INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 6, p. 431-439, 2016

RESEARCH PAPER OPEN ACCESS

Conyza aegyptiaca (L.) Dryand ex. Aiton extracts exhibite antioxidant activity and prevent hepatic glucose liberation *in vitro*

B. Huguette Akakpo[*1,2], Casimir D. Akpovi[3], Thierry C. M. Medehouenou[3], Fidèle M. Assogba[2], Pierre H. Dansou[1], Joachim D. Gbénou[2]

[1]*Laboratory of Effort Physiology, National Institute of Youth, Physical and Sport Education, University of Abomey-Calavi, Cotonou, Republic of Benin*

[2]*Laboratory of Pharmacognosy and Essential Oils, University of Abomey-Calavi, Cotonou, Republic of Benin*

[3]*Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Benin.*

Key words: *Conyza aegyptiaca*, glucose, liver, hepatic glucose liberation, Benin

http://dx.doi.org/10.12692/ijb/9.6.431-439 Article published on December 31, 2016

Abstract

Also called ahlonmè in Ewe, *Conyza aegyptiaca* (L.) Aiton (*C. aegyptiaca*) is one of the plants used for diabetes treatment in West Africa. In type 2 diabetes patients, enhanced glucose output by liver contributes to hyperglycemia and inhibition of hepatic glucose production favors glycemic control. In this study, we analyzed the effect of *C. aegyptiaca* on hepatic glucose release. Freshly collected liver from rats sacrificed under anesthesia was cut into small pieces. The pieces were thoroughly rinsed and incubated under stirring with appropriate dilutions of the extracts of *C. aegyptiaca*. Glucose level was determined in the incubation medium over time by Glucose Oxidase method. Aqueous and ethanolic extracts of *C. aegyptiaca* showed no larvae toxicity. The liver glucose liberation test showed that glucose levels decreased significantly ($p < 0.005$) at all tested concentrations of extracts compared to control. Glucose level increased over time in the control medium without extract. When liver pieces were incubated with extract, glucose was kept at significantly ($p < 0.005$) lower level compared to control. The maximum rate of inhibition of hepatic glucose release was achieved 20 minutes ($p < 0.001$) and 30 min ($p < 0.005$) after incubation respectively for the ethanolic and aqueous extracts. Glucose level measured in the incubation medium with the ethanolic extract is significantly ($p < 0.05$) lower than that of the aqueous extract. Our results show that *C. aegyptiaca* extracts prevent hepatic glucose liberation and suggest that this property contributes to the antdiabetic effect of the plant.

* **Corresponding Author:** Huguette B. Akakpo ✉ baihugg@yahoo.fr

Introduction

There is a definite renewed interest in the plant usage for medicinal purposes around the world. The empirical traditional plant usage, especially in rural areas in Africa, is enhanced by the high cost of modern medicine. However, little is known regarding the scientific assessment of their effects.

The world Health Organisation encourages research on new ways to fight against these diseases. In recent years, many researchers have achieved major scientific studies on plants (Govindappa, 2015). In the sub-Saharan in Africa, most of the medicinal plants are used empirically to overcome disease like diabetes with no knowledge on involving mechanisms of action. The management of the diabetic patient requires lifelong care using a combination of several expensive treatments.

The liver plays an important role in controlling carbohydrate homeostasis by maintaining glucose concentrations in a normal range over time. In type 2 diabetes, alterations in hepatic glucose metabolism are reported (Trinh *et al.*, 1998). These include increased post-absorptive glucose production and impaired suppression of glucose production together with diminished glucose uptake following carbohydrate ingestion. Hyperglycemia in type 2 diabetes is characterized by enhanced glucose production in the liver and kidney. Fasting blood glucose is determined by de novo glucose production (in liver and kidney) and glucose deposition in peripheral tissues.

In the presence of insulin resistance, enhanced glucose output by liver contributes to hyperglycemia together with reduced glucose deposition in skeletal muscle, heart and adipose tissue. Inhibition of hepatic glucose production contributes to glycemic control in the diabetic patients by insulin sensitizers (Xia *et al.*, 2011).

Conyza aegyptiaca (L.) Dryand ex. Aiton, of Asteraceae family, is a well known plant used by the population of Togo and Benin (West Africa). Also called *Ahlonme* in Ewe (local dialect), *Conyza aegyptiaca* (*C. aegyptiaca*) is widely used to overcome malaria, sickle cell disease, sore throat, diabetes (Akpagana *et al.*, 1996).

In this study, the aqueous and ethanolic extracts of *C. aegyptiaca* were tested for their effects on hepatic glucose liberation in diabetes-free Wistar rats in order to better understand the plant properties on diabetes.

Material and methods

Plant material

The plant material was composed of aerial parts of *C. aegyptiaca* bought at Palimè (Republic of Togo). The leaves and stem of the plant were shade-dried and ground into a fine powder. This powder is then extracted with water and ethanol. .

Extractions

For the aqueous extraction, decoction was done on a heating plate during 30 minutes by addition of 1000 ml distilled water to 100g of powder. The mixture was filtered with Whitman paper and evaporated with rota vapor at 65-70°C. The ethanolic extraction was done by macerating 100g of powder in ethanol diluted at 50°C with distilled water, during 24 hours mixed by homogenizer. After the filtration with Whitman paper, the product was evaporated with the rota vapor at 40-45°C. Extracts were conserved at 4°C. The yield of each extract is calculated with the formula: r = Extract mass x 100 / Powder mass.

Animal material

Male and female albinos Wistar rats weighting 150 to 200 g at the age of six weeks were used for the study. Animals were housed in polypropylene cages and maintained under standard conditions with an alternated cycle of twelve hours light and twelve hours dark. They had free access to food and water. Room temperature was maintained at 25°C with a relative humidity of 35-60%.

Phytochemical screening

Phytoconstituents of *Conyza aegyptiaca* were determined by the color reactions and precipitations of the major groups of chemical compounds in the plant of Houghton and Rama (1998), a qualitative chemical analysis.

Quantification of bioactive molecules

Polyphenols compounds

The method of Singleton and Rossi (1965) slightly modified was used to determine the total polyphenols compounds. A volume of 125µL of the sample diluted in 625µL of the folin-Ciocalteu reagent was used. After 5 min incubation, 500µL of sodium carbonate (Na 2CO3) at 75mg/ml was added. The mixture was incubated in darkness for 2 hours and measured at 760 nm in spectrophotometer. A solution of gallic acid was used as standard. The results were expressed in mg of gallic acid equivalent/g (mg GAE/g) of sample.

Flavonoids compounds

This was essayed following the method described by Zhishen *et al.*, 1999 and Kim *et al.*, 2003). A mixture of 500µL of aluminium trichlorid $AlCl_3$ (2%) with 500µL of sample plus 3 ml of methanol was used. Quercetine (10mg/ml) was used as reference molecule. The flavonoids compounds are expressed in mg of quercentine equivalent/g of sample.

Condensed tannins

Sulfuric vanillin method described by Broad Hurst and Jones (1978) and modified by Heimler *et al.* (2006) was used to quantify condensed tannins in the extracts; 500µL of sample or standard were added to 3ml of the solution of vanillin (4%) in methanol and 1.5ml of concentrated chlorhydric acid and 2ml of methanol. After 15 min incubation, the absorbance was measured at 500 nm. The condensed tannins are expressed in µg of catechine equivalent/mg of sample.

Antioxidant activity

Antioxidant activity is related to the capacity of plant extract to trap the free radical. Total antioxidant activity was quantified by the method described by Lamien-Meda *et al.* (2008). Briefly, 1.5 ml of diluted extract solution was added to 3 ml of methanolic solution of 2, 2-diphenyl-1-picrylhydrazyl (DDPH) (Cm = 0.04mg/ml). The mixture was incubated 15 min at room temperature and the absorbance was measured at 517nm using ascorbic acid as standard. Ascorbic acid standard curve (0-10mg/ml) was used to determine the antioxidant capacity of extracts.

The total antioxidant activity was expressed as mmol Equivalent of ascorbic acid per gram of extracts (m mol EAA/g) with the formula: C = (Cread x D) x 100 / M x Ci, where C is antiradicalar concentration, Cread is extract concentration read on the curb, extract diluted factor, M is ascorbic acid molar mass and Ci is initial extract solution concentration. In order way, we calculated the 50% inhibition concentration (IC_{50}), the 50% effective concentration (EC_{50}) and the antiradicalar power (APR) of the extracts. The concentration with 15% of DPPH radical was capted (IC_{50}) by extract and calculated with the formula: $IC_{50} = A_c - A_s / A_c \times 100$, where A_c is absorbance of control and A_s is absorbance of sample. The efficient concentration of the extracts was determined (Mensor *et al.*, 2001). The antiradicalar power (APR) was $1/EC_{50}$ (Prakash *et al.*, 2007).

Larval toxicity

We make the larvae toxicity based on the survey of larvae in sea water in presence of the tested solution proposed by Michael *et al.* (1956). A positive correlation between the toxicity on the larvae shrimp and cytotoxicity on cells 9PS and 9KB (human nasopharyngeal carcinoma) has been showed (José *et al.*, 2002).

Hepatic liberation activity

A modified procedure of the washed liver achieved by Claude Bernard (Grmek, 1997) as described by Adam Sakine (2012) was used. Briefly, a rat was sacrificed under anesthetic and the liver was harvested and immediately dropped in a flask containing Mac Ewen solution. Then, the liver was cut into pieces of 300mg average weight and washed with the Mac Ewen solution. Each piece was incubated under agitation in five different solutions according to the concentration of extracts: Solution A, 1ml of the Mac Ewen (control); Solution B, 1ml Mac Ewen + 62.5mg/ml of aqueous or ethanolic extract (extract); Solution C, 1ml Mac Ewen + 125mg/ml of extract; Solution D, 1ml Mac Ewen + 250mg/ml of extract; Solution E, 1ml Mac Ewen + 500mg/ml of extract. After 20 min of incubation, glucose level was measured in the supernatant by enzymatic method.

Statistical analysis

Data obtained from the experiments are expressed as mean ± standard error of the mean (SEM) and evaluated by Student's t-test using the Sigma Plot statistical analysis software (Systat Software, Inc. San Jose, CA, USA). A level of $p < 0.05$ was set as significant.

Results

Extraction yields

Extraction yield was 24.94% for the aqueous extract, and 16.88 for the ethanolic extract.

Phytochemical screening

The phytochemical screening was realized three times (Table 1).

Quantification of bioactive molecules

Table 1. Chemical compounds of C. aegyptiaca.

Chemical Compounds	C. aegyptiaca (L.) Dryand ex. Aiton
Alkaloïds	+
Catechiques Tannins	+
Galliques Tannins	+
Flavonoïds (flavone)	+
Anthocyans	+
Leuco-anthocyans	-
Quinonics Compounds	-
Saponosids	+
Triterpenoïds	+
Steroïds	+
Cardenolids	+
Cyanogenics Compounds	-
Mucilages	+
Coumarins	+
Reducing compounds	+
Free Anthracenics	-
O-Heterosids	+
C-Heterosids	+

Legend:- Absent, + Present.

Table 2. Measures of Bioactive compounds in C. aegyptiaca aqueous and ethanolic extract.

	C. aegyptiaca aqueous extract	C. aegyptiaca ethanolic extract
Polyphenolic extract preparation Cm=1mg/ml: 120µL + 620µL FCR + 500µL Na2C03		
Mean OD of polyphenols	1.5± 0.215	2.611±0.002
Read GA (µg/ml)	20.959±3.011	36.498±0.024
Polyphenols in mgGAE/g	721.921±103.703	1257.153±0.835
Flavonoids extract preparation Cm=1mg/ml : 500µL+500µL AlCl3 + 3 ml méthanol		
Mean OD of flavonoids	0.980± 0.019	1.029±0.007
Read Quercetine (µg/ml)	2.495±0.049	2.621±0.018
Flavonoids in mg quercetine/g	3.992±0.077	4.194±0.028
Condensed tannins extract preparation Cm=1mg/ml: 50µL+3ml Vanilline + 1.5ml HCl +2ml méthanol		
Mean OD of condensed tannins	0.266±0.001	0.21±0.000
Read Catéchine (µg/ml)	23.645±0.138	18.554±0.000
Condensed tannins mg Catechine/g	189.888±1.108	148.432±0.000

Cm = Mass Concentration, OD = Optical Density, GA = Gallic Acid, GAE = Gallic Acid Equivalent, HCl = Hydrochloric Acid, FCR = Folinciocalteau Reagent, Na_2Co_3 = Sodium Carbonate, $AlCl_3$ = Aluminum Chloride.

Antioxidant activity

Fig. 1. showed the standard curve which equation was used to determine the antioxidant capacity C of the extracts. The aqueous extract antioxidant capacity, $C_{Aq.\ Ext.}$ = 224.52mmol/g and the ethanolic extract antioxidant $C_{Eth.\ Ext.}$ = 207.06mmol/g.

The concentration at which 50% of the DPPH radical were trapped by the aqueous extract (IC_{50}Aq. Ext.) is 7.2µg/ml. The IC_{50} of the ethanolic extract (IC_{50} Eth. Ext.) is 10.1µg/ml. The aquous extract 50% effective concentration (EC_{50}Aq. Ext.) was 213.49 µg/mg and the ethanolic one was EC_{50} Eth. Ext. was 299.88 µg/mg. Finaly, the aqueous extract antiradicalar power $APR_{Aq.\ Ext.}$ was 0.005 and the ethanolic extract one $APR_{Eth.\ Ext.}$was 0.003 (Table 3).

All these values were compared to ascorbic acid one : IC_{50} AA was estimated to 0.11µg/ml, EC_{50} AA = 0.55µg/mg and APR_{AA} = 1.81.

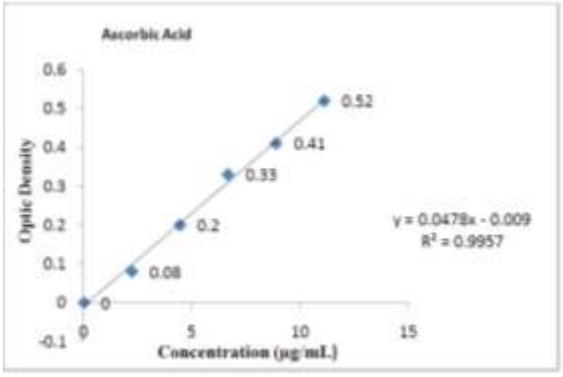

Fig. 1. Standard curve of ascorbic acid. *C. aegyptiaca* extracts antioxidant compounds dosage with DPPH method 2 ml of extracts solution (200µg/ml) was

added to 0.8 ml of DPPH (0.1183mg/ml) and incubated within 30 minutes in darkness. Ascorbic acid was used as standard. The concentration at which fifteen of DPPH radicals were trapped (IC_{50}) was calculated with the formula: $IC_{50} = (A_c - A_s / A_c) \times 100$, where A_c is absorbance of control and A_s is absorbance of sample. The absorbance was measured at 517nm.

Table 3. Antioxidant activity of ascorbic acid, aqueous and ethanolic extracts.

	IC50	Parameters	
Extract	(µg/ml)	EC50 (µg/mg)	APR
Aquous extract	7.2	213.49	0.005
Ethanolic extract	10.1	299.88	0.003
Ascorbic acid	0.11	0.55	1.81

IC_{50} = concentration at which Fifty percent of DPPH radical were trapped, EC_{50} = 50% effective concentration, APR = antiradicalar power, µg/ml = microgram per millimeter, µg/mg = microgram per milligram.

Larval toxicity

The logarithmic adjusted curves in Fig. 2 showed that the number of dead larvae increases when extracts concentration increases in a dose-response relationship manner. The concentration that causes the death of half of the 16 introduced larvae (LC50) corresponds to 2.13 mg/ml for the aqueous extract ($LC50_{Aq. Ext.}$) and 0.53mg/ml for the ethanolic extract ($LC50_{Eth. Ext.}$) (Fig. 2).

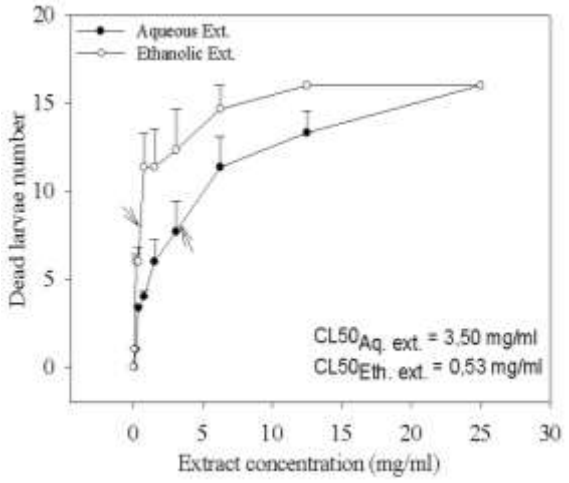

Fig. 2. Larvae toxicity test. *Artemia salina* eggs were incubated in 1000 ml of sea water at laboratory temperature (17°C) 48 h on an agitator.

After larvae hatching, eighty larvae were incubated 24 hours in each tube. Dead larvae were counted. The concentration at witch 50% of larvae were dead, named lethal concentration (LC50), was calculated. LC50 of aqueous extract (Aq. Ext.) was 3.50mg/ml, and that of ethanolic extract (Eth. Ext.) was 0.53 mg/ml.

Hepatic glucose liberation

We tested four doses of the aqueous and ethanolic extracts in order to determine the efficient extract concentration. All four tested doses decreased significantly ($p < 0.005$) glucose level in the incubation medium after 60 min compared to control (Fig. 3A).

Glucose level was significantly ($p < 0.05$) lower in ethanolic extract medium than in aqueous extract medium at each dose (Fig. 3A). Both aqueous extract and ethanolic extract showed the lowest level of glucose at the dose of 250mg/ml of extract (Fig. 3A).

The results showed in Fig. 3A. indicated that the dose of 250mg/ml was the most efficient in inhibiting of hepatic glucose liberation. Therefore, that dose was chose to analyze time course inhibition of hepatic glucose release.

With the aqueous extract, glucose level increased significantly ($p < 0.01$) after 10 min post incubation followed by no further changes. In the control however, glucose level increased significantly ($p < 0.02$) after 10 min post incubation and reached later the maximum at 60 min (Fig. 3B).

In opposite to the aqueous extract, glucose level showed no variation when liver was incubated with the ethanolic extract (Fig. 3B). Glucose level was significantly ($p < 0.005$) lower in the medium containing the aqueous extract compared to control throughout the experiment. When compared to aqueous extract medium, glucose was kept at significantly ($p < 0.05$) lower level in the incubating medium containing ethanol (Fig. 3B).

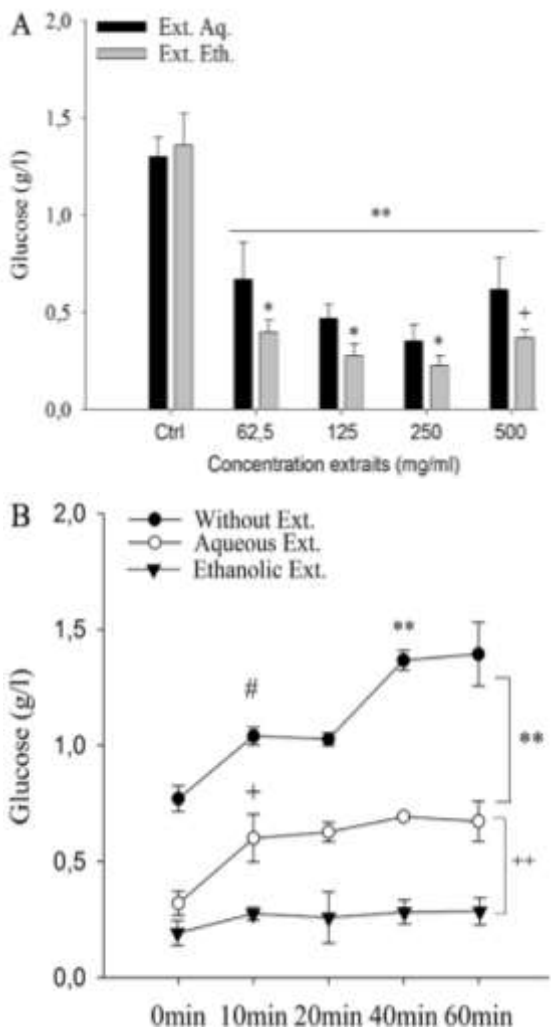

Fig. 3. Liver glucose liberation. (A) Small pieces of freshly harvested rat liver were incubated in buffer containing the extracts at concentrations of 62.5, 125.0, 250.0 and 500.0mg/ml at 37°C under agitation. Control was made by incubating liver pieces with Mac Ewen buffer. Glucose level was measured in the incubation medium after 60 min. Results are presented as mean ± standard error of five independent experiments. Dose dependent hepatic glucose release is analyzed and the concentration of 250mg/ml was determined to be more efficient. (B) Nest, liver pieces were incubated with the aqueous extract (—○—) and the ethanolic extract (—▼—) at a dose of 250mg/ml at 37°C under agitation. After 20 min incubation, glucose level was measured in the incubation medium at 0-, 10-, 20-, 40- and 60 min post incubation. The results were compared to the control made with Mac Ewen buffer only (—●—) (*p <0.05; **p <0.005; +p <0.01; #p <0.02).

Discussion

The use of plants as alternative therapy for controlling diseases like diabetes mellitus is growing today's. In the present study, we aimed to examine the antdiabetic effect of *C. aegyptiaca* plant on hepatic glucose liberation. This plant material, after stewing, is traditionally used by West African people as a tea in contrast with other medicinal plant materials which are infused in local alcohol. The performance of *C. aegyptiaca* aqueous extraction was compared to that of ethanolic extraction in the present study. The results showed that water favored better extraction yield compared to ethanol at 50°C. This suggested that the type of solvent is important for the solubility of some chemical compounds (Mada *et al.*, 2012).

The phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, steroids, triterpenoïds, cardenolids, saponosids, heterosids, mucilages, anthocyans, coumarins, and reducing compounds. Alkaloïds have an immunosuppressive activity which can help to inhibit auto-reactive T-cells in autoimmune reaction of beta cells from antdiabetic plants (Yessoufou *et al.*, 2013). Tannins are useful for their antioxidant activity (Yessoufou *et al.*, 2013). Flavonoïds are anti inflammatories, hepato protectors and diuretics (Gbénou *et al.*, 2011). Mucilages are mechanic laxatives (Bruneton, 2009). Steroids, triterpenoïds and saponosids are also anti inflammatories (Bruneton, 2009) which will be useful if the diabetes complications lead to foot injury. The absence of cyanogenics compounds is good because they are toxics for the organism. The presence of Alkaloïds, tannins, flavonoids is in concordance with the study of Batawila *et al.* (2002) who test the antifungal activity of *C. aegyptiaca* in Togo. We showed here that ethanolic extract has a high level of polyphenols (1257.153±0.835 vs 721.921±103.703mg GAE/g) and flavonoids (4.194±0.028 vs 3.992±0.077mg quercetine/g) compared to the aqueous extract. In contrast, the aqueous extract content condensed tannins more than the ethanolic one (189.888 ± 1.108 vs 148.432 ± 0.000 mg Catechine/g).

These results showed that the quality of *C. aegyptiaca* extraction depend on the solvent used. We showed here that *C. aegyptiaca* extracts have an important antioxidant activity which can be explained by the presence of phenolic counpounds (Adedapo *et al.*, 2008) and flavonoids ones (N'guessan *et al.*, 2007; Zhi at al., 2008). Our results also confirmed Bidie *et al.*'s ones (2011) on antioxidant activities of ten ivory's medicinal plants. Besides been rich in antioxydants and anti-inflammatory compounds, *C. aegyptiaca* aqueous and ethanolic extracts showed no larvae toxicity since the LC50 of both extracts was higher than 0.1mg/ml (Mousseux, 1995).

Glucose production in the liver and kidney is increased in type 2 diabetes (Abdul-Ghani and De Fronzo, 2008). In the presence of insulin resistance, elevated glucose output by liver contributes to hyperglycemia (Postic *et al.*, 2004). Inhibition of hepatic glucose production contributes to glycemia control in diabetic patients by insulin sensitizers (Agius, 2007). Here, we showed that *C. aegyptiaca* extracts inhibit liver glucose release. Aqueous extract and ethanolic extract concentrations assayed were both efficient in liver glucose secretion blocking. Aqueous and ethanolic extract of *C. aegyptiaca* at 125mg/ml reduced significantly liver glucose secretion into the incubation medium during time course incubation.

This suggests that *C. aegyptiaca* extract used in this study may counter liver glucose release from glycogen. Many medicinal plants used to overcome diabetes were reported to have anti-glycogenolysis effect trough glucose-6-phosphatase inhibition (Dentin *et al.*, 2007; Adeneye *et al.*, 2014). The fact that *C. aegyptiaca* extract reduced significantly glucose liberation from liver could be explained by a direct activation of glucose-6-phosphatase (Kolawole and Akanji, 2014). The last step in both glycogenolysis and glyconeogenesis is catalyzed by glucose-6-phosphatase which cleaves phosphate from glucose-6-phosphate to free glucose into the bloodstream (Adeneye *et al.*, 2014). Further studies are underway to analyze C. aegyptiaca extract effect in vivo on glucose transport and liver glucose metabolism pathways.

Conclusion

Results present here showed that *C. aegyptiaca* plant has potential therapeutic properties as liver glucose liberation inhibition activities. It composed of antioxidant and anti-inflammatory compounds. Further studies are needed to analyze C. *aegyptiaca* extract effect *in vivo* on glucose transport, to understand the liver glucose metabolism pathways mechanism, and its toxic properties.

References

Abdul-Ghani MA, De Fronzo RA. 2008. Inhibition of renal glucose reabsorption: a novel strategy for achieving glucose control in type 2 diabetes mellitus. Endocr Pract **14,** 782-790. DOI:10.4158/EP.14.6.782.

Adedapo AA, Jimoh OF, Afolayan JA, Masika JP. 2008. Antioxidant activities and phenolic contents of the methanol extracts of the stems of Acokanthera Oppositifolia and Adenia gummifera. BMC Complem. Altern. med. **8(54),** 1-7. DOI:10.1186/1472-6882-8-54.

Agius L. 2007. New hepatic targets for glycemic control in diabetes. Best Pract Res Clin Endocrinol. Metab **21,** 587-605.

Akpagana K, Koumaglo KH, Bouche PH, Gbeassor M. 1996. Un cas de sauvegarde passive d'une espèce utile et en voie de disparition au Togo: Conyza aegyptiaca var. lineariloba. Revue Med. Pharm. Afr **10(1),** 99-102.

Batawila K, Kokou K, Akpagana K, Koumaglo K, Bouchet P. 2002. Activité antifongique d'une espèce en voie de disparition de la flore togolaise: *Conyza aegyptiaca* (L.) Ait. Var. lineariloba (DC.) O. Hoffm. (Asteraceae). Acta Botanica Gallica: Botany Letters **149,** 41-48.

Bidie P, N'Guessan BB, Yapo AF, N'Guessan JD, Djaman AJ. 2011. Activités antioxydantes de dix plantes médicinales de la pharmacopée ivoirienne. Sci. Nat **8,** 1-11.

Broadhurst RB, Jones WT. 1978. Analysis of condensed tannins using acidified vanillin. J Sci Food Agr **29(9),** 788-794.
DOI: 10.1002/jsfa.2740290908.

Bruneton J. 2009. Pharmacognosie, Phytochimie, Plantes Médicinales (3ème édition). Tec & doc, 1292.

Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos MD. 2002. A comparison between Two brine shrimp assays to detect in vitro cytoxicity in marine natural products BMC Biotechnoloy **2,** 17-354.

Djrolo F, Houinato D, Gbary A, Akoha R, Djigbénoudé O, Sègnon J. 2012. Prevalence of diabetes mellitus in the adult population at Cotonou, Benin. Med. Metab. Diseases **6,** 167-169.

Gbénou JD, Ahounou JF, Ladouni P, Agbodjogbé WKDD, Tossou R, Dansou P, Moudachirou M. 2011. Propriétés anti-inflammatoires des extraits aqueux de Sterculia setigera Delile et du mélange Aframomum melegueta K. Schum-Citrus aurantifolia Christm et Panzer. Int. J. Biol. Chem. Sci **5,** 634-641.

Govindappa M. 2015. A Review on Role of Plant(s) Extracts and its Phytochemicals for the Management of Diabetes. J Diabetes Metab **6,** 565.

Grmek MD. 1997. Le legs de Claude Bernard. Fayard. Paris.

Heimler D, Vignolini P, Din MG, Vinueri FF, Ronani A. 2006. Antiradical activity and polyphenol composition of local Brassiccaceae edible varieties. Food Chemistry **99,** 464-469.

Houghton JP, Rama A 1998. Laboratory Handbook for the Fractionation of Natural Extract. Pharmacognosy Research Laboratories, Department of Pharmacy, King's College, London 212 p.

Kim DO, Chun O, Kim Y, Moon H, Lee C. 2003. Quantification of phenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem **51,** 6509-6515.

Lamien-Meda A, Lamien CE, Compaoré MMH, Meda NTR, Kiendrebeogo M, Zeba B, Millogo JF, Nacoulma OG. 2008. Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. Molecules **13,** 581-594.

Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TC, Coube CS, Leitão AG. 2001. Screening of Brazilian plant extracts for antioxidant isoactivity by the use of DPPH free radical method. Phytother. Res **15,** 127-130.

Michael AS, Thompson CG, Abramovitz M. 1956. Artemia salina as a test organism for a bioassay. Science **123,** 467-505.

Mousseux M. 1995. Test de toxicité sur les larves d'artémia salina entretien d'élevage de balanes. Université française de Pacifique. Centre universitaire de Nouvelle Calédonie. Duest. Aquaculture p. 20.

N'guessan JD, Zirihi GN, Kra AKM, Kouakou K, Djaman AJ and Guede-Guina F. 2007. Free radical scavenging activity, flavonoid and phenolic contents of selected Ivoirian plants. International Journal of Natural and Applied Sciences **4,** 425-429.

Postic C, Dentin R, Girard J. 2004. Role of the liver in the control of carbohydrate and lipid homeostasis. Diabetes Metab **30,** 398-408.

Prakash D, Upadhyay G, Brahma N, Singh HB. 2007. Singh antioxidant and free radical Scavenging activities of seeds and agri-wastes of some varieties of soybean (Glycine max). Food Chemistry **104,** 783-790.

Sakine MNA, Mahmout Y, Dijoux-Franca MG, Gbenou J, Mansourou M. 2012. In vitro anti-hyperglycaemic effect of glucocapparin isolated from the seeds of Boscia senegalensis (Pers.) Lam. ex Poiret. African Journal of Biotechnology **11(23),** 6345-6349. DOI: 10.5897/AJB11.3445.

Singleton VL, Orthofer R, Lamuela-Raventos RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol **299,** 152-179.

Tekao T, Watnabe N, Yagi I, Sakata K. 1994. A sample screening method for antioxidant and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotechnol. Biochem **58,** 1780-1783.

Trinh KY, O'Doherty RM, Anderson P, Lange AJ, Newgard CB. 1998. Perturbation of fuel homeostasis caused by over expression of the glucose-6-phosphatase catalytic subunit in liver of normal rats. J Biol Chem **273,** 615-620.

Xia X, Yan J, Shen Y, Tang K, Yin J, Zhang Y, Yang D, Liang H, Ye J, Weng J. 2011. Berberine Improves Glucose Metabolism in Diabetic Rats by Inhibition of Hepatic Gluconeogenesis. PLoS ONE **6,** e16556.
DOI:10.1371/journal.pone.0016556.

Yessoufou A, Gbenou J, Grissa O, Hichami A, Simonin A-M, Tabka Z, Moudachirou M, Moutairou K, Khan AN. 2013. Anti-hyperglycemic effects of three medicinal plants in diabetic pregnancy: odulation of T cell proliferation. BMC Complementary and Alternative Medicine **13,** 1-13. www.biomedcentral. com/1472-6882/13/77.

Zhi PR, Liang LZ, Yi ML. 2008. Evaluation of the Antioxydant Activity of Syzygium cumini Leaves. Molecules **13,** 2545-2556.

Zhishen J, Mengcheng T, Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry **64,** 555-559.