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Research Article

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Phytochemical and nutritional analyzes of *Solanum macrocarpon* leaves harvested in Benin

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Abstract: Vegetables are very important sources of protein and minerals for people. This study aims to promote *Solanum macrocarpon* on diet in Benin through phytochemical analysis and assessment of nutritional potential its leaves. Ash following standard ISO 749:1977, proteins according to ISO 5983:2005; content of mineral and trace elements by atomic absorption spectrophotometry and secondary metabolites following staining and precipitation reactions specific to each metabolite family. Determination of total phenolic compounds was made by Folin-Ciocalteu reagent. Aluminum trichloride method has been used to quantify total flavonoids, while the determination of condensed tannins was carried out by hydrochloric vanillin method. The results revealed that *Solanum macrocarpon* leaves contain flavonoids, tannins, proteins and saponosides. The yield of ethanol extract is 19.60%; the ash content is 15.22% and 3759 mg/Kg of proteins. The content of mineral and trace elements showed 428.90 mg/kg of potassium, 31.75 mg/kg phosphorus; 700.25 mg/kg of iron; 6.91 mg/kg of magnesium, 2199.49 mg/kg of sodium, 601.44 mg/kg of nitrogen and 157.10 mg/kg of calcium. These results therefore reflect a high nutritional potential of *Solanum macrocarpon* leave for people in Benin.

Keywords: *Solanum macrocarpon*, alkaloids, flavonoids, tannins, proteins and saponosides.

1. INTRODUCTION

Nutritionally, the fresh immature fruits of okra plays an important role in the human diet because it contains carbohydrates, protein. In African countries in general and in Benin particularly, the market gardening sector has experienced a boom in recent years which has made this sector an important activity both in agriculture, trade and in the processing of agricultural products ^{[1],[2]}. The growing importance given to vegetable cultivation is due to critical role played in agricultural economies in most African countries. In fact, market gardening constitutes an important source of income and employment for unemployed women, children and youth. Nutritionally, vegetable crops play an important role in food security through the diversification and improvement of the diet, thanks to the supply of essential vitamins and minerals ^{[3],[4]}. Given the socio-economic and dietary importance of vegetable crops, FAO has initiated actions in recent years to improve the production of vegetables ^[5]. *Solanum macrocarpon* L (Solanaceae) is an important native African vegetable, especially in west Africa where both leaves and fruits are eaten ^[6]. It is called gboma in Benin. Its taste is more or less bitter and very appreciated. The leaves, fruits and roots have great medicinal use. *Solanum macrocarpon* is one of the most widely cultivated and consumed fruit vegetables in tropical Africa.



Figure1: Mature *S. macrocarpon* leaves grown in Benin

It would occupy the 4th place in volume of consumption after tomatoes, onions and okra ^[7]. Foods which contain high quantity of antioxidants are very important for prevention of chronic diseases such as cardiovascular and cancer ^[8]. Despite its importance as a vegetable and its widespread occurrence, little study has been done until now on this plant. It is to fill this scientific gap that this study aims to enhance *Solanum macrocarpon* through the determination of its nutritional potential and the phytochemical analysis of its leaves.

2. EXPERIMENTAL

2.1. Materials

2.1.1. Plants Materials: The fresh immature fruits of twelve varieties of *Solanum macrocarpon* leaves used in this study were collected from Abomey-Calavi in Benin.

2.1.2. Chemicals: Methanol, Folin–Ciocalteu reagent, quercetin, gallic acid, aluminum chloride, potassium acetate, sodium acetate, aspirin, catechin, hydrochloric acid and sulfuric acid were purchased from Sigma-Aldrich. All reagents and chemicals were analytical grade.

2.2. Methods:

2.2.1. *Solanum macrocarpon* leaves pretreatment: After harvesting, the samples were dried at laboratory temperature until their plant mass stabilized and then reduced to powder.

2.2.2. Plant extracts: The extraction was made with ethanol and hydroethanolic under ultrasounds. Briefly, 10g of powdered biomass were mixed with 100 mL solvent and sonicated for two hours at 50°C with Bandelin (Sonorex Digitech device). Further, all the extracts were filtered through Whatman No.1 filter paper and concentrated under vacuum (Buchi R215, heating bath B-491, rotation 280 rpm, vacuum controller V-850 of 290 mbar) at (50±1)°C. The residues were dried to constant weight and stored in the darkness at 4°C to avoid the degradations until use ^{[9], [10]}

2.2.3. Preliminary phytochemical screening: Secondary metabolites were carried out by coloration and precipitation reactions specific to each family of metabolites ^{[11], [12], [10]}

Table1: Methods for identification secondary metabolites of *Solanum macrocarpon* leaves

Secondary metabolites	Chemical test
Alkaloids	Mayer's test and Dragendroff's test
Anthocyanes	test with hydrochloric acid and ammonia
Anthraquinones	Borntranger's test
Coumarins	Fluorescence at 365 nm
Flavanoids	shinoda test and magnesium powder
Tannins	stiasny test, Ferric chloride and sodium acetate test
Saponins	Frothing test
Leuco anthocyanins	Bate-Smith and metcalf
Mucilages	flaky test
Cyanogenic derivatives	picric acid test
Reducing compound	Fehling's test
Sterols and terpenes	Liebermann-Burchard's test
proteins	Biuret test

2.3. Determination of phenolic compound

2.3.1. Total phenol content: Total phenolic content was determined using the Folin-Ciocalteu colorimetric method Lupoe *et al.*^[9] with some modifications. This method consisted on using a mixture of phosphotungstic and phosphomolybdic acids, which were reduced during the oxidation of phenols into a mixture of tungsten blue oxide and molybdenum. Finally, the absorbance was measured at 760 nm using a spectrophotometer and the total phenol content are expressed in micrograms of gallic acid equivalence per milligram of dry matter (µgGAE/mgDM) ^{[9][13], [14]}

2.3.2. Total flavonoids content: The method of aluminum trichloride (AlCl₃) was used to quantify total flavonoids. This technique was based on the formation of aluminum complex flavonoids. The absorbance was read at 415 nm using a spectrophotometer and total flavonoid content are expressed in micrograms quercetin equivalence per milligram of dry matter (µg QE/mgDM) ^[15].

2.3.3. Condensed tannin content: Vanillin and hydrochloric acid method was used to determine total condensed tannins content. The absorbance was measured at 500 nm using spectrophotometer and tannin content was expressed in micrograms catechin equivalence per milligram dry matter (µgEC/mgDM) ^[16].

2.3.4. Ash content measuring: The ash content has been evaluated following the standard ISO 749 ^[17].

The ash was obtained from the seed powder by incineration at $550^{\circ}\text{C} \pm 15^{\circ}\text{C}$, in a muffle furnace until a constant mass was obtained. Crude ash content (Ta) expressed in % (m/m) of dry matter was obtained using equation :

$$T(\%) = \frac{m_3 - m_1}{m_2 - m_1} * 100$$

Ta: ash content (% m/m) dry matter ; m_1 : mass of the empty crucible (g); m_2 : mass of the crucible + sample (g) ; m_3 : mass of the crucible + ash (g).

2.3.5. Protein Content : The nitrogen content was obtained by the KJELDAHL method from the micro-distillation of the mineralized material of the sample digested with sulfuric acid in the presence of a selenium-based catalyst according to ISO 5983-1:2005. This micro-distillation was done by steam distillation in the presence of a normal solution of sodium hydroxide. In order to obtain the nitrogen content of the sample, the distillate collected in boric acid was titrated with sulfuric acid in the presence of methyl red . The nitrogen content obtained was multiplied by 6.25 to obtain the sample protein content expressed in % of dry matter ^[18].

$$\%N = \frac{1.4 * V * n * d}{100 * P}$$

N: Nitrogen content (%); V: volume of the sulfuric acid solution used during the titration (ml) ; n: normality of the sulfuric acid solution used during titration ; d: dilution of the mineralizer; P: mass of the sample (mg) ; %Proteins= %N * 6.25.

2.3.6. Mineral and Trace Elements Content : Five (05) grams of the sample were cremated following the procedure indicated above. The ash obtained was dissolved in 5 ml of hydrochloric acid (6N) and then evaporated on a hot plate at 125°C . The resulting residue was dissolved and recovered with HNO_3 (0.1M) in a 100 mL flask. This solution was used to obtain the content of the minerals by spectrophotometer Atomic Absorption. The results are expressed in relation to the dry matter ^{[20],[21]}.

3. RESULTS AND DISCUSSION

The secondary metabolites identified in the leaves of *Solanum macrocarpon* are listed in Table 2. The results of the preliminary phytochemical screening revealed the presence of flavonoids, tanins saponosids and proteins in the *Solanum macrocarpon* leaves..In the leaves of *Solanum macrocarpon* collected in Nigeria the alkaloids were identified contrary to the sample from Benin ^[22].

Table 2. Secondary metabolites identified on *Solanum macrocarpon* leaves

Secondary metabolites	Present/absent	Secondary metabolites	Present/absent
Alcaloids	-	Coumarins	-
Tannins	+	Cyanogenic derivatives	-
Flavonoids	+	Saponosids	+
Anthocyanes	-	Anthraquinones	-
Leuco anthocyanins	-	Sterols and terpenes	-
Reducing compound	-	Proteins	+
Mucilages	-		

Legends: +: present; - : absent

The variation of secondary metabolites observed at the level of our samples compared to previous work could be related to the harvest period, the nature of the soil or climatic factors ^{[23],[24]}. The bitter taste of *Solanum macrocarpon* leaves could be explained by its tannin content

3.1. Extraction yield, ash and protein content of *Solanum macrocarpon* leaves: Table 3 shows the yield of hydroethanolic extract, ash and protein contents of *Solanum macrocarpon* leaves. The yield of the hydroethanolic extract of *Solanum macrocarpon* leaves is 19.60%. The ash content of *Solanum macrocarpon* leaves is 15.22%. This ash content is lower than the content obtained from ^[25] in Cameroon (18%) for the leaves of *Solanum macrocarpon* from certain regions. Compared to the ash contents of tubers and cereals which vary only between 2 and 10% (FAO), the leaves of *Solanum macrocarpon* constitute an important reserve of minerals. The protein content of *Solanum macrocarpon* leaves is 3759 mg/ kg. The amount of protein present in the leaves and fruits of *Solanum macrocarpon* is quite high and explains why it is so much appreciated by consumers. The leaf protein content of *Solanum macrocarpon* is higher than those reported for most leafy vegetables such as *Momordica balsamina* (11.29%) and *Moringa oleifera* (20.72%) considered to be important sources of protein ^[26]. They are molecules essential to the life of cells. According to ^[27], with more than 12% protein in plants, they are a good source of protein and can help ensure a balanced diet.

Table 3: Extraction yield, ash and protein content of *Solanum macrocarpon*

Extraction yield	Ash content	Protein content
19.60 %	15.22 %	3759.00 mg/Kg

3.2. Phenolic compound content: The calibration curves for determining the contents of total phenols, total flavonoids and total tannins are shown in Figure 2. The calibration curves for measuring the total phenols content is obtained with the equation $y = 0.1129x$ with the coefficient of determination of $R^2 = 0.9829$. The calibration curves for determining the content of total flavonoids is $y = 4.7156x$ with the coefficient of determination $R^2 = 0.9946$ while that of tannins is $y = 12.857x$ with the coefficient of determination of $R^2 = 0.9968$.

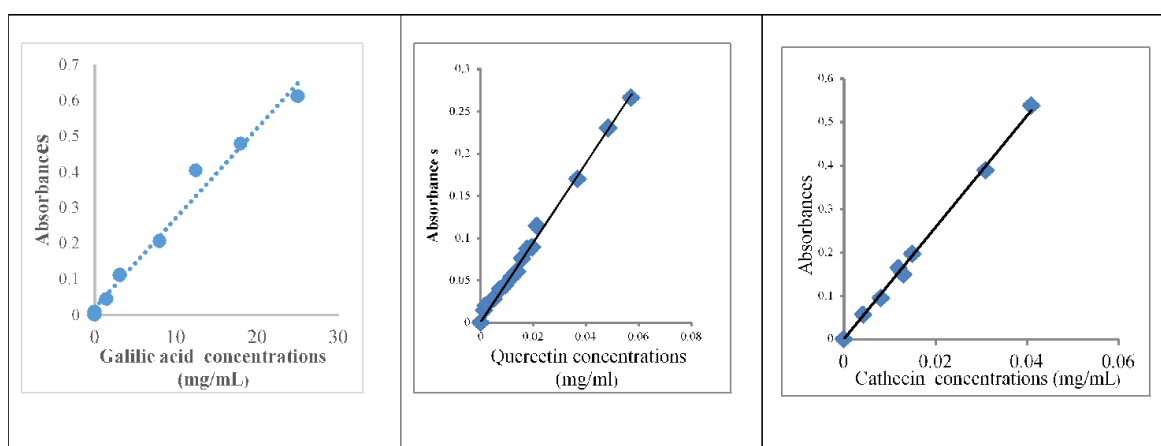


Figure 1: Calibration curves for the evaluation of the levels of phenolic compounds

Table 4 reports the content of phenolic compound in the ethanolic extract of the leaves of *Solanum macrocarpon*. It emerges from the analysis of this table that the content of total phenols in the

ethanolic extract of the leaves of *Solanum macrocarpon* is (2.35±0.02) µg GAE/mgDM and that the content of total flavonoids is (13.77±0.55) µg QE/mg DM with a content of (0.16±0.01) µg CE/mg DM for condensed tannins. The high tannin content of *Solanum macrocarpon* leaves could explain its bitter taste.

Table 4. Phenolic compound content of the ethanolic extract of *Solanum macrocarpon* leaves

Phenolic compound	TPC (µg GAE/mgDM)	TFC (µgQE/mgDM)	CTC (µgCE/mg DM)
hydroethanolic extract	2.35±0.02	13.77±0.55	0.16±0.01

Legends: µgGAE/mgDM: microgram Gallic acid equivalent per gram of dry matter; µgQE/mg DM: microgram Quercetin Equivalent per milligram of dry matter; µgCE/mgDM: microgram catechin Equivalent per milligram of dry matter; TPC: Total phenol content; TFC: Total flavonoids content ; CTC: Condensed tannin content

3.3. Mineral salt content of the *Solanum macrocarpon* leaves: Table 5 shows the calcium, iron, potassium, phosphorus, sodium, nitrogen and magnesium contents of leaves of *Solanum macrocarpon*. The phosphorus content of *Solanum macrocarpon* leaves is 31.75 mg/Kg. It is higher than the content obtained in the sample from Côte d'Ivoire which is 22.29 mg/kg [28]. The potassium and sodium contents are respectively 428.90 and 2199.49 in the leaves of *Solanum macrocarpon*. These levels are much higher than the levels obtained in previous work in Benin, which are 1760.15 mg / kg for sodium and 45.960 mg/kg for potassium [29]. Regarding the calcium content, it is 157.10. This content is higher than the contents obtained in Bangladesh [30],[31], but much lower than the content obtained in Cameroon [32]. As for the iron content of *Solanum macrocarpon* leaves, it is 700.25 mg/Kg. This content is higher than those obtained in previous work [33]. Given these high levels of iron in the leaves of our different cultivars, it is of utmost importance for the population to consume it because iron plays an important role in the formation of hemoglobin in the blood and in the course of reactions metabolism [34]. Regarding the magnesium content, it is 6.91mg/Kg at the level of *Solanum macrocarpon*. This content is lower than that obtained in the literature [29]. As for the nitrogen content, it is 601.44 mg/Kg. The discrepancy noted in the mineral salt content could be explained by the nature of the soil, the harvest period, the fertilizers used, the climate or the methods of analysis. *Solanum macrocarpon* leave is a real reservoir of nutrients. So the leaves of this plant constitute a significant nutritional value for people.

Table 5. Mineral salt content

MSC (mg/Kg)	Ca	Fe	K	P	Na	N	Mg
	157.10	700.25	428.90	31.75	2199.49	601.44	6.91

MSC: mineral salt content ; **K:** Potassium ; **Ca:** Calcium ; **Na:** sodium; **P:** phosphorus; **Mg:** Magnesium ; **Fe:** Iron ; **N :** nitrogen.

CONCLUSION

Consumption of local leafy vegetables is a common practice for most Beninese population in general and especially in the southern Benin. Micronutrients have assumed great public health importance. This research work studied phytochemical and nutritional composition of *Solanum macrocarpon* leave. Phytochemical analysis revealed that samples contain chemical compound like tannins, saponins, flavonoids and proteins. *Solanum macrocarpon* leaves are rich on mineral salts such as

potassium, calcium, sodium; phosphorus; magnesium, nitrogen and iron. *Solanum macrocarpon* leaves contain chemical groups that give them interesting nutritional and medicinal properties.

Disclosure of conflict of interest

The authors of this publication declare that there is no conflict of interest regarding the publication of this research paper.

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