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SPECTROPHOTOMETRY (UV) AND THIN LAYER CHROMATOGRAPHY (TLC) OF TOTAL ALKALOIDS AND GLYCOSIDES OF CUCURBITACINS EXTRACTED FROM A PLANT USED IN THE TREATMENT OF UROGENITAL DISEASES: *LAGENARIA SICERARIA*

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ABSTRACT

Medicinal plant extracts have always played an essential role as medicines to preserve our health. Lagenaria siceraria is a plant of the cucurbitaceae family widely used in the traditional Nigerian pharmacopoeia. Within the framework of the valorization of this plant, this study focused on the seeds and pulps of six (6) regions of Niger. This work was devoted to the selective extraction of total alkaloids and glycosides of cucurbitacins, thin layer chromatography (TLC) and spectrophotometry of these extracts. The yield of extracts in total alkaloids ranges from 0.2 to 10.51%. The highest yield is obtained with Maradi seeds (10.51%). For cucurbitacin glycosides, the yield varies from 1.88 to 24.80%. The highest yield is obtained with Tillabery pulp (24.80%). TLC and spectrophotometry have shown a wide diversity of chemical compounds with different maximum wavelengths and frontal references. The variation in the yields of total alkaloids and glycosides of cucurbitacins is significant not only according to the parts (seeds and pulp) but also according to the regions.

KEYWORDS: Lagenaria siceraria, cucurbitacins, Tillabery pulp.

INTRODUCTION

For many years, man has always used plants to heal himself. Indeed, there are about 500,000 plant species on earth, 80,000 of which have medicinal properties.[1] These intrinsic therapeutic properties of plants are attributed to secondary metabolites. The latter are chemical substances, sometimes with a very complex structure, produced by plants to protect themselves against aggressors (insects for example) and to adapt to their environment.

Despite the progress of modern medicine, the use of medicinal plants in traditional ways is very present in some countries of the world and especially in developing countries.[2] According to the WHO (World Health Organization), 80% of the world's population treat their health problems with traditional remedies, on the one hand because they often do not have access to the drugs prescribed by modern medicine, and on the other hand because these plants are often truly effective.[3] They are also used in pharmaceutical industries.[4] Among these medicinal plants are plants of the cucurbitaceae family. This family of herbaceous plants has adapted to arid regions.[5] It presents interesting nutritional and pharmacological interests.[6,7] About eighteen (18) species are encountered in Niger.[8] *Lagenaria siceraria* (Mol) Stand, which is the subject of this study; belongs to this family.

Numerous studies have shown the use of this plant in traditional medicine.[9] It is used in the preparation of recipes to treat various conditions: prostate cancer, diabetes, intestinal worms, headaches, etc.[10, 11, 12, 9] However, there is little data on the chemical composition of this plant in Niger. Our objective is to make a chemical study of seed and pulp powders from six (6) regions of Niger. This will involve the extraction of total alkaloids and glycosides of cucurbitacins; TLC of these extracts; UV visible spectrophotometric reading and finally to compare the yields of the extracts and the variation of the chemical composition according to the regions.

MATERIALS AND METHODS

Material

Plant material

The plant material consists of fruits of Lagenaria siceraria collected in six (6) regions of Niger during a survey. In the laboratory, the fruits were crushed and the seeds separated from their pulp. After washing and drying the seeds, both parts were ground into powder using a mechanical grinder. Twelve samples were obtained and coded as follows: Dosso seed (GD), Maradi seed (GM), Niamey seed (GN), Tahoua seed (GT), Tillabery seed (Gti), Zinder seed (GZ), Dosso pulp (PD), Maradi pulp (PM), Niamey pulp (PN), Tahoua pulp (PT), Tillabery pulp (Pti), Zinder pulp (PZ).

Methods

Extraction of total alkaloids

Alkaloids are compounds widely present in plants are either in the form of alkaloid salts or alkaloid bases. Their extraction is based on the difference of their solubilities in acidic and basic medium on the one hand and in polar and apolar organic solvents on the other hand.

To extract these compounds the method of Harborne, [13] is used with minor modifications. It involves several steps noted as follows:

- Maceration under reflux with stirring, for 24 hours, of 45g of seed powder or 45g of *Lagenaria siceraria* pulp powder in the presence of 250mL of 2% HCl and 110mL of ethyl acetate ;
- Then the mixture is filtered and the filtrate is recovered;
- The pH of the filtrate is adjusted to 10 using NH_4OH 10% ;
- Liquid-liquid extraction of the filtrate (3 to 4 times) with 50mL ethyl acetate until the aqueous phase is exhausted;
- Removal of traces of water in the organic phase by addition of Na_2SO_4 ;
- Filtration
- Finally, concentration of the dry organic phase in the rotavapor at a temperature below 40°C to avoid denaturing the alkaloids (Figure 1).

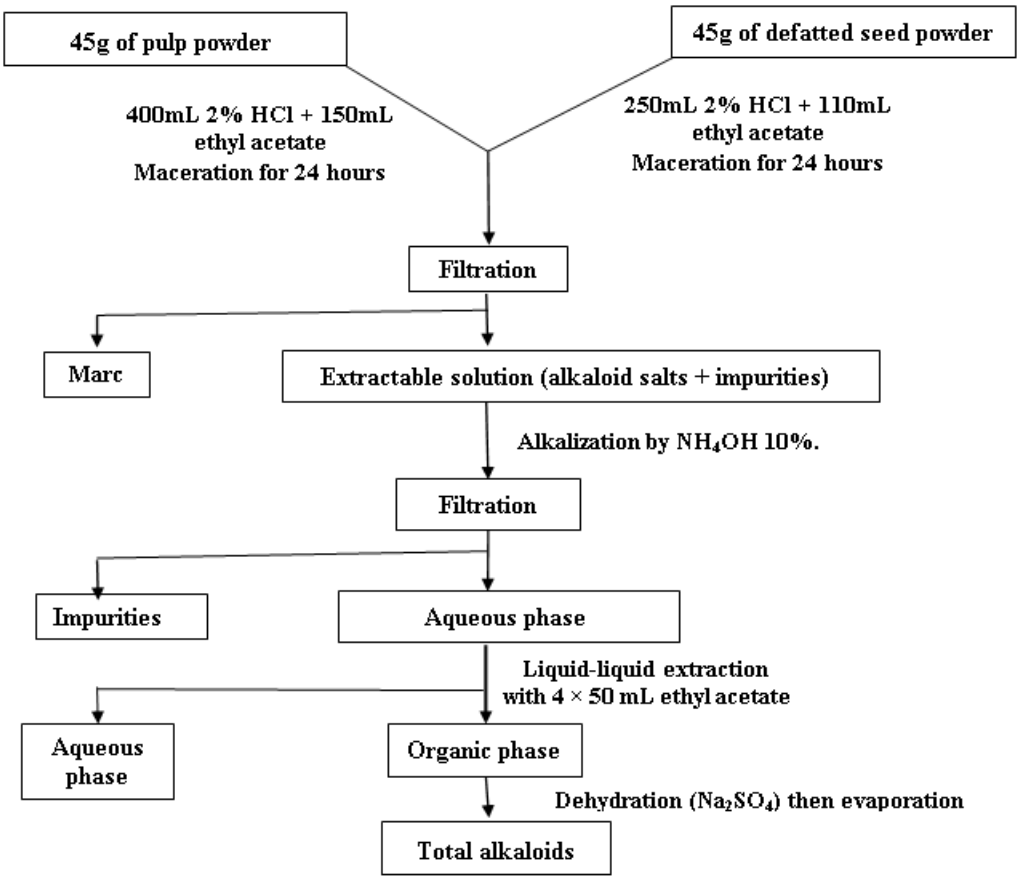

Figure 1: Diagram of acid extraction of total alkaloids.[13]

Chloroformic and ethanolic extraction of cucurbitacin glycosides

The method of Natiq *et al.* [14] was used for the extraction of these compounds. It allows the chloroformic and ethanolic extracts of cucurbitacin glycosides to be obtained respectively. This method is described as follows:

Chloroformic extraction of glycosides

- 25g of seed powder or 25g of pulp powder is extracted under reflux for 6 hours in the presence of 100mL of chloroform ;
- The mixture is filtered and the filtrate is recovered;
- Traces of water present in the phase are removed using Na2SO4 ;
- Filtration;
- The organic phase is concentrated to dryness in the rotavapor (Figure 2).

Ethanolic extraction of glycosides

- The pomace retained at the end of the above reaction is taken up with 100mL of 80% ethanol for 6 hours;
- Filtration of the mixture and recovery of the filtrate ;
- The ethanol is evaporated in a water bath;
- Liquid extraction of the filtrate (3 to 4 times with 50mL ethyl acetate) ;
- Removal of traces of water with sodium sulfate (Na_2SO_4) ;
- Filtration ;
- Concentration of the dry organic phase with rotavapor (Figure 2)

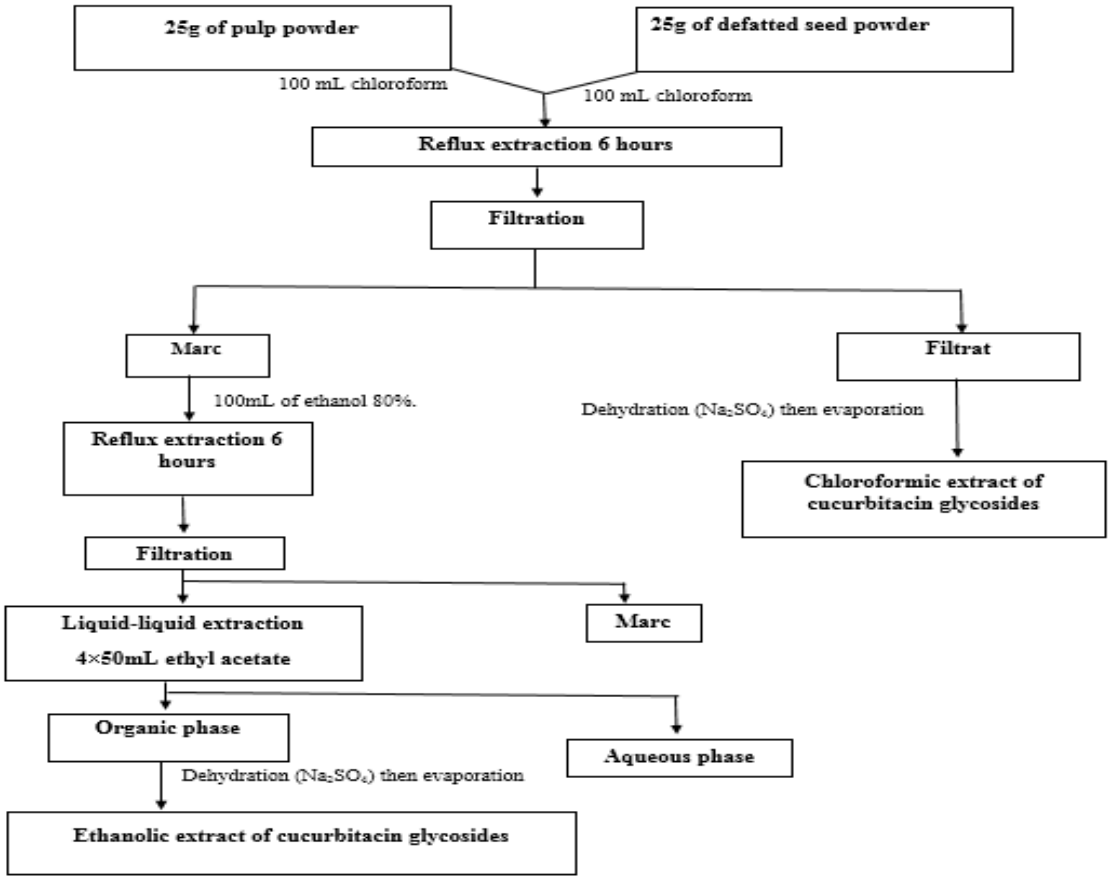

Figure 2: Diagram of glycoside extraction from cucurbitacins.[14]

The yield of total alkaloids and cucurbitacin glycosides in the samples is determined in relation to the mass of the sample.

$$r = \frac{mass\ of\ extract}{mass\ of\ sample} \times 100$$

Thin Layer Chromatography (TLC)

TLC of total alkaloid extracts

The protocol described below is used to establish the TLC of the total alkaloids extracted from our samples.

- Selection of the stationary phase :
- Activation of the TLC plates in the study at 100°C for 30min;
- Choice of the mobile phase: After several trials with the chloroform/methanol system, finally the 18/1 ratio was the best eluent ;
- Saturation of the cell with the eluent solvents;
- A mixture of 10mg of alkaloid sample in 0.5mL of chloroform is prepared;
- Deposition of the sample in small spots 3 to 4 times;
- Introduction of the plate into the saturated cell;
- Follow the development of the chromatogram until the solvent reaches the upper front;
- Revelation: UV lamp (λ = 254nm and 366nm), iodine vapor and sulfuric acid-water 50% ;
- Calculation of the frontal ratio Rf for each component:

$$Rf = \frac{Distance\ travelled\ by\ the\ constituent}{Distance\ travelled\ by\ the\ front\ of\ the\ eluent}$$

TLC of ethanolic and chloroformic extracts of cucurbitacin glycosides

The same steps cited above for TLC of total alkaloids were applied. The only difference is the use of the 17/1 chloroform/methanol system obtained after the trials.

Spectral scanning of total alkaloids and cucurbitacin glycosides

The spectral reading of glycoside extracts of cucurbitacins and total alkaloids was performed in methanolic solution. The apparatus used is a UV-visible spectrophotometer controlled by a computer system and managed by an application software.

Statistical analysis

Statistical analysis was performed using Minitab 16 software. Results are expressed as mean ± standard mean error (m ± s.e.m.). The single-factor ANOVA statistical test coupled with Fisher's comparison method was used.

RESULTATS

Yield and appearance of the various extracts of seeds and pulps of cucurbitacin glycosides and total alkaloids

Yield and appearance of the different extracts of the seeds and pulps of the total alkaloids

Table 1 shows the total alkaloid yield of seeds and pulps. It varies between 0.2 and 10.51%. The highest yield is obtained with Maradi seeds (10.51%) followed by Dosso seeds (7.69%). The lowest yield is obtained with Dosso pulp (0.2%). The seeds are richer in total alkaloids than the pulp. The color of the total alkaloid extracts is brown for the seeds and orange for the pulp. It is also observed that the appearance of these extracts is crystalline.

Table 1: **Extraction yield of total alkaloids from seeds and pulps.**

TOTAL ACALOIDS				
Samples	**Color**	**appearance**	**mass of extract(g)**	**Yield (%)**
MS	Brown	Crystal	4.73	10.51
ZS	Brown	Crystal	0.42	0.93
NS	Brown	Crystal	1.76	3.91
TiS	Brown	Crystal	0.14	0.31
TS	Brown	Crystal	0.4	0.89
DS	Brown	Crystal	3.46	7.69
TP	Orange	Crystal	2.9	6.44
MP	Orange	Crystal	0.15	0.33
TiP	Orange	Crystal	0.21	0.47
NP	Orange	Crystal	0.35	0.78
DP	Orange	Crystal	0.09	0.20
ZP	Orange	Crystal	0.31	0.69

TS: Tahoua seed; tiS: Tillabery seed; ZS: Zinder seed; DS: Dosso seed; MS: Maradi seed; NS: Niamey seed; TP: Tahoua pulp, tiP: Tillabery pulp, ZP: Zinder pulp; MP: Maradi pulp; NP: Niamey pulp; DP: Dosso pulp.

Yield and appearance of the various extracts of seeds and pulps of cucurbitacin glycosides.

Table 2 shows that the chloroformic glycoside extracts of cucurbitacin glycosides from seeds and pulp are all orange in color and viscous in appearance. While the ethanolic extracts of cucurbitacin glycosides (seeds and pulps) are Brown in color with the exception of Maradi, Dosso, Tillabery and Dosso seed pulp extracts which are orange in color.

Table 2: Crude extracts of cucurbitacin glycosides.

	CUCURBITACIN GLYCOSIDES					
	Chloroformic extract			Ethanolic extract		
Samples	**Color**	**appearance**	**mass of extract(g)**	**Color**	**appearance**	**mass of extract(g)**
MS	orange	viscous	1.36	Brown	cristallin	0.03
ZS	orange	viscous	0.23	Brown	cristallin	0.24
DS	orange	viscous	1.08	orange	viscous	0.31
tiS	orange	viscous	1.67	Brown	cristallin	0.8
NS	orange	viscous	1.44	Brown	cristallin	0.95
TS	orange	viscous	0.24	Brown	cristallin	0.24
MP	orange	viscous	0.15	orange	cristallin	0.36
ZP	orange	viscous	0.14	Brown	cristallin	1.23
DP	orange	viscous	0.07	orange	cristallin	0.26

tiP	orange	viscous	0.15	orange	cristallin	2.33
NP	orange	viscous	0.29	Brown	cristallin	0.39
TP	orange	viscous	0.19	Brown	cristallin	1.14

TS: Tahoua seed; tiS: Tillabery seed; ZS: Zinder seed; DS: Dosso seed; MS: Maradi seed; NS: Niamey seed; TP: Tahoua pulp, tiP: Tillabery pulp, ZP: Zinder pulp; MP: Maradi pulp; NP: Niamey pulp; DP: Dosso pulp.

Pulp yields of cucurbitacin glycosides are shown in Figure 3. Thus, the highest yield of chloroformic extract is obtained with Tahoua pulp (3%) followed by Niamey pulp (2.90%). As for ethanol extracts, the highest yield is obtained with Tillabery pulp (23.30%) followed by Tahoua pulp (16%). It is also important to note that the yields of ethanolic extracts of cucurbitacin glycosides are higher than those of chloroformic extracts. However, when comparing the total yield (sum of chloroform yield and ethanolic yield) of our samples, Tillabery pulp with the highest yield (24.80%) followed by Tahoua (19%) and Dosso pulp with the lowest yield (3.30%).

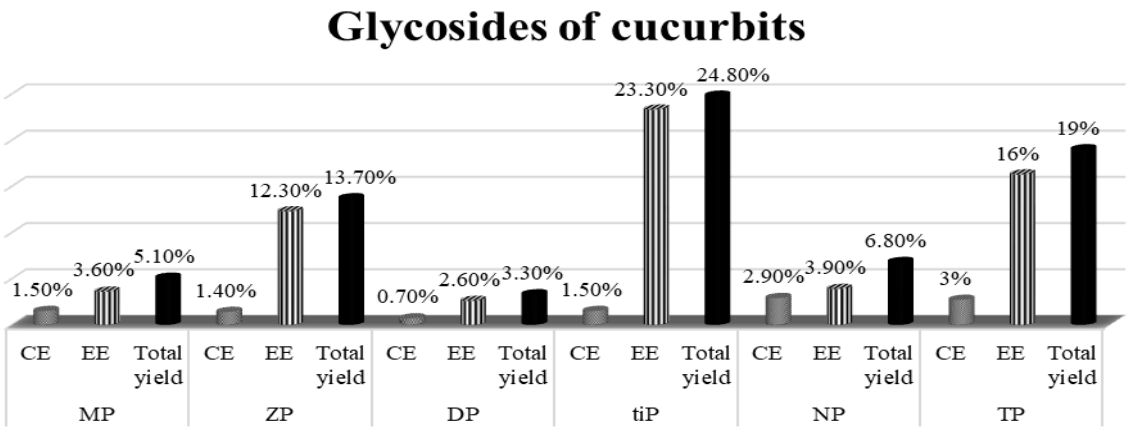

Figure 3: Yield of pulp extracts in cucurbitacin glycosides.

Figure 4 shows the yield of cucurbitacin glycoside extraction from seeds in our different samples. For chloroformic extracts, the highest yield is obtained with Tillabéry seeds (6.68%) followed by Niamey (5.76%). For ethanol extracts, the highest yield is obtained with Tahoua seeds (4%). On the other hand, the lowest yield of chloroformic extracts was obtained with Zinder seeds (0.92%) and the lowest yield of ethanolic extracts was obtained with Maradi seeds (0.12%). Comparison of total yield shows that Tillabery seeds are the richest in cucurbitacin glycosides (9.88%) followed by Niamey seeds with (9.56%).

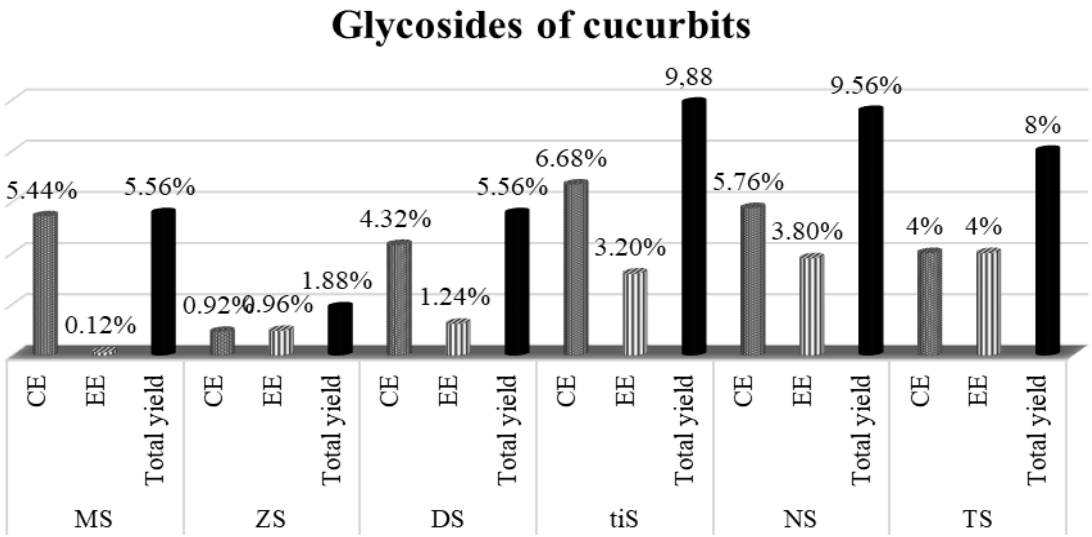

Figure 4: Yield of seed extracts in cucurbitacin glycosides.

Seed and pulp yield variation in total alkaloids and cucurbitacin glycosides by region

Table 3 shows the variation in yields (seed and pulp) of total alkaloids and cucurbitacin glycosides by region. Analysis of this table shows a significant difference in extraction yields across regions.

Depending on the part used, seed yield in total alkaloids and cucurbitacin glycosides (chloroformic and ethanolic)

are significantly different between regions except for the ethanolic extracts of cucurbitacin glycosides from GZ and GD.

For pulps, extraction yields are significantly different. On the other hand, they are insignificant for chloroformic extracts of cucurbitacin glycosides from PM, PZ and Pti.

Table 3: Variation in extraction yields of total alkaloids and cucurbitacin glycosides.

Parties	Total alkaloids	Chloroformic extracts of cucurbitacin glycosides	Ethanol extracts of cucurbitacin glycosides
MS	$10,52 \pm 0,02^{a}$	$5,44 \pm 0,02^{c}$	$0,12 \pm 0,01^{h}$
ZS	$0,92 \pm 0,01^{e}$	$0,92 \pm 0,01^{h}$	$0,96 \pm 0,03^{g}$
DS	$7,67 \pm 0,02^{b}$	$4,32 \pm 0,01^{d}$	$1,24 \pm 0,02^{g}$
tiS	$0,3 \pm 0,04^{i}$	$6,68 \pm 0,04^{a}$	$3,2 \pm 0,04^{e}$
NS	$3,91 \pm 0,03^{d}$	$5,76 \pm 0,01^{b}$	$3,8 \pm 0,01^{d}$
TS	$0,88 \pm 0,01^{e}$	$0,96 \pm 0,01^{h}$	$0,96 \pm 0,01^{g}$
MP	$0,33 \pm 0,01^{i}$	$1,5 \pm 0,01^{g}$	$3,6 \pm 0,03^{d}$
ZP	$0,68 \pm 0,02^{g}$	$1,4 \pm 0,03^{g}$	$12,3 \pm 0,3^{b}$
DP	$0,20 \pm 0,01^{j}$	$0,7 \pm 0,01^{i}$	$2,6 \pm 0,3^{f}$
tiP	$0,46 \pm 0,02^{h}$	$1,5 \pm 0,03^{g}$	$23,3 \pm 0,2^{a}$
NP	$0,77 \pm 0,02^{f}$	$2,9 \pm 0,05^{e}$	$3,9 \pm 0,02^{d}$
TP	$6,44 \pm 0,04^{c}$	$1,9 \pm 0,4^{f}$	$11,4 \pm 0,4^{c}$

TS: Tahoua seed; tiS: Tillabery seed; ZS: Zinder seed; DS: Dosso seed; MS: Maradi seed; NS: Niamey seed; TP: Tahoua pulp, tiP: Tillabery pulp, ZP: Zinder pulp; MP: Maradi pulp; NP: Niamey pulp; DP: Dosso pulp.

TLC of total alkaloids and chloroformic and ethanolic extracts of cucurbitacin glycosides

Total alkaloids

TLC analysis of the total alkaloids in our various samples identified several compounds indicated by their front references. The number of stains per sample is shown in Table 4. The analysis of this table shows that GM seed and Pti and PN pulps represent a large number of spots. On the other hand, Gti, GD, GN seeds and PT and PD pulps have only one spot. Comparison of the number of spots according to regions shows that Gz and GM seeds have more spots than their pulps and conversely, Pti and PN pulps have more spots than their respective seeds. Thus, in Zinder and Maradi, Lagenaria siceraria stores most of its alkaloids at the seed level while in Tillaberi and Niamey, it stores its alkaloids at the pulp level.

Table 4: **TLC of crude extract of total alkaloids from seeds and pulp of *Lagenaria siceraria.***

Samples	Eluent	Number of stains	Rf
ZS	hloroform/méthanol 18/1	2	0.79-0.88
MS		3	0.66-0.79-0.91
TiS		1	0.68
DS		1	0.2
NS		1	0.9
TS		3	0.9-0.9-0.9
MP		2	0.36-0.68
ZP		1	0.67
TiP		4	0.72-0.8-0.93-0.79
TP		1	0.92
DP		1	0.9
NP		7	0.19-0.4-0.66-0.83-0.26-0.9-0.4

TS: Tahoua seed; tiS: Tillabery seed; ZS: Zinder seed; DS: Dosso seed; MS: Maradi seed; NS: Niamey seed; TP: Tahoua pulp, tiP: Tillabery pulp, ZP: Zinder pulp; MP: Maradi pulp; NP: Niamey pulp; DP: Dosso pulp.

Cucurbitacin glycosides

For the TLC of cucurbitacin glycosides, tables 5 and 6 allow us to see not only a difference in frontal reference between chloroformic and ethanolic extracts of the same sample but also the number of spots varies according to regions).

For the chloroformic extracts (Pti, PT, PD, PZ), we find the highest number of spots than at the level of their respective seeds. This table also allows us to observe that the ethanolic extract of Tahoua (GT) seeds has more spots than its pulp.

Overall, cucurbitacin glycosides are more stored in the pulp.

Table 5: TLC of the chloroformic extract of cucurbitacin glycosides from seeds and pulp of *Lagenaria siceraria*.

Samples	Eluent	Number of stains	Rf
ZS	Chloroforme/méthanol 17/1	3	0.82-0.83-0.84
MS		4	0.84-0.95-0.84-0.82
TiS		2	0.08-0.88
DS		3	0.1-0.82-0.89
NS		3	0.09-0.93-0.9
TS		3	0.09-0.93-0.9
MP		3	0.44-0.74-0.73
ZP		4	0.41-0.09-0.81-0.78
tiP		6	0.1-0.4-0.76-0.06-0.75-0.76
TP		6	0.09-0.67-0.81-0.53-0.81-0.93
DP		6	0.89-0.11-0.89-0.45-0.12-0.89
NP		6	0.11-0.45-0.06-0.85-0.12-0.84

TS: Tahoua seed; tiS: Tillabery seed; ZS: Zinder seed; DS: Dosso seed; MS: Maradi seed; NS: Niamey seed; TP: Tahoua pulp, tiP: Tillabery pulp, ZP: Zinder pulp; MP: Maradi pulp; NP: Niamey pulp; DP: Dosso pulp.

Tableau 6 : CCM de l'extrait éthanolique de glycosides des cucurbitacines des graines et pulpes de *Lagenaria siceraria.*

Samples	Eluent	Number of stains	Rf
ZS	Chloroform/méthanol 17/1	3	0.63-0.77-0.87
MS		3	0.26-0.59-0.85
tiS		5	0.8-0.54-0.82-0.1-0.7
DS		2	0.7-0.9
NS		4	0.64-0.72-0.88-0.11
TS		3	0.09-0.9-0.7
MP		3	0.63-0.89-0.13
ZP		5	0.63-0.64-0.76-0.88-0.2
tiP		5	0.1-0.64-0.75-0.89-0.51
TP		1	0.9
DP		2	0.89-0.66
NP		4	0.1-0.65-0.75-0.89

TS: Tahoua seed; tiS: Tillabery seed; ZS: Zinder seed; DS: Dosso seed; MS: Maradi seed; NS: Niamey seed; TP: Tahoua pulp, tiP: Tillabery pulp, ZP: Zinder pulp; MP: Maradi pulp; NP: Niamey pulp; DP: Dosso pulp.

Spectral scanning of extracts of total alkaloids and cucurbitacin glycosides (determination from λmax)

The UV spectrum of the extracts of total alkaloids and cucurbitacin glycosides, from six (6) regions, performed in methanolic solution by a UV/visible spectrophotometer allowed to obtain the maximum absorption peaks. The analysis in Table 7 shows that the wavelength of the total alkaloids of the seeds varies from 219.69 to 275nm. For cucurbitacin glycosides the maximum absorption wavelengths vary from 196.47 to 283nm.

For the total alkaloids of the pulps, the wavelengths vary from 198 to 279nm. And for glycoside extracts, they range from 196 to 275nm (Table 8).

This method has also shown this variation in the absorption of the molecules of the extracts according to the regions. This shows that there are several different chemical compounds (total alkaloids and cucurbitacin glycosides) present in these extracts.

Table 7 : Absorption wavelengths of total alkaloids and cucurbitacin glycosides in the pulps.

λmax extracts of total alkaloids					
Seeds					
Dosso	**Maradi**	**Niamey**	**Tahoua**	**Tillabéry**	**Zinder**
202 ; 220 ; 271,8	219,69 ; 270	221,95 ;269	219,93 ;267,8	221 ; 275	220,274
λmax chloroformic extracts of cucurbitacin glycosides					
221,280	204, 221,277	221 ; 278	204, 221,273	218,277	200 ; 220 ; 283
λmax ethanol extracts of cucurbitacin glycosides					
197,47 ; 203,8 ;277,23	198, 64, 272,11	200 ; 221,63 ;277,27	196,47 ; 217,47 ; 274,11	219,63 ; 273,11	220 ; 275,11

Tableau 8 : Longueurs d'ondes d'absorption des alcaloïdes totaux et glycosides de cucurbitacines des pulpes.

λmax extracts of total alkaloids					
Pulps					
Dosso	**Maradi**	**Niamey**	**Tahoua**	**Tillabéry**	**Zinder**
200 ;221 ;279	221 ;278	198 ;221 ;275	220 ;275	220 ;280	220 ;273
λmax chloroformic extracts of cucurbitacin glycosides					
219,36 ;272	203 ;220,63 ;277	276,9 ;217,3	202,63 ;221 ;274,77	222 ;276	220,63 ;273,61
λmax ethanol extracts of cucurbitacin glycosides					
220 ;225 ;275	200 ;225 ;277	196,47 ;268,94	199,64 ; 218,46 ; 275	217,47 ; 275	197,47 ;203,8 ;272

DISCUSSIONS

Yields of *Lagenaria siceraria* seeds and pulp in total alkaloids range from 0.31 to 10.51% for seeds and 0.2 to 6.46% for pulp. This variation in total alkaloid extraction yield is also observed according to regions.

The appearance and color of the extracts vary according to the regions. The color is brown and the aspect is pasty for the seed extracts. Pulp extracts appear orange and with a crystalline appearance.

This difference in yield, appearance and color of the seed and pulp extracts could be due to their chemical compositions. Indeed, the chemical composition of a plant is influenced by the mineral salt content of the soil.[15]

The presence of these secondary metabolites in these parts of the plant (sheaths and pulps) could be due to the defensive roles they play for the plant.[16] Also, according to Pathak *et al*.[17] the role of secondary metabolites is related to their location within the plant. The experiment of Zobel and Brown[18] showed that furanocoumarins accumulated in the leaves constitute a kind of 'first chemical barrier'.

The variation in yields of total alkaloids and cucurbitacin glycosides observed according to region could be due to the adaptation of the plant to its environment. Indeed, according to Chadhaey *et al*. [19] plants produce specific secondary metabolites in order to adapt to adverse environmental changes. The alleophatic phenomenon could also justify this difference in chemical composition.[20,21]

Chromatographic and spectrophotometric analyses have shown a significant number of spots and maximum wavelengths (λmax). Those that demonstrate the structural diversity of the total alkaloids and cucurbitacin glycosides that make up this plant family. Several experiments have corroborated, with other plants, these results. Indeed, an experiment carried out by Takeda *et al*,[22] on strawberry cells showed that at 45°C this species produces a large part of the anthocyanin molecule. This diversity of chemical compounds could also be justified by the type of soil where the plant developed. According to Pundarikashu and Bhasvar[23] soil salinization increases alkaloid content.

CONCLUSION

The results obtained by the extraction of total alkaloids and cucurbitacin glycosides indicate that the seeds are richer in alkaloids and the pulps in cucurbitacin glycosides. This variability in yield is observed not only according to the parts (seeds and pulps) but also according to the regions. A great diversity of compounds is observed through their frontal references and absorption wavelengths. It would be important to identify these compounds and to carry out biological tests in order to select active ingredients with effective therapeutic properties.

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