Phytochemical Study, Mineral Salt Content and Evaluation of the Antihyperglycemic Activity of *Psathyrella tuberculata* (*Psathyrellaceae*) an Edible Mushroom

Fofie Ybn[1]*, N'Cho Al[2], Coulibaly T[3], Kouassi G[1], Kouassi M[1], Maiga-Ngouandi[1], Kone-bamba Diénéba[1,] and Kouakou Th[4]

[1]Faculty of Pharmaceutical and Biological Sciences, University of Félix Houphouët Boigny Cocody Abidjan, Ivory Coast
[2]Centre National de Recherche Agronomique (CNRA), Station de Recherche de Frekessédougou, Côte d'Ivoire
[3]Laboratory of Biology and Health, UFR Biosciences, University of Félix Houphouët-Boigny (UFHB), Abidjan, Côte d'Ivoire
[4]University of Nangui Abrogoua, Faculty of Natural Science, Laboratory of Biology and Improvement of Plant Productions, Côte d'Ivoire

Corresponding Author*

Fofie Ybn
Faculty of Pharmaceutical and Biological Sciences,
University of Félix Houphouët Boigny Cocody Abidjan
Abidjan, Côte d'Ivoire
E-mail: yvettefofie2021@gmail.com

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Abstract

Introduction: *Psathyrella tuberculata* is recognized in Africa and particularly in West Africa for its many therapeutic properties. For decades, this mushroom has been the subject of several scientific studies in order to highlight its various therapeutic properties, proven in traditional medicine, and to understand its mechanism of action.

Objective: It is within this framework that this research work falls, which is a contribution to the study of *Psathyrella tuberculata* a food mushroom.

Material and methods: The material was the whole mushroom (*Psathyrella tuberculata*) including the stem and the cap. The experimental study first concerned the identification of the drug by macroscopic and micrographic description. Then, the secondary metabolites were sought and then assayed by HPLC. Then the biochemical constants were calculated after trials in triplicate. The experimental study focused on the total aqueous extract obtained by the decoction method. After extraction and phytochemical analyzes, the antihyperglycemic effect *in vivo* in Wistar rats was evaluated. This hyperglycemia was induced by oral overload with 30% glucose. Also, other parameters

Results: The phytochemical analysis of *Psathyrella tuberculata* based on staining and/or precipitation reactions revealed the presence of heterogeneity of chemical groups (saponins, terpenes, sterols, alkaloids, quinonics, polyphenols, flavonoids). The results obtained on rat blood glucose levels showed that the product caused a significant drop in blood sugar levels after oral glucose overload. *Psathyrella tuberculata* is therefore active in rats given glucose overload, but its action on fasting blood sugar is not significant. The pharmacological study on the activity of *Psathyrella tuberculata* shows a decrease in blood glucose in rats given an oral glucose overload (post-meal glucose).

Conclusion: Consequently, *Psathyrella tuberculata* could be recommended as food to diabetics as well as in a person wanting to prevent diabetes.

Keywords: *Psathyrella tuberculata* • Traditional medicine • Phytochemical study • Antihyperglycemic activity

Introduction

According to the World Health Organisation (WHO), traditional medicine has always existed: it is the sum total of knowledge, skills, and practices which are based on culturally specific theories, beliefs, and experiences and which are used to maintain human beings in good health, as well as to prevent, diagnose, treat and cure physical and mental illnesses. The use of plants for healing is a question of culture and tradition in Africa. It should be noted that for primary health needs, a large segment of the African population uses traditional medicine, the remedies of which are essentially based on plants [1,2]. The art of healing with plants has been known and practiced in Africa for a long time because it makes use of knowledge transmitted orally from generation to generation to certain categories of trained individuals such as traditional health practitioners and herbalists [3].

Like other pathologies, diabetes is also treated in traditional medicine. In fact, diabetes is a potentially fatal disease responsible for nearly 4 million deaths worldwide each year [4]. It is a disease considered by WHO to be an epidemic and the prevalence of which has increased dramatically in recent years. Currently, nearly 425 million people worldwide have diabetes [5]. Diabetes mellitus is one of the most common no communicable diseases in both industrialized and many developing countries where it tends to take the form of an epidemic [6]. The prevalence in Côte d'Ivoire is currently estimated at nearly 5% [5]. In addition, a poor balance in the functioning in the organism of the diabetics is responsible for the occurrence of cardiovascular complications by alteration of the blood vessels, and represents [7].

- 3 to 6 times more risk of developing heart disease
- 25% of kidney failure requiring dialysis
- the leading cause of blindness
- 50% of lower limb amputations

Diabetes is the fourth leading cause of hospitalization and death worldwide [6]. Based on these findings and in view of the considerable increase in the number of diabetics in the world and the high cost of drugs, it is a wise decision to undertake a study on medicinal plants, in particular edible plants. It is with this in mind that we have embarked on the study of diabetes in Côte d'Ivoire using *Psathyrella tuberculata*, an edible fungus considered to have anti-diabetic properties.

We intend to contribute towards improving the traditional African pharmacopoeia by evaluating, through scientific tests, the action of macerated *P. tuberculata*, an edible fungus of the Psathyrellaceae family, renowned in traditional African medicine, for its antidiabetic activity [8].

The general objective of this study was to evaluate the hypoglycemic activity of this fungus on the blood sugar levels of rats.

Thus, we set up the following specific objectives:

- determine quantitatively and qualitatively the different chemical groups contained in this fungus
- evaluate the activity of the extract on glycemia of the fasting rat then on the glycemia after an oral glucose overload

Materials and Methods

Plant material

The plant material consists of *Psathyrella tuberculata*. It's a species of true fungi of the *Psathyrellaceae* family. *P. tuberculata* is an edible mushroom found in West Africa. The material was dried in an oven at 60°C then powdered using a grinder.

Micrograph

It consists of the powder of *Psathyrella tuberculata*. The fresh-picked mushroom is dried in the open air for two weeks, then crushed with a wooden mortar and pestle. A powder was obtained which will be observed under a microscope. A very small quantity of fine powder was observed between slide and coverslip and mounted in a drop of distilled water. The examination and

identification of the different elements were done at the magnification (10x) and to draw the different elements, the magnification (40x).

Determination of biochemical characteristics

The method as described by Coruh [9] was used for the determination of humidity. Crude protein was determined from total nitrogen, according to Kjeldhal method [9]. The lipid content was determined according to the method described by AFNOR. The energy value was calculated using the specific coefficients of [10] for proteins, fats, and carbohydrates. The method of [9] was used for the determination of ash content.

Mineral salts

Measurement of mineral salts: The qualitative and quantitative analysis of minerals are made using punctual microanalysis by energy dispersive spectrometry coupled with Scanning Electron Microscope/ Energy Dispersion Spectrometry (SEM/EDS), variable pressure DC/AR (SEM FEG Zeiss Supra 40 VP). This device is equipped with an X-ray detector (Oxford Instruments) connected to a micro analyzer platform Energy-Dispersive X-Ray Spectroscopy EDS (Inca Cool Dry, without liquid nitrogen) [10].

Procedure: Two grams of sample were incinerated (ASTM D 482), then cooled in a dryer. Then 10 mg of ash are homogeneously spread with double-sided adhesive carbon onto a primed pad and attached to the object holder of SEM/EDS. The whole was introduced into the SEM chamber for microanalysis-RX (EDS). The desired minerals are primarily calcium, chromium, magnesium, manganese, sodium, potassium, and zinc. Results are proportions, averages of three trials (Table 1).

Phytochemical study

Mushroom extract preparation for the specific determination of total phenols and total flavonoids

A sample of 50 mg powder of *Psathyrella tuberculata* was placed in 10 mL of methanol (96%) contained in a hemolysis tube for 12 h. The filtrate was centrifuged at 2000 rpm for 10 min, and the supernatant represented the crude extract.

Determination of total phenolic content

Folin Ciocalteu reagent was used for the analysis of total phenolics content [8]. Briefly, 0.1 mL of the crude extract was mixed with 0.5 mL of Folin-Ciocalteu reagent. The solution was kept at 25°C for 5 min before adding 1.5 mL of sodium carbonate solution 20% (w/v) and adjusting the volume to 0.9 mL with water. After 30 min, the absorbance was measured at 765 nm. Gallic acid was used as a standard for the calibration curve. Total phenolic content was expressed as mg gallic acid equivalents per gram of sample (mg/g).

Determination of flavonoid content

The total flavonoid content was measured by a colorimetric assay [9]. A volume of 0.1 mL of crude extract was added to 0.9 mL of distilled water, 0.5 mL of aluminum chloride and after 5 min, 0.5 mL of potassium acetate was added. Immediately, the mixture was diluted by the addition of 2 mL of distilled water and mixed thoroughly. The absorbance was determined at 415 nm against a blank. rutin was used as a standard for the calibration curve. The total flavonoids content of the extract was expressed as mg rutin equivalents per gram of sample (mg/g).

***In vitro* antioxidant activity**

The anti-free radical activity was measured by the degradation of DPPH, 2,2'-diphenyl-1picrylhydrazyl, which is a synthetic radical exhibiting an intense purple coloration. The DPPH test makes it possible to measure the anti-free radical power of pure molecules or plant extracts in a model system (organic solvent, room temperature) according to the protocol described by Coruh **[**9].

Table 1. Search for major chemical groups.

Chemical groups	Reagent	Reactions
Sterols and polyterpenes	Sulfuric acid	Interface of a red ring
Polyphenols	Ferric chloride	Green coloring
Flavonoides	Cyanidine	Color change
Catechin tannins	Stiasny's reagent	Flake precipitation
Gallic tannins	Ferric chloride	Blue-black coloring
Quinonic substances	Ammonia diluted to half	No red coloration
Alcaloïdes	Dragendorff/Bouchardât	Orange/reddish brown precipitation
Saponosides	Stir	Presence of foam 10 minutes after the physical test

% Inhibition (DPPH) = [$A0 - A1$] $A0 \times 100$

A0=Absorbance of white,

A1=Absorbance of the extract at a given concentration

Qualitative analysis by HPLC

Extraction and purification of phenolic compounds

Extraction of the total phenols was carried out as in the previous experiment. For purification, 4 mL of the crude phenol extract was evaporated at Speed Vac (Savant, USA). The sample was taken up in 1 mL of methanol/water (30/70, v/v) and then chromatographed on a mini-column of C18 (Sep pack®) scraped silica in the Supelco Visiprep™ system. Beforehand, the conditioning of the columns is carried out by successive washing with 100% methanol (2 mL), with 50% methanol (2 mL), and with distilled water (6 mL). After the sample was removed, awash with 2 mL of distilled water was performed and the phenolic compounds were eluted with 4 mL of methanol/water (90/10, v/v). The eluate obtained is evaporated at Speed Vac, taken up in 1 mL of methanol/water (50/50, v/v), and then filtered on a Millipore membrane (0.45 µm) before being injected into high-performance liquid chromatography (purified phenolic extract).

Analysis conditions

High-Performance Liquid Chromatography (HPLC) is performed according to the modified method of [11]. It is used for the separation and quantification of the various phenolic compounds of cotton leaves treated with the fungal fraction. The analysis of the samples was carried out on two HPLC chains; the first chain (Agilent LC 1100 series) is equipped with a degasser, an automatic injector, a high-pressure binary pump, and a UV-visible detector. The second chain (Agilent LC 1200 series) includes a quaternary pump and is connected to an iodine array detector and a nuclear magnetic resonance spectrometer (Bruker Avance III, 600 MHZ). The column used with the two chains was a reverse phase C18 (Zorbax Eclipse XDB-C18, 150 mm × 2,1 mm: 1,8 µm). Elution is carried out with a binary gradient composed of solvent A: Trifluoroacetic Acid (TFA) 1% / water (2.5/97.5; v/v) and solvent B: acetonitrile / solvent A (80/20, v/v). The profile of the elution gradient was : 0 min-5 min (5%solvant B), 5 min-10 min (10%solvant B), 10 min-15 min (50%solvant B), 15 min-20 min (80%solvant B), 20 min-25 min (5%solvant B). The chromatograms were detected at 254 nm with a flow rate of 1.3 ml/min.

Separation and identification of phenolic compounds by HPLC

The separation and the determination of the phenolic compounds are carried out in HPLC whose control is managed by a microcomputer (Workstation system). A reference library of phenolic compounds was made with compounds purified and identified by nuclear magnetic resonance (Raman). This library contains the retention times and Raman spectra of these compounds. The chromatograms obtained were used for the identification of the compounds contained in the injected samples. The structure of the phenolic compounds was verified by Raman.

Assessment of anti-hyperglycemic activity

This is evaluated by inducing temporary hyperglycemia in non-diabetic rats and then verifying the effect of the studied plant extracts on induced hyperglycemia. In practice, the rats chosen for this test were allowed to fast for 18 hours, weighed to constitute homogeneous batches before being marked to differentiate them. Then, the basal glycemia Thyroxine Binding Globulin (TBG) of each animal was measured using the Accu-Chek® Active Go glucometer (Roche). The weight of each animal made it possible to administer a glucose overload in order to create hyperglycemia with a 30% glucose solution, i.e a dose of 3 g/kg of body weight. This administration was made by gavage [12]. The rats having exhibited hyperglycemia greater than or equal to 0.5 mmol/L relative to the basal glycemia after 30 minutes were selected and then regrouped into a homogeneous batch. Each batch consisted of 6 rats. Immediately after the state of hyperglycemia, demonstrated by the measurement of blood glucose, the batches were treated by gavage with distilled water, aqueous extract of the drug, or a reference oral antidiabetic agent. The administration volume was 10 mL/ kg body weight.

The glycemia after treatment of each animal was measured every 30 minutes (t) for 90 minutes. The blood glucose was determined by applying a drop of blood from the tail vein to an Accu-Chek® Active Go (Roche) test strip. The reading was taken in mg/dl then multiplied by the factor 5.55 (inverse of the weight of glucose (180)). Thus, the values used are in mmol/L [13]. The anti-hyperglycemia activity was expressed as a percentage reduction (% reduction) in induced hyperglycemia over time compared to hyperglycemia Oral Glucose Tolerance Test (OGTT) in the control group.

OGTT: Oral hyperglycemia value of the control group

TG: Blood glucose value at time t

Evaluation of anti-hyperglycemic activity

Principle

This test consists of provoking temporary hyperglycemia in non-diabetic rats and then verifying the effect of different doses of the aqueous preparation of the drug under study on hyperglycemia.

Method

The rats chosen for this test, fasted for 18 hours, are weighed to form homogeneous batches before being marked to differentiate them. Then, the Basal Glycemia (BGT) of each animal is measured using the Accu-Chek Active Go glucometer (Roche). After induction of hyperglycemia by 30% glucose as mentioned above, the rats presenting hyperglycemia greater than or equal to 0.5 mmol/L compared to the baseline glycemia were retained and reorganized into homogeneous batches. Each batch consisted of 6 rats. Immediately, after the state of hyperglycemia, objectified by the measurement of glycemia, the batches were treated by force-feeding with distilled water, aqueous drug preparations, or a reference oral antidiabetic.

The volume of administration is 10 mL/kg body weight.

The blood sugar after treatment of each animal was measured every 30 minutes (t) for 90 minutes. Blood glucose determination was made by applying a drop of blood from the tail vein to an Accu-Chek Active Go(Roche) test strip. The reading was made in mg/dL and the values used in g/L. The anti-hyperglycemic activity was expressed as the percentage reduction (% reduction) of induced hyperglycemia over time compared to fasting hyperglycemia (OGTT) of the observed rats. Below are the different batches created for our study:

Batch I: Normoglycemic rats (controls) receiving distilled water

Batch II: Hyperglycemic rats treated with distilled water

Batch III: Hyperglycemic rats treated with Gliclazide 60 mg/kg

Batch IV: Hyperglycemic rats treated with Metformin 500 mg/kg

Batch V: Hyperglycemic rats treated with the aqueous preparation at 100 mg/kg

Batch VI: Hyperglycemic rats treated with the aqueous preparation at 200 mg/kg

Batch VII: Hyperglycemic rats treated with the aqueous preparation at 500 mg/kg

Batch VIII: Hyperglycemic rats treated with the aqueous preparation at 1000 mg/kg

Batch IX: Normoglycemic rats treated with the aqueous preparation at 1000 mg/kg

Treatment of rats

The hyperglycaemic rats of batch III were treated with gliclazide at 60 mg/kg.

The hyperglycemic rats of batch IV were treated with metformin at 500 mg/kg.

Hyperglycemic rats from batches V to VIII were treated with the aqueous preparation of the drug at different doses.

The normoglycemic rats of batch IX were treated with a dose of 1000 mg/kg.

Statistical analysis

The data obtained from the antidiabetic hypoglycemic activities study are expressed as mean ± standard deviation. The comparison between the different groups was carried out by Analysis of Variance (ANOVA), and Student's t-test was used to separate the means. A p-value <0.01 is considered highly significant and a p-value <0.05 is significant [14] (Table 2).

Statistical analysis method

The data relating to the study of blood glucose were subjected to a repeated measure analysis of variance α=5% and expressed as the mean ± standard deviation in the Tables 3-5 that we present. The median was also determined in each of the samples in order to assess the dispersion of the glycemia of each of them.

In addition, a comparison of glycemia means was carried out on the basis of the t-student test at risk α=5% in order to assess the significance of the differences in glycemia.

In addition, a comparison of glycemia means was carried out on the basis of the t-student test at risk α=5% in order to assess the significance of the differences in glycemia [15].

Results and Discussion

Macroscopic description

The distribution of *Psathyrella tuberculata* is cosmopolitan but is mainly found in forest areas. In the adult stage, the carpophore or aerial part of the fungus is dark brown in color (Figure 1). The flesh of the cap is thin, spindly, brittle, and tender. The taste is bland with the smell of decaying wood. The uniform stipe, of less marked color than the cap, is centered, cylindrical, thin, hollow, and fistulous (Figure 2).

Micrographic description

Micrographic study of *Psathyrella tuberculata* powder reveals the presence of numerous spores (Figure 3).

Biochemical Constants Result

Determination of biochemical characteristics

The Biochemical composition of *Psathyrella tuberculata* samples was

Figure 1. Whole dried *Psathyrella tuberculata.*

Figure 2. Different views of *Psathyrella tuberculata.*

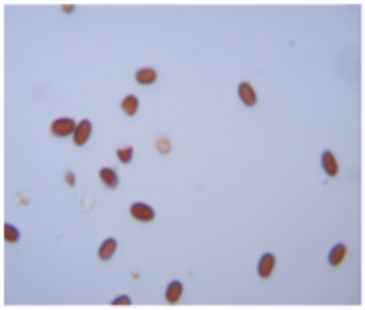

Figure 3. Micrograph of *Psathyrella tuberculata.*

determined. The results obtained are shown in Table 2.

Mineral salts result

Phytochemical screening

Identification of the *Psathyrella tuberculata* chemical compounds by the HPLC method:

From this figure 4, 3 peaks are distinctive. The following table 6 allowed an identification

Effect of the decocted *Psathyrella tuberculata* on blood sugar

Analysis of basal blood sugar

In this study, the normoglycemic rats were treated with the highest dose of 1000 mg/kg (Figures 4-5). During the first 30 minutes, the blood glucose levels remain relatively stable both in the control batch and in the batch treated with the dose at 1000 mg/kg (batch IX). After treatment with the aqueous preparation at 1000 mg/kg, a slight increase in blood sugar was observed 30 minutes later in the rats of batch IX, while that of the control batch that received nothing dropped slightly to an average of 105.3 g/L. One hour after the treatment in batch IX, the glycemia of the rats of this batch tends to stabilize to return to its initial level at T0. In the control batch, the blood sugar also remains stable.

Evaluation of the activity of the treatment applied to normoglycemic rats

The Tables 3-7 below shows the different variations in blood glucose at 30 and 60 minutes after treatment at a dose of 1000 mg/kg. The changes observed over the period are not significant at a threshold of 5%. Thus, no significant variation in blood glucose was recorded either in the control batch or in the batch administered with the dose of 1000 mg/kg. Moreover, the comparison of the final blood glucose at T90 relative to the basal blood sugar (fasting sugar evaluated at T0) showed that the final blood glucose remains significantly identical to that observed on an empty stomach in batch IV (p-value>0.05). It can thus be deduced that no hypoglycemic activity was observed at the 5% threshold in normoglycemic rats.

Evolution of the average glycemia (g / L) in hyperglycemic rats treated with *Psathyrella tuberculata* according to time.

The figure below traces the evolution of the blood sugar levels in hyperglycemic rats over time-based on the products administered. In 30 minutes after glucose overload at T0, blood glucose increases in all the batches observed, with a much higher level in the batch treated with the dose at 200 mg/kg. After 60 minutes, the blood glucose drops in all the batches following the treatment applied, both in the batches treated with Gliclazid (batch III) and Metformin (batch IV) and in the batches treated with the different doses of *Psathyrella tuberculata*. Thus, the blood sugar in batch III changed from about 150 g/L to 75 g/L. In

Table 2. Average chemical composition of *Psathyrella tuberculata.*

Sample Parameters	Decoction of *Psathyrella tuberculata*
Humidity (%)	7.5
Total ash (%)	21.39
Sulfuric ash (%)	296.07
Chloric ash (%)	730.41
Carbohydrate (%)	66.08
Fat (lipid%)	1.88
Protein (%)	2.57
Energy Value (kcal/100 g)	290.38

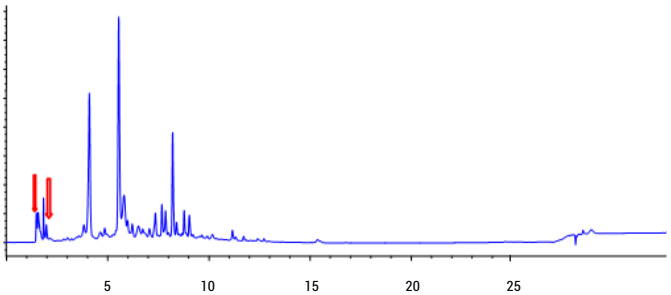

Figure 4. Chromatogram of phenolic compounds extracted from samples.

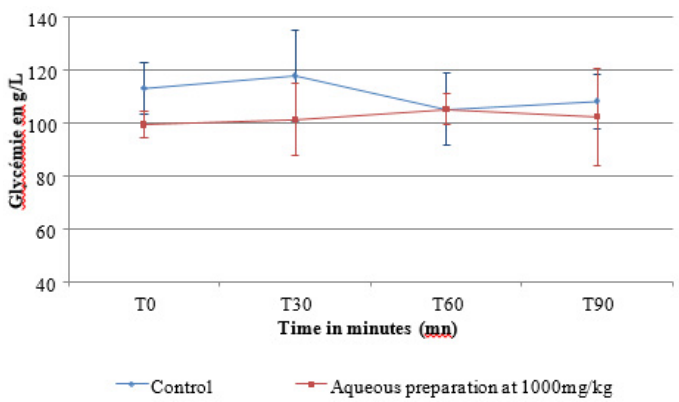

Figure 5. Evolution of the glycemia in normoglycemic rats.

Table 3. Mineral salts composition in the percentage of weight in the drug. We noticed the presence of many mineral salts including Phosphorus, Sulphur, Chlorine, Potassium, Calcium, Chromium, Manganese, and Iron

Z	Symbole	Elément	Norm. Int.	Concentration	Erreur Abs.
1		Somme		79.35%	
2	Na_2O	Sodium	76.4916	2497%	0.035%
3	MgO	Magnesium	369.7753	3420%	0.017%
4	Al_2O_3	Aluminium	1128.7347	3525%	0.010%
5	SiO_2	Silicium	5742.3797	9095%	0.010%
6	P_2O_5	Phosphorus	21971.5692	17.46%	0.01%
7	SO_3	Sulfur	3837.4693	1965%	0.002%
8	Cl	Chlorine	5896.8233	5753 ppm	6 ppm
9	K_2O	Potassium	8314.5245	36.80%	0.03%
10	CaO	Calcium	251.4383	1578%	0.016%
11	TiO_2	Titanium	64.3145	0.3017%	0.0036%
12	V_2O_5	Vanadium	23.894	78 ppm	18ppm
13	Cr_2O_3	Chromium	15.9764	65.8 ppm	2.2ppm
14	MnO	Manganese	68.8575	0.05543%	0.00064%
15	Fe_2O_3	Iron	3074.4471	1778%	0.003%
16	CoO	Cobalt	25.099	<14 ppm	(11) ppm
17	NiO	Nickel	25.9480	55.6 ppm	1.2 ppm
18	CuO	Copper	177.7845	301.6ppm	1.9 ppm
19	ZnO	Zinc	715.2041	915.2ppm	2.7 ppm
20	Ga	Gallium	10.6580	8.5 ppm	0.5 ppm
21	Ge	Germanium	19.597	1.2 ppm	0.3 ppm
22	As_2O_3	Arsenic	0.0000	< 0.7 ppm	(0.0) ppm
23	Se	Selenium	25.728	1.0 ppm	0.2 ppm
24	Br	Bromine	38.6495	14.7 ppm	0.3 ppm
25	Rb_2O	Rubidium	2840.1661	697.7 ppm	1.0 ppm
26	SrO	Strontium	588.1838	140.7 ppm	0.5 ppm
27	Y	Yttrium	0.0000	< 0.5 ppm	(0.0)ppm
28	ZrO_2	Zirconium	82.9171	340.4 ppm	2.8 ppm
29	Nb_2O_5	Niobium	29.739	9.9 ppm	0.8 ppm
30	Mo	Molybdenum	11.925	1.7 ppm	0.4 ppm
31	Ag	Silver	0.0000	< 2.0 ppm	(0.0) ppm
32	Cd	Cadmium	0.9995	1.6 ppm	0.8 ppm
33	SnO_2	Tin	0.0000	< 3.9 ppm	(0.0) ppm

Table 4. Major chemical compounds groups are found in the decoction of Psathyrella.

Extract	Chemical constituants								
				Tanins			Alc.		
Decoction	S/P	Pol.	Flav	TG	TC	Quin	D	B	Sapo
	++	++	+	++	++	-	++	++	++

(+): positive reaction. The number of (+) varies according to the positivity of the reaction (intensity of the coloration or of the foam obtained); (-): negative reaction

S / P. sterols/polyterpenes; Pol: polyphenols; Fla.: Flavonoids; T.C: Catechic tannins, T.G: Gallic tannins, Alc:

Alkaloids; Quin: Quinonic Substance, Sapo: Saponosides, D: Dragendorff; B: Bouchardât

addition, if the decrease observed on the batch treated with Gliclazide is the most important, that of Metformin is almost identical to the decreases obtained after the treatment with the aqueous preparations of *Psathyrella tuberculata*. Finally, 90 minutes later, blood sugar levels stabilize at a level relatively close to T0 blood sugar (Figure 6). Only the batch treated with Gliclazide has an average blood sugar level lower than the fasting blood sugar level [16].

Comparison of the activity of the different treatments applied to hyperglycemic rats

The following table summarizes the various changes as well as their level of significance. Analysis of the results shows different activities over time.

Activity after 30 minutes: 30 minutes after the application of the various treatments, a significant drop in the blood sugar level was observed in all the batches.

Treatment with Glicazide 60 mg/kg causes the greatest decrease of 48.7%. Likewise, treatment with Metformin at 500 mg/kg dose causes a significant decrease of 27.9%. Distilled water results in a significant decrease of 17%. Depending on the dose applied, the aqueous preparation of *Psathyrella tuberculata* produces an effect ranging from 16.6% at 1000 mg/kg dose to 22.2% at 200 mg/kg.

Activity after 60 minutes: One hour later, the decreases continue in all batches. Gliclazide showed an overall decrease in blood glucose by half (53%) in rats, while that of metformin was 31%. Treatment with distilled water results in a reduction of 21%. The different doses of *Psathyrella tuberculata* produce an almost identical reduction in blood sugar levels of about 27% overall, even if the first 30 minutes suggested a stronger action of the dose at 200 mg/kg.

Table 5. Phenolic compound composition and antioxidant activity of *Psathyrella tuberculata* decoction.

Parametres	Total Phenol (mg/g MS)	Total flavonoides (mg/g MS)	Percentage (%) of inhibition
Psathyrella tuberculata	71.67	34.98	57.5
Vitamine c		100	

The decoction of *Psathyrella tuberculata* has an inhibiting capacity that is half that of vitamin C

Table 6. Identification of phenolic compounds in the chromatogram.

Phenolic group	Compounds searched	
Flavonoïdes	1 Catechin	D
	5 Cyanidin	D
	7 Kaempferol	D
	8 Myricetin	D
	11 Quercitin	D
	12 Quercetin	D
	13 Cyranosid	D
Phenolic acid	2 Protocatechic acid	D
	3 Vanilic acid	D
	4 P-coumaric acid	D
	9 Cinnamic acid	D
	10 Salicylic acid	D
	14 Ferulic acid	Nd
	16 Ellagic acid	Nd

D : Detected / Nd : Not detected

Table 7. Rate of changes in blood sugar level in normoglycemic rats and level of significance.

Evolution of blood sugar		T30-T60	T30-T90	T0 - T90
Control	Evolution rate	-10.6%	-8.1%	-4.3%
	P-value	0.69	0.14	0.38
	T-stat	0.43	1.77	0.96
Aqueous preparation at 1000 mg / kg	Evolution rate	3.9%	1.2%	3.0%
	P-value	0.46	0.85	0.63
	T-stat	0.80	0.20	0.51

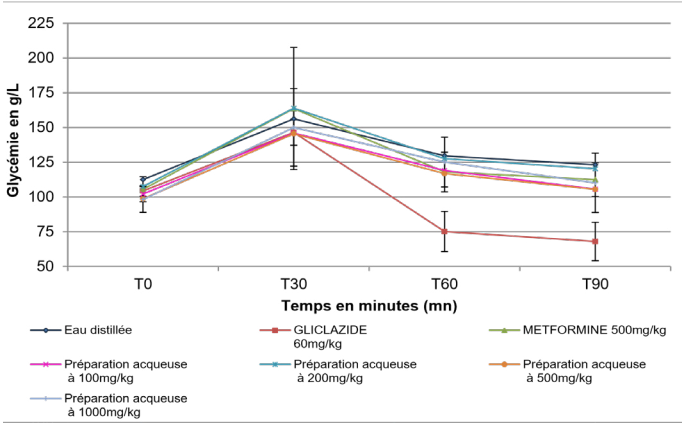

Figure 6. Evolution of the blood sugar level of hyperglycemic rats over time according to the products administered.

Table 8. Rate of change in glycemia in hyperglycemic rats and level of significance.

Evolution of blood sugar		T30-T60	T30-T90	T0-T90
Distilled water	Evolution rate	17.0%	21.1%	9.5%
	P-value	0.03*	0.01*	0.01*
	T-stat	3.10	4.11	4.31
GLICLAZIDE 60 mg/kg	Evolution rate	48.7%	53.6%	34.8%
	P-value	0.00*	0.00*	0.00*
	T-stat	15.50	11.58	5.90
METFORMINE 500 mg/kg	Evolution rate	27.9%	31.3%	6.6%
	P-value	0.04*	0.01*	0.23
	T-stat	2.69	3.90	1.38
100 mg/kg	Evolution rate	18.5%	27.6%	3.4%
	P-value	0.00*	0.00*	0.50
	T-stat	5.25	8.29	0.73
200 mg/kg	Evolution rate	22.2%	26.6%	11.9%
	P-value	0.03*	0.03*	0.26
	T-stat	2.91	2.97	1.27
500 mg/kg	Evolution rate	19.7%	27.5%	6.9%
	P-value	0.01*	0.00*	0.21
	T-stat	4.35	5.02	1.44
1000 mg/kg	Evolution rate	16.6%	26.6%	12.2%
	P-value	0.01*	0.00*	0.22
	T-stat	4.45	5.93	1.40

* Significant at the 5% level

Return to basal blood sugar after 60 minutes: The comparison of the final glycemia at T90 relative to the basal glycemia (fasting blood sugar evaluated at T0) shows that only the aqueous preparations and Metformin lead to a decreased blood glucose level significantly identical to that observed in the fasting state (p-value>0.05). The gliclazide-based treatment causes a significant drop (34.8%) resulting in blood sugar below basal blood sugar, and the treatment based on distilled water leaves blood sugar levels significantly higher than basal blood sugar (+ 9.5%) (Table 8).

Discussion

Wild edible fungi are valuable non-wood forest products with a high potential for commercial expansion. They are widely exploited by rural African populations mainly as food resources [17, 18] and/or as a source of income [15, 19], thus contributing to the substantial reduction of poverty in rural areas. Once considered to be a food of low nutritional value, edible mushrooms are of particular interest today. To this end, local populations consider edible mushrooms a "healthy" food and do not hesitate to include them in a diet aimed at good cardiovascular health [20]. Like medicinal plants, mushrooms are recognized for their biological properties; anticancer, cholesterol-lowering drugs, immunostimulants, antioxidants [21].

In this study, the results of the chemical tests carried out on the stems of *Psathyrella tuberculata* made it possible to detect the presence of several parameters making it possible to enhance this fungus species. They are the moisture content, ash, fat, carbohydrate, energy, polyphenols, and antioxidant power of this fungus.

The biochemical compositions of *Psathyrella tuberculata* species have been determined in order to understand their importance in the treatment of diabetes. This study indicated that the *Psathyrella tuberculata* species has a water content of 7.5% of the dry weight. However, the water content of this studied fungus is believed to be due in part to variations in drying methods. Indeed, the mushrooms are preserved for trading purposes and subsequent consumption [22]. This situation also makes it possible to cope with periods of scarcity when other animal protein resources are scarce. Sun-drying is an easy, quick, safe method that retains certain sensory and nutritional properties of fungi, and may be responsible for the different variations in moisture content [23]. Our study result is similar to that of [24] who reported a water content of around 9%. The presence of proteins (2.57%) and total carbohydrates (66.08%) confirms that the studied fungus would provide a nutritional value in proteins and carbohydrates comparable to that of milk, soya, and beans [25].

Indeed, the work carried out by some authors has revealed that wild edible mushrooms are rich in protein and fibre. The work of [26] maintains that the proteins contained in our mushrooms constitute an interesting source of amino acids which are essential for health. These are explained by its protein and carbohydrate content and its bioavailability to the body. Thus, the energy value of about 290.38 kcal/100 g is an indicator of this. Indeed, according to the work of [27], the energy value of edible mushrooms could vary from 250 kcal/100g to 400 kcal/100g. In addition, the fat content (1.88%) of our mushrooms is relatively low than that obtained by the work carried out by [28]. In addition to lipids, proteins, and carbohydrates, *Psathyrella tuberculata* would be rich in mineral elements (phosphorus, potassium and magnesium, Iron, Copper, Zinc, Iodine, Calcium, Chromium, Manganese, Iron, and selenium). This would explain the high ash content in *Psathyrella tuberculata* and their contribution to health. These minerals according to Fofie et al. [29] Also intervenes in blood glucose metabolism to prevent hyperglycemia.

In addition to the presence of proteins, lipids, minerals, carbohydrates, these edible mushrooms contain polyphenols and provide very important antioxidant activity. Indeed, antioxidants and phenols reduce the oxidative actions of free radicals which could be responsible for cardiovascular diseases. To reduce oxidative damages, our body then needs a diet rich in exogenous antioxidants, including phenolic compounds [30]. Polyphenols and alkaloids are also believed to be involved in the treatment of diabetes. Indeed, Alkaloids are stimulators of hepatic glycogenogenesis [31] and sterols and polyterpenes are stimulators of the release of insulin, hormones responsible for lowering blood glucose levels [32].

The results obtained on rat blood sugar levels showed that the extract caused a significant drop in blood sugar levels after oral glucose overload. This is especially observed with the highest concentrations dose of extracts. Decoction of *Psathyrella tuberculata* is therefore active in rats with glucose overload, but its action on fasting blood sugar is not significant.

These results suggest that the decoction of our fungus causes a rapid decrease in blood sugar, then it returns to its initial value after 60 minutes of treatment. The significant decrease and progressive of blood glucose levels in rats after oral glucose loading may suggest the use of Psathyrella tuberculate in the management of diabetes. These results could be explained due to the presence of chemical compounds in this fungus.

Conclusion

At the end of our study, we can say that our study aimed to assess the hypoglycaemic and antihyperglycaemic effect of *Psathyrella tuberculata* on the blood sugar level of rats. Also, we conducted a phytochemical study in order to determine the chemical group's constituent and to deduce those responsible for the antihyperglycemic effect.

The results revealed the presence of proteins, carbohydrates, lipids, energy, and phenolic compounds. The presence of these chemical compounds in *Psathyrella tuberculata* extract is believed to participate in its antioxidant activity, which testifies to its abundant contribution in the treatment of diseases often mentioned by local populations such as diabetes.

The pharmacological study on the activity of macerated *Psathyrella tuberculata* showed a decrease in rats' blood sugar level, being given an oral glucose overload (postprandial blood sugar), however, its activity on fasting blood sugar is not significant. We can therefore say that *Psathyrella tuberculata* has normoglycemic activity, confirming the traditional use of the plant.

However, before recommending it for the management of diabetes mellitus, subsequent, more complete studies on pharmacology and toxicology will make it possible to provide more details and to propose an adequate dosage.

Finally, our study could be a contribution to improving the diets of diabetics, in the sense that the edible mushroom could be introduced into their diet, given its anti-hyperglycemic effects.

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