IN VITRO ANTIOXIDANT AND BIOLOGICAL ACTIVITIES OF HYDROETHANOLIC EXTRACTS OF *UAPACA PALUDOSA*

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ABSTRACT

Introduction: Diabetes mellitus is a chronic metabolic disorder characterized by abnormalities in carbohydrate, lipid, and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis.

Materials and Methods: The purpose of the present study was to measure the in vitro antioxidant and biological activities of hydroethanolic extracts of *Uapaca paludosa.* Phytochemical screening, Antioxidant capacity (Polyphenolic content, Free radical scavenging activity, Nitric oxide scavenging activity and Metal chelating activity) and biological activities (Blood sugar assessment, Glucose tolerance test, antiamylasic activity test and Low grade postprandial inflammation) have been done.

Results: Phytochemical studies of hydroethanolic extracts of *U. paludosa* revealed the presence of tannins, phlobatannins, glycosids, phenols, reducing sugars, flavonoids, saponins, and alkaloids. Antioxidant activity has revealed that *U. paludosa* was riched in polyphenols and radical inhibitory effect was best with DPPH° radical. In glucose tolerance test, hydroethanolic extracts 400mg/kg BW allowed inhibition of glycemia than 200mg/kg BW. Hypoglycemic test revealed that hydroethanolic extracts 400mg/kg BW inhibit glycemia (6.511±1.152%) than 200mg/kg BW (0.283±0.151%). The results of low grade inflammation showed that postprandial endothelium dysfunction and oxidative stress appear 2 hours after consumption of deleterious diet. But extracts has significantly inhibited endothelium dysfunction and oxidative stress ($p<0.05$); *U. paludosa* present a reduction of postprandial nitric oxide but only hydroethanolic extract 400mg/kg BW has presented a maximum reduction of postprandial oxidative stress (postprandial hydroperoxides).

Conclusion: Hydroethanolic extracts of *U. paludosa* have effect on inflammation but haven't no effect on antiamylasic activity; because of hyperosmolar diabete

after 30min, it will be not necessary to use these extracts to cure diabete.

Key words: Diabetes, metabolic disorder, Uapaca paludosa, anti oxidant activity

RESUMÉ

Introduction: Diabètes mellitus est une desordre metabolique chronique caracteriser par les anomalies en glucides, lipids, et metabolisme lipoptoteines, qui aboutis non seulement a hyperglycemie mais aussi provoque beaucoup des complications tels que hyperlipidemie, hyperinsulinemie, hypertension, et l'atherosclerose.

Matériels et Méthodes : Le présent travail avait pour but de mesurer l'activité antioxydante in vitro et les propriétés biologiques des extraits hydroéthanoliques de *Uapaca paludosa.* Le screening photochimique, l'activité antioxydante (teneur en polyphénols, activité antiradicalaire DPPH, activité piégeuse du radical NO et activité chélatrice des métaux) et l'évaluation des activités biologiques (test de tolérance au glucose, test d'hypoglycémie, test d'activité antiamylasique et test d'inflammation à bas bruit en période post prandiale) ont été réalisés.

Résultats : L'étude phytochimique a revélé que les extraits hydroéthanoliques de *Uapaca paludosa* sont riches en tannins, phlobatannins, glycosides, phénols, sucres réducteurs, flavonoïdes, saponines, and alcaloïdes. L'activité antioxydante a revélé que ces extraits sont riches en polyphénols et ont eu un effet sur le radical DPPH. Les doses 400mg/kg PC ont eu un meilleur effet sur la réponse glycémique en période postprandiale que les doses 200mg/kg PC. Il en est de même des résultats du test d'hypoglycémie (6,511±1,152% pour la dose 400mg/kg PC et 0,283±0,151% pour la dose 200mg/kg PC). Les résultats de l'inflammation à bas bruit en

période postprandiale ont revélé que la dysfonction endothéliale et le stress oxydant sont apparus 02h après la consommation du repas délétère. Par ailleurs, tous les extraits ont eu un effet réducteur de la dysfonction endothéliale postprandiale (oxyde nitrique postprandial) mais seules doses 400mg/kg PC ont présenté un maximum de réduction (p<0,05) du stress oxydant postprandial (hydropéroxides postprandiaux).

Conclusion : Les extraites hydroethanoliques de *U. paludosa* ont l'effet sur l'inflammation mais effet de activités antiamylasique a cause de diabètes hyperosmolaire après 30 mins, et donc par nécessaire a l'utiliser l'extrait pour le traitement de diabète.

Mots Clés : diabètes, désordres metabolique chroniques, activités antioxydante, *Uapaca paludosa*

INTRODUCTION
Diabetes mellitus is a chronic metabolic disorder characterized by abnormalities in carbohydrate, lipid, and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis (Qiong *et al*., 2004). Control of diabetes mellitus normally involves exercise, diet and chemotherapy. Development and utilization of antidiabetic plants have attracted increasing interest (Qiong *et al*., 2004). The plant kingdom is a wide field to search for natural effective oral hypoglycemic or hypolipidemic agents that have slight or no side effects. The implication of oxidative and free radical–mediated reactions in degenerative processes related to aging and chronic disease conditions is important. Free radicals in the form of reactive oxygen species (ROS) and reactive nitrogen species are implicated in numerous diseases such as inflammation, metabolic disorders, reperfusion damage, atherosclerosis, and carcinogenesis (Agbor et al., 2006). Aerobic respiration, stimulated polymorphonuclear leukocytes, macrophages, and peroxisomes are the main endogenous sources of most of the oxidants produced by cells (Agbor et al., 2006). In order to protect themselves against free radicals, organisms are endowed with endogenous (catalase, superoxide dismutase, glutathione peroxidase/ reductase) and exogenous (C and E vitamins, β-carotene, uric acid) defences; yet these defence systems are not sufficient in critical situations (oxidative stress, contamination, UV exposure, etc.) where the production of free radicals significantly increases (Mondon *et al*., 1999). Thus, compounds that can scavenge free radicals can play a role in improving health in oxidative stress-related disorders.
The purpose of the present study was to measure the in vitro antioxidant and biological activities of *Uapaca*

paludosa which is a Cameroonian medicinal plant used to cure malaria, hypertension, B viral hepatitis and diabetic.

MATERIAL AND METHODS
Collection and identification of plant material
Leaves of *Uapaca paludosa* was harvested at Nkolbisson mount Kala around the Yaoundé neighbourhood, and the specimen sample deposited at the National Herbarium Centre for plant identification..

Preparation of hydroethanolic extract
The leaves were dried in drying room and ground into uniform powder. Hydroethanolic extracts was obtained by maceration. 250g of leaves were dissolved on 2L of hydroethanolic mix (1:1) during 48h. Mix was filtered and extracts were obtained after evaporation under oven.

Phytochemical screening: Chemical tests were carrying out on the aqueous and hydroalcoholic extracts for the qualitative determination of phytochemical compounds using standard procedures: Alcaloids and reducing sugars (Odebeyi and Sofowora, 1978); Saponins (Wall et al., 1954); Flavonoids, tannins, glycosids and cardiac glycosids (Trease and Evans, 1978); Polyphenols and anthocyans (Harbone, 1976) and phlobatannins (Trease and Evans, 1989)

Antioxidant capacity
Polyphenolic content: It was determined by Folin – Ciocalteu reagent as previously described by Singleton and Rossi (1965**)**. Phenol content value was obtained from regression curve **y= 0.00015x+0.094** and expressed as mg/g catechine equivalent.

Free radical scavenging activity: It was carried out according method of Katalinie *et al*., 2003. 20µl of extract are added with 1000µl of DPPH methanol solution after reading wavelength at 517nm, percentage antioxidant capacity was obtained by formula:
AA%= ((Abs sample – Abs control)/Abs sample) x 100.
Where Abs1 is the absorbance of the control, and Abs2 is the absorbance of the plant extract.

Nitric oxide scavenging activity: It was carried out according to the method of Sreejayan and Rao, 1997. At 1ml of crude extract (10 – 1000µg/ml in methanol 0.2%) it was added 2ml of sodium nitroprusside 10mM (in phosphate buffer pH 7.4; 50mM). the mixture was homogenized and incubated at room temperature during 15 min. after incubation 0.5ml of mix reaction is added with 1ml of sulfanilic acid (0.33% ice acetic acid) after incubation during 05 min at room temperature (complete

diazotation), 1ml of NED (Naphtylethylen diamine dichloride (0.1%) was added. Solution was shaken and incubated at room temperature during 30min. Absorbance was read at 540nm. Control was doing like extract except that extract was replaced by methanol. Blank was done for control and extract; it contained all reagents except nitroprusside.

% NO inhibition= ((Abs sample – Abs control)/Abs sample) x 100.

Metal chelating activity: It was carried out according to Dinis et al., 1994. 25µl of 2mM $FeCl_2$ and 100µl of ferrozine was added to 0.5ml of the plant (10 – 1000µg/ml). The mixture was vigourously shaken and left at room temperature for 10min. the absorbance was measured at 562nm. A control was similarly prepared except that methanol replaced extract. **% metal chelating = ((Abs sample – Abs control)/Abs sample) x 100**.

Hypoglycemic activity

Blood sugar assessment: Blood samples of normal rats were drawn after an overnight fasting (12 hr.) from rat's tail vein at different time intervals at 0 min, 60min, 120min, 240min and 300 min for determination glycemia level using commercial test strips (SD Check).

Glucose tolerance test: A glucose tolerance test is the administration of glucose to determine how quickly it is cleared from the blood. The rats were tested in a fasting state (having no food or drink except water for at least 12 hours but not greater than 16 hours). An initial blood sugar was drawn and then the rats were fed extracts and glucose (2g/kg BW) 30 minutes after the consumption of extracts. The rats then had their blood tested again 30 minutes after administration of glucose, 60 min, 90 min and 150 min after drinking the high glucose drink using commercial test strips (SD Check) (Komaki *et al.,* 2003).

In vivo antiamylasic activity test: antiamylasic activity test is the administration of starch to determine how quickly it is cleared from the blood. The rats were tested in a fasting state (having no food or drink except water for at least 12 hours but not greater than 16 hours). An initial blood sugar was drawn and then the rats were fed extracts and starch (1g/kg BW) 30 minutes after the consumption of extracts. The rats then had their blood tested again 30 minutes after administration of glucose, 60 min, 90 min and 150 min after drinking the high glucose drink using commercial test strips (SD Check) (Komaki *et al.,* 2003).

Low grade inflammation

Low grade postprandial inflammation: this test is the administration of 5ml of emulsion of deleterious diet (60% palm oil, 20% casein and 20% saccharose) to predict the time of low grade postprandial inflammation and how hydroperoxide and nitric oxide levels are cleared from the blood. The rats were tested in a fasting state (having no food or drink except water for at least 12 hours. An initial blood nitric oxide and hydroperoxide were drawn and then the rats were fed extracts and deleterious emulsion 30 minutes after extracts. The rats then had their blood tested again 2 hours, 4hours and, 6 hours.

(Magné *et al.*, 2009).

Statistical analysis: The data obtained in the animal experiments was subjected to statistical analysis. All values are expressed as Mean ± S.E.M (Standard Error of Mean). The data were assessed by the *t* test of Student and the group means were evaluated by test of Kolmogorov Smirnov. Mean values were considered significantly different if $p < 0.05$.

RESULTS
Phytochemical screening
Phytochemical studies of hydroethanolic extracts of *U. paludosa* revealed the presence of tannins, phlobatannins, glycosids, phenols, reducing sugars, flavonoids, saponins, and alkaloids.

Antioxidant activity

Table 1 reveals IC_{50} value of the extract which was 781.999 µg/ml with DPPH° radical, 1388.068 µg/ml with NO° radical and 9669 µg/ml with metal chelating.

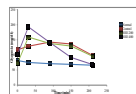| Antioxidant Tests	
Polyphenols content	1296.673±28.559 mg of catechine equivalent
Inhibitory concentration$_{50}$ (IC_{50})	
DPPH° radical scavenger	781.999µg/ml
NO° radical scavenger	1388.068µg/ml
Metal chelating	9669µg/ml

Hypoglycemic activity

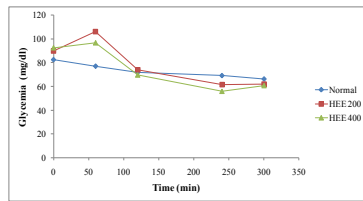**Figure 1: Antihyperglycemic effect of hydroethanolic extracts of U. *paludosa***

Figure 2: Hypoglycemic effect of hydroethanolic extracts of *U. paludosa*
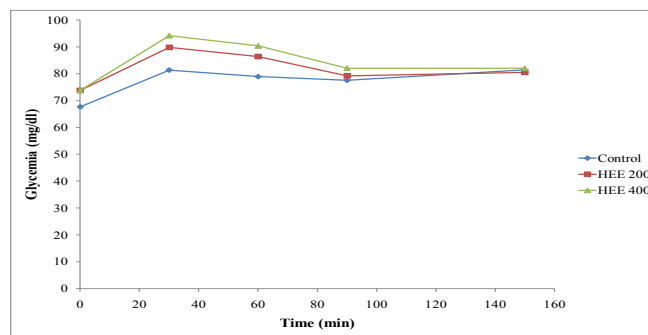

Figure 3: Antiamylasic effect of hydroethanolic extracts of *U. paludosa*

Figures 1, 2 and 3 showed that glycemic peak of extracts is very significantly ($p \leq 0.05$) high than control after 30min. moreover, the glycemia of extracts is very high than control. In glucose tolerance test, hydroethanolic extracts 400mg/kg BW allowed inhibition of glycemia than 200mg/kg BW (4.065±1.419% respectively). Hypoglycemic test revealed that hydroethanolic extracts 400mg/kg BW inhibit glycemia (6.511±1.152%) than 200mg/kg BW (0.283±0.151%)

The results of low grade inflammation showed that postprandial endothelium dysfunction and oxidative stress appear 2 hours after consumption of deleterious diet. But extracts significantly inhibit endothelium dysfunction and oxidative stress ($p < 0.05$) (Table 2 and table 3).

Table 2: Effect of hydroethanolic extracts of *U. paludosa* on postprandial hydroperoxides

Time (hours)	0	2	4	6
Control	9.889±0.354[a]	12.303±0.651[a]	12.555±0.875[a]	10.484±0.354[a]
HEE 200mg/kg PC	12.788±0.953[b]	11.528±1.107[b]	13.678±0.817[b]	11.687±0.604[b]
HEE 400mg/kg PC	12.986±0.734[b]	13.515±0.883[c]	12.114±1.118[a]	10.387±0.553[a]

Values are mean ±SEM, (n = 5); *Different letters mean p<0.05*

Table 3: Effect of hydroethanolic extracts of *U. paludosa* on postprandial nitric oxide level

Time (hours)	0	2	4	6
Control	106,153±2,348[a]	169,230±2,449[a]	243,076±0,402[a]	101,282±2,205[a]
HEE 200mg/kg PC	91,948±1,847[b]	88,769±1,245[b]	92,410±1,089[b]	92,512±1,450[b]
HEE 400mg/kg PC	92,307±1,919[b]	93,179±1,567[b]	88,615±1,150[b]	88,410±1,563[b]

Values are mean ±SEM, (n = 5); *Different letters mean p<0.05*

Tableau 4: Inhibition effect of hydroethanolic extracts of *U. paludosa* on low grade inflammation

	Postprandial hydroperoxides	Postprandial nitric oxide
HEE 200mg/kg PC	5.236±1.630[a]	40.798±1.333[a]
HEE 400mg/kg PC	11.814±2.600[b]	41.950±1.948[a]

Values are mean ±SEM, (n = 5); *Different letters mean p<0.05*

This table showed that *U. paludosa* present a reduction of postprandial nitric oxide but only hydroethanolic extract 400mg/kg PC presents a maximum reduction of postprandial oxidative stress (postprandial hydroperoxides).

DISCUSSION
In vitro antioxidant activity

DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. From the results, it may be postulated that extracts of *U. paludosa* have hydrogen donors thus scavenging the free radical DPPH (IC50= 781.999 μg/ml).

The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. The transition metal ion, Fe2+ possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals (Aboul-Enein et al., 2003). *U. paludosa* chelates iron extract interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. IC_{50} of the extract for chelating activity was 9669 μg/ml.

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities (Balakrishnan *et al*.; 2009). Preliminary Phytochemical studies of hydroethanolic extracts of *U. paludosa* barks show the presence of tannins, phlobatannins, glycosids, phenols, reducing sugars, flavonoids, saponins, and alkaloids. Suppression of released NO may be partially attributed to direct NO scavenging, as the extracts of *U. paludosa* decreased the amount of nitrite generated from the decomposition of SNP *in vitro*. The scavenging of NO by the extracts was increased at very high dose (*U.paludosa* were IC_{50} is 1388.068μg/ml).

Hypoglycemic activity

Likewise the anti-hyperglycaemic effect that was found with glucose load may be due to delayed absorption of glucose in the gut, or enhanced disposal of glucose by increased insulin sensitivity (Abu *et al*., 2011). The results after administration of hydroethanolic extracts of *U. paludosa* showed significant increasing of glycemia (p<0.05) 30 min

after loading of glucose and starch, this would be due to presence of reducing sugars and glycosids which are probably responsible of hyperosmolar diabete. The extracts of *U. paludosa* do not inhibit glycemic response with starch load.

In blood glucose assessment, the dose 400 mg/kg body weight produced maximum reduction of 5.522%. These observations suggest that hydroethanolic extracts of *U. paludosa* wouldn't have hyperglycemic and antidiabetic activities.

Low grade inflammation

Vascular endothelial function is an integrative marker of vascular homeostasis. The alteration of vascular endothelial function is the key event in the early pathophysiology of atherosclerosis (Davignon and Ganz, 2004) and predicts a wide range of cardiovascular diseases (Brunner *et al*., 2005). High-saturated fat and high-sucrose intake are considered nutritional risk factors for cardiovascular diseases (Mozaffarian *et al*., 2008), not only in patients at high risk, but also in healthy individuals (Celermajer and Ayer, 2006), and adverse effects have been documented in both chronic and acute exposures. Interestingly, in healthy humans, a single high-fat and/or high-sucrose meal induces a series of transient metabolic and physiological dysregulations (Poppitt, 2005), including impaired vascular reactivity (Jackson *et al*., 2007), low-grade inflammation (Sies *et al*., 2005), and generation of reactive oxygen species (ROS) (Celermajer and Ayer, 2006). Extracts of *U. paludosa* present inhibition of postprandial oxidative stress (hydroperoxides) and postprandial endothelial dysfunction (nitric oxide). This suggest that these extracts could delay or inhibit atherosclerosis and complications.

CONCLUSION

Hydroethanolic extracts of *U. paludosa* have shown a promising effect on inflammation but there was no evident effect on antiamylasic activity which could have been due to hyperosmolar diabetes after 30min. This study confirmed that the *U. paludosa* show no potential activity for diabetes therapy.

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