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Evaluation of antidiabetic activity of the ethanol extract of *Momordica charantia* L. and the identification of charantine by gas chromatography coupled with Mass spectrometry

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The aerial parts of *Momordica charantia* L. are very often used in traditional Congolese medicine for their multiple virtues. Our study focused on the identification of a well-known antidiabetic molecule: the charantine and also the antidiabetic properties of the ethanol extract of *M. charantia* L. After being treated with MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) and using gas chromatography-Mass spectrometry, the analysis of the extract of the leafy stem of *M. charantia* L., var abreviata, harvested in Brazzaville (Congo) led to the identification of a recognized antidiabetic molecule stigmasterol glucoside or β sitosterol: the charantine. When testing the ethanolic extract on albino Wistar rats that were made diabetic by injecting the streptozotocin, this reduces glycemia significantly by 51.62% after three hours. The significant results of the antidiabetic tests and the identification of the charantine in the plant justify its use in the traditional medicine in Congo Brazzaville.

Key words: *Momordica charantia* L, gas chromatography, mass spectrometry, charantine, antidiabetic.

INTRODUCTION

Diabetes is a metabolic and hereditary disease due to lack or insufficiency or misuse of insulin production by the body (Altman et al., 2012). Nowadays, it is considered as a major public health problem. In 2015, there were nearly 441 million of diabetics in the world (Cho et al., 2018). In the republic of Congo, many people suffer from diabetes, although a lot of cases remain undiagnosed. On the occasion of the World Diabetes Day, the latest survey of the Ministry of Health revealed a prevalence of 9%. According to the World Health Organisation (WHO) more than 80% of diabetes related deaths occur in low- and middle-income countries because of a poor medical

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Figure 1. *Momordica charantia* L.

monitoring (Jaffiol, 2011).Many plants are traditionally used as antidiabetic, some of them are on the basis of producing of drugs. This is the case of metformin and other antidiabetic drugs belonging to the class of biguanides, all inspired by galegine isolated from *Galega officinalis* (Fabaceae) (Khodadadi, 2016).

Momordica charantia L. is a plant of the Cucurbitaceae's family, found in African wild flora. It is widely spread throughout tropical African area and is taken for the wild form of the species. It is harvested in a natural state and used as a vegetable or medicinal plant (Schmelzer and Gurib-Fakim, 2008) (Cantwell et al., 1996).

In Congo, this plant is used in traditional medicine as antidiabetic and for its many other medicinal virtues (antimalarial, deworming, laxative, etc...). The ethnobotanical survey conducted in Brazzaville (Congo) by Ampa and al showed that *M. charantia* L. is a widely used antidiabetic plant (Ahombo et al., 2012; Adjanohoun et al., 1988). Although Congolese traditional healers prescribe the plant to diabetics, no antidiabetic and phytochemical studies have been made on the species present in Congo Brazzaville. Studies performed on this plant in China and India have revealed the presence of flavonoids, tannins, alkaloids, cardiac glycosides, saponins and steroids. More molecules like momordicosides, momordenol, momordicin, cucurbitacin, charantine were identified and isolated and among them some tested antidiabetic compounds such as antidiabetic activities of vicine, polypeptide P, charantine and many other triterpenoid (Singh et al., 2012; Majekodunmi et al., 1990; Sabira et al., 1997; Kimura et al., 2005; Sonal and Pratima, 2015; Haixia et al., 2004). Charantine, stigmasterol and β-sitosterol glucoside steroidal saponins with effective antidiabetic properties. Some studies have shown that *M*. *charantia* has insulin secretory properties (Kedar and Chakrabarti, 1982). Several Works in Asia highlight that it is possible to extract charantine in seeds, in fruits, and in leaves of *M. charantia* L., by chromatographic and spectral methods such as high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), ultraviolet spectrometry, infrared spectrometryHaixia Z, Xiaozuo Z, Yawei W, Mancanq L, Zhide H, 2004).

This research focuses on evaluating the antidiabetic activity of the ethanolic extract of the *M. charantia* L. in Congo and to verify the presence of the charantine (antidiabetic molecule) by a method using gas chromatography coupled with mass spectrometry. The purpose of this paper is to confirm the antidiabetic activity of *M. charantia* species found in Congo Brazzaville.

MATERIALS AND METHODS

Plant material

Aerial parts from *M. charantia* L. (Figure 1) were collected in MFILOU a southern district of Brazzaville (Congo). Botanical identification of the plant was made in the Research Institute of Exact and Natural Sciences (IRSEN). The voucher specimen deposited in the national herbarium is Mampouya C. Mounsambote JM # 201 August 10, 2017.

Animal material

Male albino Wistar weight from 200 to 350 g were used. These rats were raised at the pet shop of the Faculty of Science and Technology of Marien Ngouabi University. They were fed with a complete diet (proteins, lipids, carbohydrates and mineral salt) and had free access to water.

Extraction and analysis equipment

This study was carried out using the following laboratory equipment:

Table 1. Analytical operating conditions in gas chromatography.

Chromatograph parameter	
Capillary column (5% phenyl -95% methylpolysiloxane)	20 m × 0.18 m × 0.15µm
Carrier gas	Helium
Pressure at the top of the column	100 kpa
Split flow	30 ml/min
Injector temperature	290°C
Temperature of the transfer line	320°C
Initial temperature	240°C
Rate	20°/min
Final temperature	340°C

Table 2. Analytical operating conditions in Mass spectrometer.

Mass spectrometer parameters	
Source temperature	290°C
Voltage	70 eV
Number of scan / s	2.1
Mass range	45 - 900

(i) for sample preparation a soxhlet extractor, a BUCHI rotary evaporator;
(ii) for sample analysis a Hewlett Packard 6890 gas chromatograph coupled with MS Engine Hewlett Packard 5973 quadrupole mass spectrometry;
(iii) for evaluation of the antidiabetic activity: a glucometer of brand one touch with strips.

Preparation of the ethanolic extract

Aerial parts of *M. charantia* L. were dried in a dry place away from the light for a week and then crushed. The powder was stored in a glass vial. 20 g of the powder obtained were put in a cellulose cartridge and then extracted for 3 h with ethanol in a Soxhlet extractor with a capacity of 250 ml. The resulting extract was dried using a rotary evaporator to obtain a dry ethanolic extract.

Biological methods

Induction of diabetes

Male rats of an average weight between 200 and 350 g were anaesthetised with diethyl ether and then made diabetic by intravenous injection of streptozotocin (Sigma Aldrich) freshly prepared in sodium chloride buffer solution (0.9 %), at 55 mg / kg. Rats' Glycemia was monitored 72 h later. Rats with glycemia higher than 1.40 g/L were selected for the experiment (Andrade-Cetto et al., 2000).

Evaluation of the antidiabetic activity

The diabetic animals were fasted for 12 h and divided into three lots of five rats each. These different lots received respectively: distilled water (10 ml/kg), Glibenclamide (10 mg/kg) and ethanolic extract (400 mg/kg). Glycemia was taken every 4 h. A drop of blood collected by making a slight incision of the distal end of the tail of the rat was deposited on the reactive range of the strip. The reading is done on the glucometer which displayed the results in mmol/ml, then they were converted into g/L.

Statistical analysis

The results of the blood glucose are presented in the average form ± average standard error. Variance analysis (ANOVA) and the Student's test were used for the statistical study. The number of experiments in each group was n = 5. At the $P < 0.05$ threshold, the values of blood glucose variation were considered significant.

Chemical methods

Methods of analysis

Treatment of the ethanolic extract: 2 mg of the dry ethanolic extract were added to 500 µl of the bypass reagent (pyridine / MSTFA) and then placed in an oil bath at 80°C for 2 h. The reagent was then evaporated to dryness using a rotary evaporator, the residue is taken up with 500 µl of hexane. The solution obtained is then ready for analysis in gas chromatography coupled with mass spectrometry (GC-MS) in split mode. 10 µl of the sample were injected. The duration of the analysis was 120 min, the acquisition began at 25 min.

Analysis of the extract in GC-MS: The operating conditions and the parameters of the chromatograph coupled with the mass spectrometer for the analysis of the ethanolic extract after derivatization are shown in Tables 1 and 2. The mass spectra are recorded in electronic impact mode with an ionization energy of 70 eV in "full-scan" mode. Chemical identification is carried out by comparing the mass spectrum obtained with that of reference. The

Table 3. Evolution of the glycemia and the percentage reduction of the blood glucose of the rat over the time after administration of the ethanolic extract of *M. charantia* L.

Time (h)	0	1	2	3	4
Water (10 ml/kg)	2.78± 0.07	2.53 ± 0.04 **(8.99 %)**	2.37± 0.01 **(14.74%)**	2.39 ± 0.02 **(14.02 %)**	2.43 ± 0.01 **(12.59 %)**
Glibenclamide (10 mg/kg)	1.54 ± 0.04	1.19 ±0.03 **(22.73%)*****	0.80 ±0.06 **(48.05%)*****	0.64 ±0.05 **(58.44 %)*****	0.62 ± 0.04 **(59.10%)*****
Ethanol extract (400 mg/kg)	2.46 ± 0.11	1.73 ± 0.02 **(29.67%)*****	1.20 ± 0.04 **(51.22%)*****	1.19 ± 0.03 **(51.62 %)*****	1.29 ± 0.03 **(47.56%)*****

Significant difference between rats that received only water (negative controls): *** p <0.05;
() = Percentage reduction in blood glucose (P.R.)

$$P = \frac{(blood\ glucose\ at\ \mathrm{t}_0 - blood\ glucose\ at\mathrm{t}_x)}{blood\ glucose\ at\mathrm{t}_0} \times 100$$

software used x callibur.

RESULTS

Effeciency of extraction

The extraction of 20 g of *M*. *charantia* L aerial parts with ethanol gave a crude weighing 2.34 g, which was a 2.34 g, and yield of 11.7%.

Effect of streptozotocin on normal rats

After injection of streptozotocin, polyuria and elevation of fasting rat glucose were observed with values between 1.40 and 2.53 g/L. These parameters justify that the rats were indeed made diabetic.

Evaluation of the antidiabetic effect of the ethanolic extract of *M. charantia*

Table 3 presents the evolution of the mean blood glucose level with percentages of glycemic reduction in diabetic rats that have been orally treated with ethanol extract, Glibenclamide and distilled water at the doses indicated above. As shown in the table, only the rats that received glibenclamide and plant extract showed significant reductions in blood glucose levels in the first hour, respectively 22.73 and 29.67% versus 8.99% for those that received distilled water. The latter showed no significant reduction in blood glucose until the fourth hour, while the other two lots of rats showed very significant reductions in blood glucose levels until the fourth hour ($p < 0.05$). At the fourth hour, the percentage reduction in glycemia of rats treated with glibenclamide continued to increase (58.44% at the third hour for 59.10% at the fourth hour), while that of the rats treated with the extract plant starts to decline (51.62 to 47.56% at the fourth hour).

GC-MS analysis of *M. charantia* L. ethanolic extract and Identification of sterol glucoside: charantin

The exploitation of the chromatogram with the database available in the laboratory enabled us to identify at different retention times several compounds and particularly β-sitosterol glucoside (charantin) with a retention time of 73.84 min (Figure 3). Mass spectrum related to the peak eluted at 73.84 is shown in Figure 2. On this mass spectrum, the following can be observed:

(i) The base peak is at m / z is 204,09 ;
(ii) Peaks of 395,34 and 451,10 are fragments of sterol and glucoside respectively
(iii) Ions at m/z : 147; 204; 217; 305 and 361 are characteristic of the carbohydrate moiety;
(iv) m/z : 129 and 255 are peaks characteristic of the sterol part;
(v) The molecular peak is that of which m / z = 861.43.

All of these spectral data are oriented towards a steroid compound whose basic skeleton is a stigmasterol; m/z = 395.34 (Figure 3) stuck to a sugar: glucose; m/z = 451.10 (Figure 4). The steryl glycoside TMSi ether allowed it to be analysed by GC/MS on a short packed column or by a capillary column operated at high temperature. The mass spectrum of steryl glycoside TMSi ether shows, molecular peak is not perceivable and it is dominated by ions arising from hexose TMSi ether moiety (m/z 361, 217, 204, 191 and 147). The base peak for a hexose pyranoside moiety is at m/z 204 but for a furanoside the prominent ion is m/z 217. The presence of stigmasterol glucoside (charantin) by

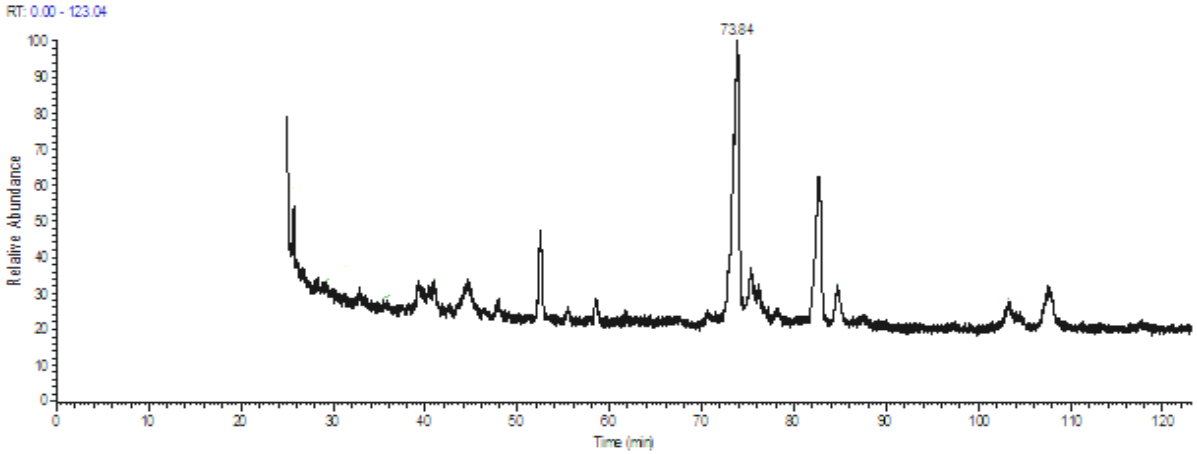

Figure 2. Chromatogram of the ethanolic extract of *M. charantia* obtained in GC-MS.

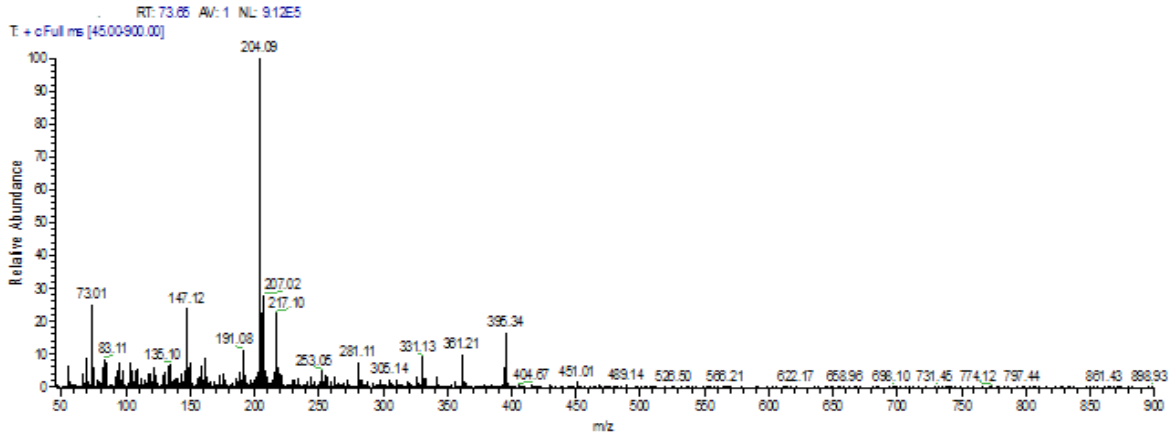

Figure 3. Mass spectrum corresponding to peak at t = 73.65 min in GC-MS analysis of ethanolic extract of *M. charantia.*

GC-MS analysis of the ethanolic extract of the leafy stems of *M. charantia* was confirmed on the basis of the comparison of the mass spectrum obtained with the work of Phillips KM. In addition, Figure 3 shows the characteristic mass spectrum of sterol glucoside peaks. Characteristic peaks of the steroid fragment were also observed in this spectrum (Phillips et al., 2005; Goad and Akihisa, 1997).

The data from the chromatogram and the mass spectrum obtained made it possible to emphasize the presence of charantine in the ethanolic extract of *M. charantia* L. with structure as shown in Figure 5.

DISCUSSION

Previous work has shown that the isolation and identification of charantine is performed using several techniques such as: HPTLC, LC-MS or HPLC-DAD (Sonal and Pratima, 2015; Thomas et al., 2012). This work identified this compound in the various organs (leaf, fruit and seed) of *M. charantia* L., a species harvested in China and India using techniques such as HPTLC, LC-MS and HPLC-DAD (Ahamad et al., 2014). However, to date, analytical and phytochemical studies to identify antidiabetic compounds in the species present in Central Africa have not yet been carried out.

The bibliographic data showed that charantine has anti-diabetic properties (Sonal and Pratima, 2015). In the Republic of Congo (RC), Chad and Benin, the plant is used in traditional medicine to treat diabetes. The presence of this molecule could justify its use in traditional medicine against diabetes (Sakine et al., 2014; Ahombo et al., 2012; Lalèyè et al., 2015). The charantine

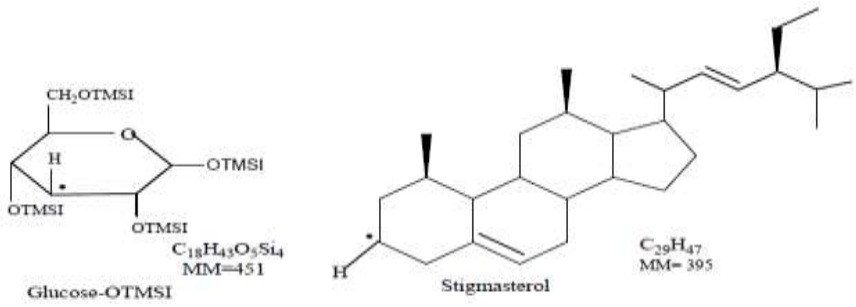

Figure 4. glucose –OTMSi and stigmasterol: fragmentation of the charantine.

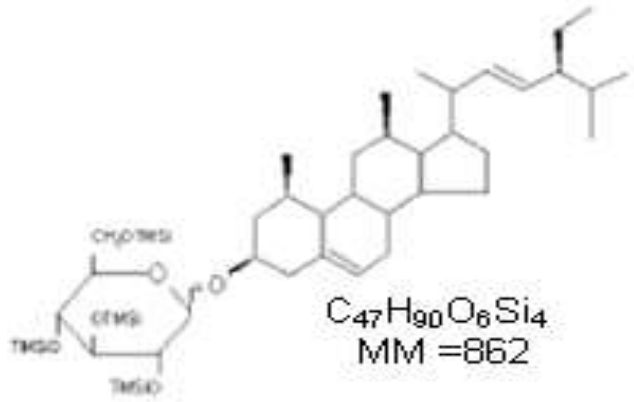

Figure 5. Charantine o-TMSi.

has been isolated in the seeds, fruits and leaves of *M. charantia*. The molecule has been tested to treat diabetes. The work by Sonal and Pratima (2015) showed that at the dose of 50 mg / kg, the analyses give a 42% reduction on glycemia percentage after 4 h, this percentage is reduced at the 5th hour by 28% (Lalèyè et al., 2015; Pitiphanpong et al., 2007). The ethanolic extract of *M. charantia* aerial parts administered at a dose of 400 mg / kg in male rats gave significant antidiabetic activity. This extract caused a drop in blood sugar until the 3rd hour but we observe at the 4th hour a slight increase in blood sugar. Previous work by Nagy (2012) has shown that the aqueous extract and even the ethanolic extract of *M. charantia* L. reduces blood glucose levels in type 2 diabetic rats. Other studies have suggested the existence of charantine having an effect on blood glucose (Nagy et al., 2012).

The results obtained make it possible to point out that the ethanolic extract of *M. charantia* decreases blood glucose at a dose of 400 mg / kg, which shows that the plant has antidiabetic effects. These effects may be due to the presence of certain chemical components such as flavonoids, alkaloids, saponins (Day et al., 1990; Coskun et al., 2005; Han et al., 2008; Neha et al., 2016). The work by Jesada et al. (2007) on the *M. charantia* was able to isolate and highlight the charantine, besides the results by Wang has shown that this molecule has antidiabetic virtues more particularly insulinosécrétrices properties (Pitiphanpong et al., 2007; Wang et al., 2014). The presence of charantine in our species could justify the reduction of glycemia in diabetic rats treated with extracts of *M. charantia* and the use of this plant as an antidiabetic in Congolese traditional medicine.

Conclusion

The aerial parts of *M. charantia* L. are in common use in traditional Congolese medicine for their multiple virtues. In addition to the current use of *M. charantia* L. decoction, the ethanol extract also has considerable antidiabetic activity. The present study based on the demonstration of the antidiabetic properties of the ethanolic extract of *M. charantia* L. and also on the identification of a recognized antidiabetic molecule: the charantine.

The charantine identification was made by gas chromatography coupled to the mass spectrometry of the extract of the aerial parts of *M. charantia* L. var abreviata harvested in Brazzaville (Congo), after treatment with MSTFA. The separation and identification of the

compounds was carried out on a column consisting of 5% phenyl-95% methylpolysiloxane. All mass spectra were recorded in electronic impact with an ionization energy of 70 eV. The ethanol extract, tested by injection of streptozotocin on albino Wistar rats made diabetic, induced a significant reduction in blood glucose (51.62%) at the 3rd hour. The significant results of the antidiabetic tests and the demonstration of the charantine justify the use of this plant in traditional medicine by the traditional healers in Congo Brazzaville.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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