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Chemical Composition of the Leaves of *Tristemma mauritianum* Consumed by the Population of Kisangani (DR Congo)

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Abstract: ***Widely found in Masako reserve (14km from Kisangani) and in the region of Kisangani (DR Congo), T. mauritianum (Melastomataceae) is a wild plant which leaves are underutilized by the population of Kisangani as vegetables. Chemical and HPLC-MS^n analyses were carried out on the leaves of Tristemma mauritianum in order to determine their nutritional value and to characterize their major phenolic compounds. These analyses showed that the investigated leaves contain proteins (8.59%), lipids (2.89%), fibers (6.03%), minerals and phenolic compounds. The mineral content (mg/100g) of these leaves was: calcium (325.17), magnesium (589.54) and phosphorus (378.1). HPLC-MS^n analyses showed the presence of six ellagitannins of molecular weigt (uma) 1066, 934 (two compounds), 906, 936 and 952. These results showed that these wild leafy vegetables are another potential source of nutrients and ellagitannins (natural antioxydants), which justify their food use by the population of Kisangani.***

Keywords: *Tristemma mauritianum*, analysis, nutrients, phenolic compouds, HPLC-MS^n, Kisangani

1. Introduction

Masako reserve is about 14 km from Kisangani (DR Congo). Rich in vegetal biodiversity including wild food plants, forests of this reserve are in the category of equatorial evergreen rainforests. It is know that plants have been very widely used by man as food sources and for their beneficial effects on his health.

Tristemma mauritianum (Melastomataceae) is a spontaneous suffrutescent herb which can reach 1 - 2 m in height. Its stem is angular with short and stiff hairs. Widely found in Masako reserve and in the region of Kisangani, *T. mauritianum* is one of the wild plants which fruits and leaves are largely neglected, unknown or underutilized by the population of Kisangani region.

The young leaves of this plant are consumed by people of Tshopo District (DR Congo) as vegetables and the whole fruits are eaten raw [1]. In Uganda, fruits and leaves of *T. mauritianum* are used as a snack and in traditional medicine respectively [2]. Moreover, [3] have reported in 2016 the use of this plant (leaves, fruits or whole plant) in Madagascar traditional medicine. It was reported in 2020 by [4] that Melastomataceae family is an important source of phenolic compouds, quinones and terpenoids.

These easily accessible leaves would be another potential source of nutrients and polyphenols and could contribute to the diversification of vegetables that can exert beneficial effects in human health.

The purpose of this work was to investigate the composition of the leaves of *T. mauritianum* in nutrients and to characterize their major phenolic compounds by HPLC-MS^n.

2. Material and Methods

2.1. Plant material

These leaves of *T. mauritianum* were collected in Masako reseve (DR Congo) and identified at the Faculty of Sciences of the University of Kisangani.

2.2. Chemical analyses

Moisture was determined by drying samples at 105°C. Kjeldhal method and Soxhlet extraction method were used respectively to determine nitrogen and lipids content. Protein content was obtained by multiplying nitrogen content by 6.25. Acid-base digestion was used for determination of fibers.

Ashes were determined by incineration of samples in a muffle furnace at 550°C. These ashes were then dissolved in hot nitric acid and the dissolved mineral elements (Ca, Mg, and P) were determined according to the methods described by [5].
Mayer and Drangedorff tests were used to detect alkaloids. Foam test and ferric chloride test were used to detect saponins and polyphenols respectively according to [6]. Gelatin test and decoloration or bleaching test were then used for detection of tannins and anthocyanins respectively. Oxalates, cyanides, nitrates and nitrites were detected by the methods described by [7].

2.3. HPLC-MS^n analysis

These analyses were carried out at the "Polyphenols BIOTECH" laboratory (France). The HPLC-MS^n system used consisted of a HPLC system Agilent technologies (Automatic injector and Diode-array detector 1200 series,

Prontosil column type C18: 4 mm x 250 mm, 5µm) and a Mass spectrometer Esquire 6000 (Bruker Daltonics Bremen) equipped with an electrospray ionization source.

Polyphenols of the leaf podwer were extracted with methanol. Their separation was performed at 25°C with solvents A (Water/Formic acid 0.1%) and B (Acetonitrile/ Formic acid 0.1%). The injection volume and the elution flow rate were 20 μL and 21.6 mL/min respectively. The following elution program was used:

Time (minutes)	0	2	15	40	44	45	50	51	60
% Solvent B	1	1	10	35	50	100	100	1	1

The detection of polyphenols was carried out at 280 nm. Mass spectra were acquired using electrospray ionization in negative mode. They were obtained in full scan MS mode from 150 to 1200. ESI-MS parameters were as follows: potential of the ESI source, 4 kV; capillary temperature, 350 °C; nebulizer, 35 psi; dry gas, 10l/min. Data were collected and treated with Hystar logicial version 3.0.

3. Results and Discussion

3.1 Chemical analyses

Chemical analyzes of the leaves of *T. mauritianum* were carried out, the results of these investigations are in tables 1 to 4.

Table 1: Proximative composition of the analyzed leaves

Humidity (%)	Proteins (%)	Lipids (%)	Ash (%)	Fibers (%)	Ca (mg/100g)	Mg (mg/100g)	P (mg/100g)
75.62	8.59	2.89	4.54	6.03	325.17	589.54	378.1

As seen in table 1, water and proteins content of the investigated leaves were 75.62% and 8.59% respectively. This content was higher than that of the the leaves of *Talinum triangulare* (3.6%), *Beta corolliflora* (3.77%) and *Basella rubra* Limn (7.8%) analyzed by [8], [9] and [10] respectively. However, it was found to be lower than the protein content of *Daucus carota* (25.19%), *Ipomoea batatas* (13.5%) and *Solanum melongena* (9.5%) analyzed by [11], [10] and [12] respectively. The observed protein content was also lower that of *Beta vulgaris* (12.33%) analyzed in 2011 by [10].

The investigated leaves showed a low lipid content (2.89%). The observed value was higher than that of the leaves of *T. triangulare* (0.15%) and *Vitex ferruginea* (1.36%) analyzed by [8] and [13] respectively. It was lower than those of *Basella rubra* Lim (11.4%) and *Ipomea batatas* Lam (6.4%) also analyzed in 2011 by [10].

The ash content (4.54%) of the investigated leaves was lower than that of *D. carota* (11.5%), *S. melongena* (9.3%) and *Brassica oleraceae* (10.5%) analyzed respectively by [12] and [14] respectively. The content obtained was lower than that of *V ferruginea*. (6.34%) or *Ricinidendron heudelotii* (11.3%) analyzed respectively by [13] and [15]. These leaves were found to contain much ash than the leaves of *T. triangulare* (1.15%) analyzed by [10].

The analyzed leaves contained more fibers than *T. triangulare* (1.82%) or *Leptadenia lancifolia* (4.65%) analyzed by [8] in 1962 and *Beta vulgaris* (4.16%) analyzed by [10] in 2011. It was observed that leaves of *Portulacaca oleraceae* (15.28%) analyzed by [10] contained much fiber than the investigated leaves.

As shown in table 1, the investigated leaves had higher content of calcium and magnesium when compared with *S. melongena* (156 and 250 mg/100g) and *D. carota* (164.11 and 15.48 mg/100g) analyzed by [12] and [11] respectively. These calcium and magnesium contents were lower than those of *Beta corolliflora* (339.30 and 367.27 mg/100g) analyzed by [9] in 2016. These leaves were less rich in calcium than *Urena lobata* (558 mg/100g) and *Leptadenia lancifolia* (398 mg/100g) analyzed by [8] in 1962.

The investigated leaves contained more phosphorus than *Urena lobata* (67 mg/100g) or *T. triangulare* (84 mg/100g) analyzed by [8] and *Primura auriculata* (237.57 mg/100g) analyzed by [9]. Phosphorus contents of the investigated plant was lower when compared with *Vitex ferruginea* (910 mg/100g) and *B. corolliflora* (636.77 mg/100g) analyzed by [9] and [13] respectively.

These results showed that the leaves of *T. mauritianum* had a nutrional potential and could contribute to the diversification of vegetables consummed in Kisangani.

Table 2: Phytochemicals of the analyzed leaves

Alkaloids	Polyphenols	Saponins	Tannins	Anthocyanins
–	+	+	+	-

Legend: + and - mean respectively presence and absence

As shown in table 2, polyphenols, saponins and tannins were present in the leaves of *T. mauritianum*. Polyphenols, as natural antioxidants, present in these leaves could help fight free radicals in human organism and may play a role in the prevention of diseases associated with oxidative stresses.

Table 3: Undesirable or toxic substances of the analyzed leaves

Oxalates	Nitrates	Nitrites	Cyanides
–	–	–	–

Legend: + and - mean respectively presence and absence

The results presented in table 3 showed that the leaves investigated were free oxalates, nitrites, nitrates and cyanides.

2.3. HPLC-MS^n analysis

HPLC-DAD-MS was used for the characterization of leaf phenolic compounds of *T. mauritianum*. The Chromatogram of the HPLC separation and the MS^n fragmentations

(negative mode) of these compounds are shown in Fig. 1 and table 4 respectively.

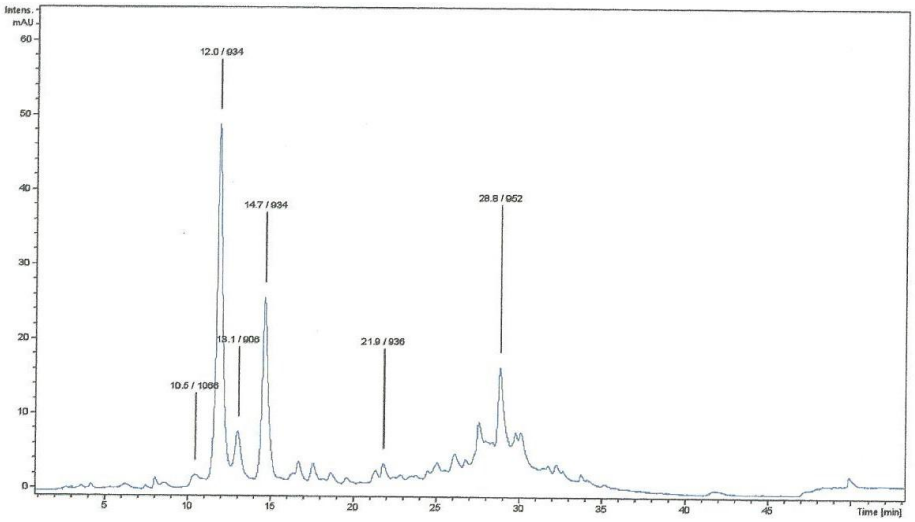

Figure 1: HPLC Chromatogram of methanolic leaf extract of *T. mauritianum* monitored at 280 nm

Table 4: Ion fragments of major phenolic compounds of T. mauritianum obtained by MS

C	Rt (min)	MS^1 ions									MS^2 ions						
1	10.5	1065 $[M-H]^-$	863	783	639	532	281				975	931					
2	12.0	933$[M-H]^-$	466								915	870	825	613	569	448	398
3	13.1	905$[M-H]^-$	783								603	423					
4	14.7	933$[M-H]^-$	783	466							915	631	569	491	425	309	
5	21.9	935$[M-H]^-$	881	708	633	467	388				917	873	659	571	383		
6	28.8	951$[M-H]^-$	951	935	860	783	718	615	505	227	933	889					

Legend : C, Rt and MS mean compoud, retention time and spectrometric mass respectively

As shown in figure 1 and table 6, the leaves of *T. mauritianum* contain six major phenolic compouds. These compounds have a high molecular weight, suggesting they belong to the class of complex phenolic compounds. The comparison of the fragments obtained and the literature data was used for their characterization.

Compound 1 (MW 1066 amu)

Compoud 1 gave in MS^1 $[M-H]^-$ a fragment ion at m/z 863 which generated a fragment ion at m/z 783 corresponding to di-HHDP-glucose. A fragment ion was generated at m/z 281 after loss of di-HHDP-glucose. In MS^2 $[M-H]^-$, a fragment ion was genarated at m/z 975 which gave a fragment ion at 931 after loss of CO_2.

The MS/MS $[M-H]^-$ of punicalagin (MW 1083 amu, molecular formula: $C_{48}H_{28}O_{30}$), an ellagitannin analyzed by [16] contained several fragment ions including 1065, 989, 931, 781 (Punicalin), 601 (gallagic acid) and 393. The $[M-H]^-$fragment ion at m/z 1065 corresponded to the loss of H_2O by punicalagin, which suggested $C_{48}H_{26}O_{29}$ as the molecular formula of compound 1.

Compoud 2 (MW 934 amu)

This compoud gave in MS^1 $[M-H]^-$ a singly fragment ion at m/z 466. In MS^2 $[M-H]^-$, it gave fragment ions at m/z 915 (loss of water), 613 (loss of HHDP) and at m/z 569 (loss of CO_2). It gave also fragment ions at m/z 870 and at m/z 569 after loss hexa-hydroxydiphenyl moiety. Compoud 2 showed some loss typical of ellagitanin. It showed also the same molecular weight and similar fragment ions at MS^1 $[M-H]^-$ and MS^2 $[M-H]^-$ as compoud 4. It was found to be isomer to compound 4 ($C_{41}H_{26}O_{26}$).

Compound 3 (MW 906 amu)

Compoud 3 gave in MS^1 $[M-H]^-$ a fragment ion at m/z 783 which correspond to di-HHDP- glucose. It gave in MS^2 $[M-H]^-$ a fragment ion at m/z 603 (loss of HHDP) which generated a fragment ion at m/z 423 (loss of glucose). It gave also a fragment ion at m/z 423 after loss of HHDP-glucose.

The observed fragment ion and loss were typical of ellagitanin. The MS^n fragmentation of this ellagitanin did not allow us to obtain its molecular formula.

Compound 4 (MW 934 amu)

This compound gave in MS^1 $[M-H]^-$ a fragment ion at m/z 783 corresponding to di-HHDP-glucose ($C_{34}H_{24}O_{22}$). In MS^2 $[M-H]^-$, it gave fragment ions at m/z 915 after loss of H_2O and at m/z 631 after loss of HHDP.

Its MS^1 $[M-H]^-$ fragmentation ressembled the MS^n fragmentation of two unknown ellagitannins (MW 934, Molecular formula: $C_{41}H_{26}O_{26}$) analyzed in 2008 by [17].

These data suggested that compound 4 was an ellagitannin with the molecular formula $C_{41}H_{28}O_{26}$.

Compound 5 (MW 936 amu)

The fragmentation of this compound generated in MS^1 $[M-H]^-$ a fragment ion at m/z 633 corresponding to galloyl-HHDP-glucose ($C_{27}H_{22}O_8$) after loss of HHDP 302. In MS^2 $[M-H]^-$, it gave fragment ions at m/z 917 (loss of H_2O), 873 (loss of CO_2) and 571 (loss of HHDP). These data suggested that compound 5 was Galloyl-diHHDP-glucose ($C_{41}H_{28}O_{26}$).

Compound 6 (MW 952 amu).

The MS^1 $[M-H]^-$ fragmentation of coumpoud 6 gave fragment ions at m/z 935 corresponding to galloyl-diHHDP-glucose after loss of oxygen, at m/z 783 corresponding to diHHDP-glucose after loss of galloyl unit and at m/z 615 corresponding to dehydro-galloyl-HHDP-glucose. It gave also a fragment ion at m/z 860 which generated a fragment ion at m/z 227 after loss of galloyl-HHDP-glucose.

In MS^2 $[M-H]^-$, this compound gave a fragment ion at m/z 933 corresponding to Castalin/Vescalagin after loss of water and at m/z 889 after loss of CO_2. These data suggested $C_{41}H_{28}O_{27}$ as the molecular formula of this ellagitannin. This compound would be isomeric to an unknown ellagitannin (Formula: $C_{41}H_{28}O_{27}$, $[M-H]^-$ MS^1 : a single fragment at m/z 951) analyzed by [17] in 2008.

The presence of these ellagitannins in the leaves of *T. mauritianum* confirms the assertion of [18] on the presence of ellagitannins in species of Melastomataceae.

As natural antioxidants, these ellagitannins could react with free radicals and prevent biological molecule damages which are responsible for various diseases such certain forms of cancer, cardiovascular and neurodegenerative diseases. Several investigators reported that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of diseases associated to oxidative stress [19, 20].

4. Conclusion

These leaves are another source of nutrients and ellagitannins (natural antioxidants) which may exert beneficial effects in human health.

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