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Screening of antioxidant and antidiabetic properties of aqueous and hydroethanolic extracts of the leaves of *Acalypha wilkesiana* Muel.Arg

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ABSTRACT

We investigated the phytochemical content, the antioxidant capacity and the anti-diabetic properties of aqueous and hydroethanolic (50%) extracts of the leaves of *Acalypha wilkesiana* Muell. Arg. A phytochemical screening was carried out to identify the different bioactive metabolites. The polyphenolic content and the antioxidant capacity of both extracts were determined using Folin-ciocalteu and FRAP (Ferric Reducing Antioxidant Power) methods respectively. *In vivo* studies were conducted in normal and streptozocin induced diabetic male rats. The effect of both extracts on postprandial blood glucose level was determined in 16 fasted normal rats. The effect of the extracts on fasting blood glucose was determined in streptozotocin induced-diabetic rats (400 mg/kg) for 5 hours. The hydroethanolic extract contained more polyphenols compared to the aqueous extract (218,51 ± 17,41 vs 111,02± 12,15 mg equivalent catechin /g of extract; $p<0,05$) . The antioxidant capacity was also higher with hydroethanolic extract (294,99 ±54,29 vs128,11 ± 7,18 equivalent catechin /g of extract; $p<0,05$). Administration of both aqueous (33.03% vs 52.58% ;$p<0.006$) and hydroethanolic extract (7.87% vs 52.58% ; $p<0.001$), 30 minutes before oral starch load suppressed the rise in postprandial glucose .The hydroethanolic extract inhibited the increase in blood glucose level compared to the aqueous extract which did not have an effect. The same extract also exhibited good hypoglycaemic activity in diabetic rats. The *Acalypha wilkesiana* Muell. Arg leaves offers an attractive therapeutic approach in the treatment of diabetes by decreasing both fasting and postprandial hyperglycaemia.

Keywords: *Acalypha wilkesiana*, antioxidant, anti-diabetic, hyperglycaemia.

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INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by a disorder of glucose regulation with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both[1,2]. Without enough insulin, body tissues in particular the liver, muscle and adipose tissues fail to absorb and utilize glucose from the blood circulation. This results in elevated blood glucose levels, a condition known as hyperglycaemia. If blood glucose levels remain high over a long period of time, this can result in long-term damage of organs such as the kidneys, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death[3,4,5].

Today, diabetes affects about 246 million people worldwide. It is estimated that this number will increase to 380 millions by the year 2025 if appropriate attention is not paid[6]. In Cameroon, recent estimations situate the prevalence rate at 4,3%, with an increased prevision of 4,7% by the year 2025[6]. Risk factors of diabetes are linked to the growing adoption of western lifestyle characterized by high energy intake coupled to a sedentary lifestyle. However, these factors are often preceded by purely environmental factors involving the concept of hereditary predisposition[7].

Currently, type 2 diabetes mellitus which is the most common type of diabetes mellitus is managed by a combination of diet, exercise, oral hypoglycaemic drugs and sometimes insulin injections[8]. However, synthetic oral hypoglycaemic drugs, which are currently the main form of treatment for type 2 diabetes mellitus have been shown to have undesirable side effects and high secondary failure [rates9,10]. In addition, these drugs cannot be afforded by the majority of people living in rural communities of developing countries such as Cameroon because of their high cost[8]. These limitations of currently available anti-diabetic pharmacological agents have prompted researchers all over the world to investigate alternative anti-diabetic remedies. In particular, consideration is given to plants and herbs used by traditional healers and herbalists as anti-diabetic remedies with the hope of discovering new natural products that can be used or developed into safe, inexpensive and effective anti-diabetic remedies. In this context, a number of medicinal plants and herbs have been studied and validated for their hypoglycaemic potential using experimental animal models of diabetes[11], as well as clinical studies involving diabetic patients[12,13]. To support the quest for alternative therapies to treat diabetes, we investigated the phytochemical content, the antioxidant capacity and anti-diabetic properties of extracts of *Acalypha wilkesiana* Muell.Arg.

MATERIALS AND METHOD

Processing of *Acalypha wilkesiana* Muell.Arg

Fresh leaves of *Acalypha wilkesiana* were harvested in the *Ngoa ekelle* neighborhood of Yaounde and dried under sunlight until a crispy texture was obtained. The dried leaves were hand-crushed then weighed giving 1kg of the powder. This powder was used to prepare the aqueous and hydroethanolic extracts.Phytochemical screening was performed according to the method described by Trease and Evans[14] and Harbone [15] using both extracts. Determination of the polyphenolic content of both extracts was done through the Folin-Ciocalteu method[16]. The antioxidant potential was assessed using the Ferric Reducing Antioxidant Power (FRAP) method [17]

In vivo antiamylase activity

Male adult albino rats of 200-245g body weight, bred in our animal house were used for the study. The animals were acclimatized for one week before the experiment. Rats were fed with normal diet during the whole experiment. Twenty male rats weighing between 200-245g were isolated from the other animals and fasted for 12 hours before the experiment.Fasting blood glucose was determined after which they were divided into four groups of five rats per cage and treated as follows:

Group1: Negative control group which received 1 mL of distilled water and another1mL of distilled water after 30 minutes,.

Group 2: Positive control group which received 1 mL of distilled water and 1 mL of starch (1g/Kg/Pc) after 30 minutes,

Group 3: The first test group which received 1mL of aqueous extract at a dose of 400 mg/Kg, and 1 mL of starch (1 g / kg / Pc) 30 minutes after).

Group 4: The second test group which received 1mL of hydroethanolic extract 400 mg/Kg, and 1 mL of starch (1 g / kg / Pc) after 30 minutes.

The postprandial blood glucose was measured at 30 min, 60 min, 90 min and 180 min after starch load. The total increment of glyceamia in each group was calculated as the sum of increases in blood glucose after administration according to the method of Madar, 1989[18].

Evaluation of the hypoglycaemic effects of the best extract in diabetes-induced rats

Induction of experimental diabetes

The baseline plasma glucose levels were determined prior to the induction of diabetes by intra-veneous injection of streptozotocin (50 mg/kg body weight in 50 mM Citric buffer, pH 4.5, NaCl

150 mM) in overnight fasted rats (average weight 197,3 g). After 5 days;15 rats with marked hyperglycaemia (blood glucose level above 200 mg/dL) were selected and and divided into three groups of 5 rats each before test.

The initial glyceamia of all the rats were measured and rats were treated as follow: Controls animals received either distilled water (group I); The reference drug group received 80 mg/kg body weight of Tolbutamide an antidiabetic reference drug (group II) and the eexperimental rats received 400 mg/kg of the hydroethanolic extract of *Acalypha wilkesiana*. Blood glucose was successively measured, 2h, and 5h thereafter using a Glucometer. Blood glucose curves of experimental rats were plotted and compared with those of control rats.

Statistical analysis of data

Data were analyzed by one-way ANOVA, and then differences among means were analyzed using the Fisher's protected LSD test.

RESULTS AND DISCUSSION

Phytochemical screening showed that the plant *Acalypha wilkesiana* Muell.Arg is rich in bioactive compounds like phenols, tannins and flavonoids. Some of these phytochemicals are believed to be responsible for the blood glucose lowering effects of these plant materials[19]

Polyphenolic content and antioxidant potential of the aqueous and hydroethanolic extracts of *Acalypha wilkesiana*

Table 1: Evaluation of the polyphenolic content of aqueous and hydroethanolic extracts of A. wilkesiana.

Extract	Aqueous extract	Hydroethanolic extract
Polyphenolic content (mg equivalent catechin /g of extract)	111,02± 12,15	218,51 ± 17,41*
FRAP (mg equivalent catechin /g of extract)	128,11 ± 7,18	294,99 ±54,29*

**p<0.05; significantly different compared to aqueous extract*

The results in table 1 above reveal the presence of large amounts of polyphenols in the hydroethanolic extract compared to the aqueous extract (218,51 ± 17,41 vs 111,02 ± 12,15mg equivalent catechin /g of extract; p<0,05) . The antioxidant capacity evaluated by FRAP method (Ferric Reducing Antioxidant) was also higher with the hydroethanolic extract (294,99 ± 54,29 vs 128,11 ± 7,18 equivalent catechin /g of extract; p<0,05).

Many studies have shown that there is a strong link between the antioxidant activity of tropical plants and the amounts of polyphenols they contain[20]. This implies that plants with high levels of phenolic compounds are good sources of antioxidants.

Effect of the aqueous and hydroethanolic extracts of *Acalypha wilkesiana* on starch digestion in normoglycaemic rats

The glyceamia obtained after adminstration of plant extracts and starch were used to draw the following curve:

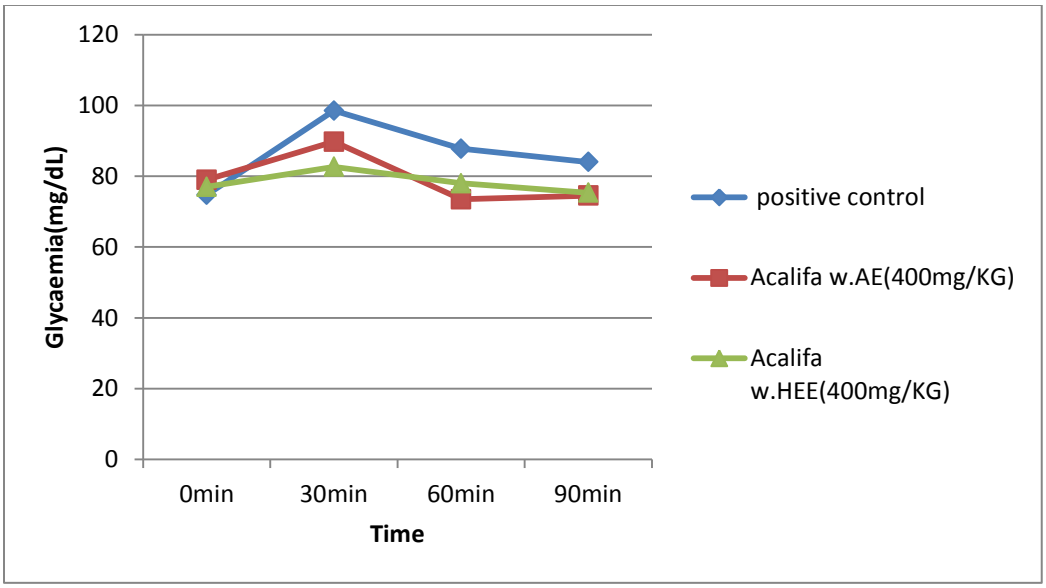

Figure 1: Effects of aqueous and hydroethanolic extracts on starch digestion in rats

AA400mg *= Aqueous extract Acalypha of dose 400mg/kg BW*

AHEE400mg *= Acalypha hydroethanolic extract of dose 400mg/kg BW*

The administration of both aqueous (33.03% vs 52.58% ;$p<0.006$) and hydroethanolic extract (7.87% vs 52.58% ; $p<0.001$), 30 minutes before oral starch load significantly suppressed the rise in postprandial glucose level compared to the positive control group. A significant difference was also noticed between the two extracts at 30 min ($p<0.001$) and 60min ($p<0.04$) following administration.

A major goal in the management of diabetes mellitus is to maintain near normal blood glucose levels in both the fasting and postprandial state[8]. One therapeutic approach to decrease postprandial hyperglycaemia is to suppress the production and/or absorption of glucose from the gastrointestinal tract through inhibition of either α-amylase or α-glucosidase enzymes[21,22]. Alpha amylase digests polysaccharides (starch) into various oligosaccharides and dissacharides. Disaccharides produced by α-amylase are futher hydrolyzed by α-glucosidases to produce glucose and other monosacharides, which are readily absorbed in the small intestine[23]

In this study, it was noticed that the hydroethanolic extract significantly reduced starch digestion probably by inhibiting enzymes involved in starch digestion. Experimental animal studies[20] and clinical studies[24] have shown that inhibitors of both α-amylase and α-glucosidase can suppress

the production and absorption of glucose from the small intestine. The observed effect may be due to the presence of polyhenols such as flavonoids which have been reported to inhibit alpha amylase[25]. The difference in structure of flavonoids contained in tested extracts may therefore explain the differences in the efficacy of the two extracts. Furthermore, some inhibitors of α-amylase and α-glucosidases such as phaseolamin, acarbose and vogiblose are currently used to suppress postprandial glucose levels in diabetic patients[26]

Effect of aqueous and hydroethanolic extracts of *Acalypha wilkesiana* on postprandial glucose level after glucose loading in normoglycemic rats

The glycemia obtained during the experiment were used to draw the curve below.

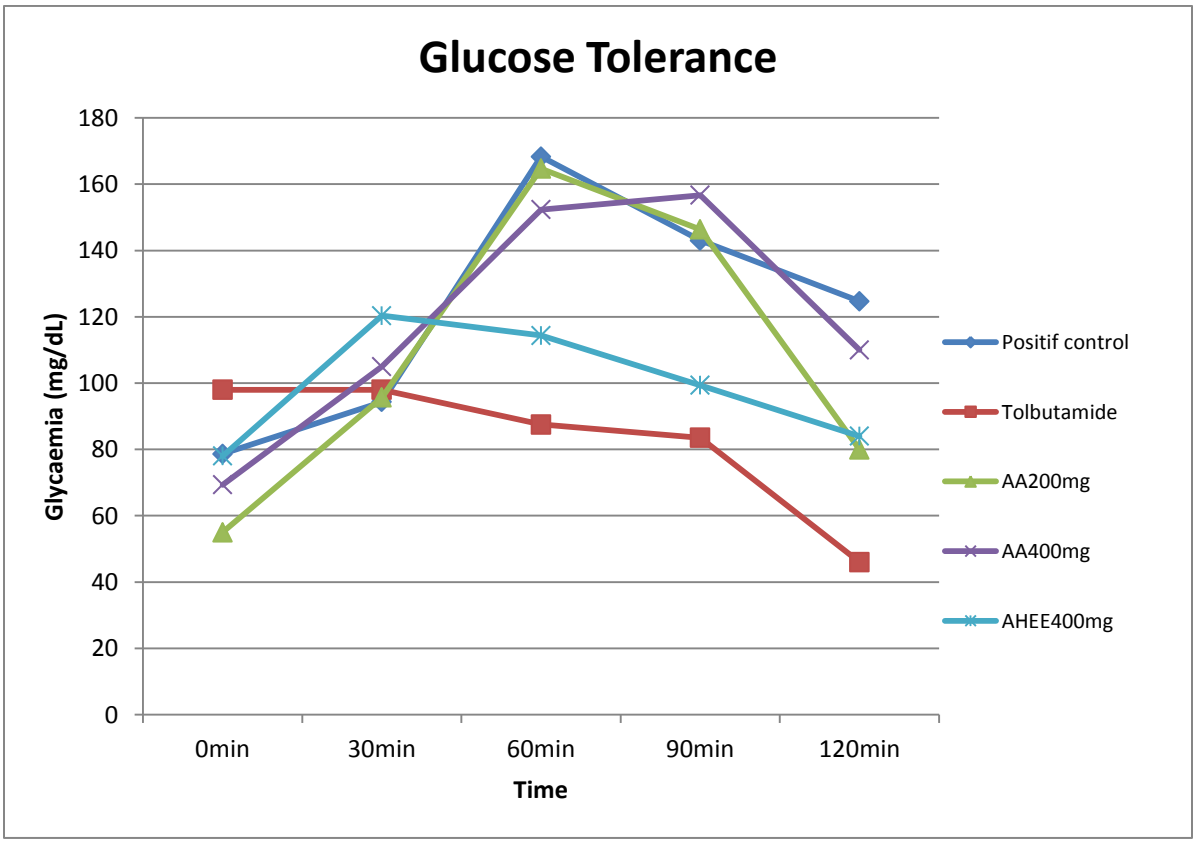

Figure 2: Effect of the aqueous and hydroethanolic extracts on glucose tolerance

AA400mg *= Aqueous extract of Acalypha at concentration of 400mg/kg BW +2glucose/kg BW*

AHEE400mg *= Acalypha hydroethanolic extract at concentration of 400mg/kg BW +2g lucose/kg BW*

The administration of hydroethanolic extract of *Acalypha wilkesiana* 30 minutes before the oral of glucose load significantly suppressed ($P < 0.01$)the rise in postprandial glucose. There was no effect observed with the aqueos extract. A significant difference ($p<0.001$) was noticed between the aqueous and hydroethanolic extracts at all time intervals. The observed effect of the

hydroethanolic extract on postprandial glucose levels showed a striking similarity with the effect exerted by Tolbutamide (the reference drug). Since Tolbutamide is known to exert its effect on postprandial hyperglyceamia through stimulation of insulin secretion, inhibiting the enzymes of neoglucogenesis, or activating the enzymes involved in the catabolism of glucose, it can be speculated from the result of the current study that the hydroethanolic extract of leaves of *Acalypha wilkesiana* also exert its postprandial glucose lowering effect through the same mechanism. The bioactive compounds like flavonoids, phenols, alkaloids and others found in the extract are known to have lowering effects on glycemia[27] These compounds are more extractible in ethanol than in water. This could partly explain why the hydroethanolic extract was more efficient.

Hypoglycemic effect of the hydroethanolic extract of *Acalypha wilkesiana* in steptozotocin diabetic rats

Figure 3 shows the time course of the hypoglycaemic effect of the the hydroethanolic extract of *Acalypha wilkesiana* in streptozotocin diabetic rats.

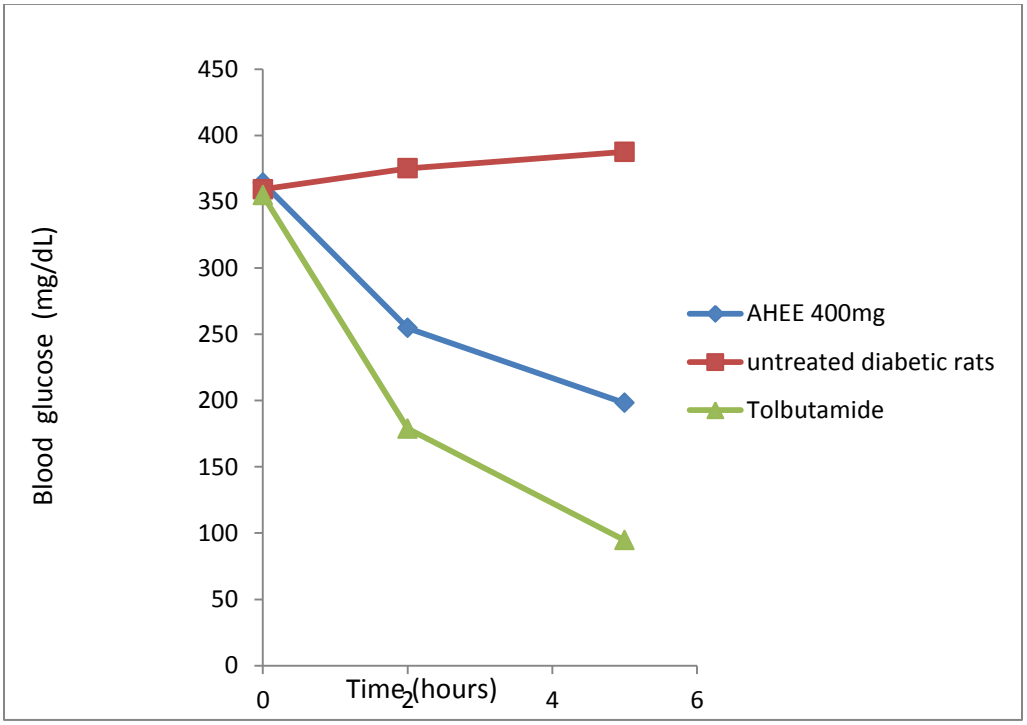

Figure 3: Time course of the hypoglycaemic effect of the hydroethanolic extract of *Acalypha wilkesiana* on streptozotocin-diabetic rats.

Streptozotocin induces diabetes by destroying the insulin secreting cells of the pancreas leading to hypoinsulinemia and hyperglycaemia. In agreement with these known effects of streptozotocin, the blood glucose levels of untreated diabetic rats significantly increased

throughout the study compared to those treated with the hydroethanolic extract *Acalypha wilkesiana* or the reference drug. The peak activity of the extract was observed at 2h (30.06%) and 5h (45, 57%) post drug administration. In this study, we found that the activity of the plant extract is comparable to the hypoglycaemic action of Tolbutamide which acts by stimulating insulin release and the inhibition of glucagon secretion[28]. Some medicinal plants with hypoglycaemic properties are known to increase circulating insulin levels in normoglycaemic rats[29]. A plausible mechanism of action is that the extract might have stimulated the residual pancreatic beta-cell function or produced the hypoglycaemia through an extra-pancreatic mechanism[30]. The hypoglycaemic effect produced by the hydroethanolic extract of *Acalypha wilkesiana* may be due to the glycosides, flavonoids, tannins and saponins present in the extract[31]

CONCLUSION

The study revealed that the aqueous and hydroethanolic extracts of *Acalypha wilkesiana* Muell.Arg contain different bioactive substances like phenols, flavonoids, and alkaloids. The hydroethanolic extract offers an attractive therapeutic approach to the treatment of postprandial hyperglycaemia by decreasing glucose release from starch, which may be potentially useful in the treatment of diabetes mellitus and obesity. The same extract also exhibited a good anti-hyperglycaemic and hypoglycaemic activity in normal and diabetic rats respectively

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