ethanolic extracts, the weak IC50 registered on cultivars Moussa 1, Poutou Poutou and Moussa 2 are 0.9, 1.24 and 2.1 mg/ml respectively. In contrast, with aqueous ethanolic and aqueous extract, the increase of IC50 value suggesting weak antioxidant activity on the leaves of the three local cultivars.

The best antioxidant activity was found with ethanolic extract on the leaves of cultivar Moussa 1(IC50 value = 0.9 mg/ml). However, the weak antioxidant activity was found with aqueous extract on leaves of cultivar Moussa 2. Antioxidant activity on leaves of three cultivars of *hibiscus sabdariffa* tested would be due to high levels of phenolic compounds. Flavonoids, particularly, stabilize the radical peroxide by hydrogen donation[20]. The results revealed that antioxidant activity on leaves of *Hibiscus sabdariffa* varied significantly according to extraction solvent and cultivar.

Table-3: IC50 value of three local cultivars of *Hibiscus sabdariffa.*

Cultivars	Extraction solvent		
	Ethanolic	Aqueous ethanolic	Aqueous
Poutou- Poutou	1.24b	2.79b	4.03b
Moussa1	0.90c	1.403c	2.40c
Moussa 2	2.10a	4.00a	6.8a

Evaluation of peroxidasic activity: On the three local cultivar of *Hibiscus sabdariffa*, the cultivar Moussa 1 revealed good peroxidasic activities (Figure-7). Peroxidasic activity (9226.66UE) was raised compared to those of Poutou Poutou (2500UE) and Moussa 2 (2000UE) respectively. The statistical analyses show a significant difference of peroxidasic activity on leaves according to cultivars local of *hibiscus sabdariffa*. They distinguish two homogeneous groups (a, b).The cultivars Poutou Poutou and Moussa 2 (group b) present no significant difference (Figure-6). Moreover, the strong peroxidasic activity noted on cultivar Moussa 1 contrasts with quantities of total polyphenolic compounds. Indeed, the highest quantities of the phenolic compounds, cultivar Poutou Poutou was presented a weak peroxidasic activity. In addition to total polyphenolic compounds, the highest peroxidasic activity found on the leaves with cultivar Moussa 1 could be explained by presence of others compounds which were a significant antioxidant capacity such as the vitamins, the enzymes, etc. Thus, peroxidasic activity can be independent of the quantities of phenolic compounds. This independence could be due to compounds having chemical structures which support peroxidasic properties.

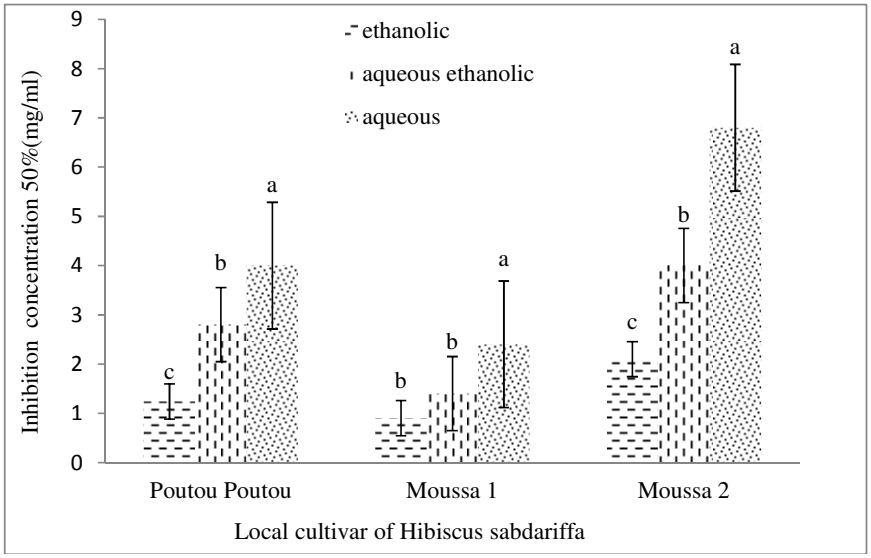

Figure-6: Evaluation of antioxidant activity of three local cultivars of *Hibiscus sabdariffa.*

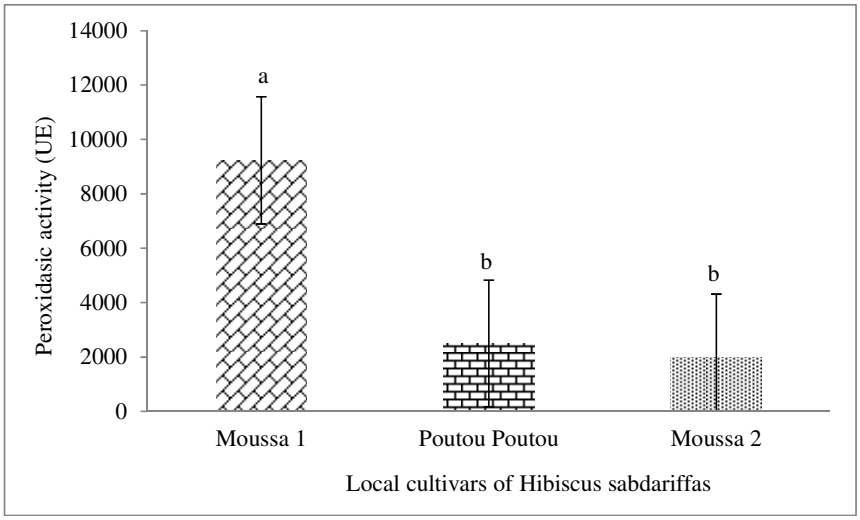

Figure-7: Evaluation of peroxidasic activity of three local cultivars of *Hibiscus sabdariffa.*

Conclusion

This investigation aimed at studying the physico-chemical composition on *Hibiscus sabdariffa* leaves in order to refine the nutritional value of three local cultivar. It results from this study that aqueous content is significant but no vary significantly according to cultivar. For total sugar contents, the most significant rate is obtained with cultivar Moussa 2 follow-up Moussa 1 and Poutou Poutou. In regard to total acidity contents, cultivar Moussa 1 presents a strong acid content by contrast, the cultivar Poutou Poutou was a low acidity and an intermediate acidity for cultivar Moussa 1. The cultivars Poutou Poutou, Moussa 1 and Moussa 2 are rich in total polyphenolic and total flavonoid compounds. However, these contents were higher in the cultivar Poutou Poutou. For extraction solvent, the ethanolic extract presents highest total polyphenolic and total flavonoid contents as well as antioxidant activity more significant compared with aqueous ethanolic and aqueous extract. The cultivar Moussa 1 presents an antioxidant capacity very significant follow-up Poutou Poutou and Moussa 2. The strong peroxidasic activity is recorded on cultivar Moussa 1 compared to cultivars Poutou Poutou and Moussa 2. For total sugar compounds, the most significant level is obtained with cultivar Moussa 2 follow-up Moussa 1 and Poutou Poutou. In regard to physico-chemical parameters, the cultivar Moussa 1 presents strong total acid contents, a low acidity for cultivar Poutou Poutou and cultivar Moussa 1, an intermediate acidity. Thus, a variability of nutritional value is proven between leaves of three local cultivar of *Hibiscus sabdariffa.*

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