

# Antihyperglycaemic Effect of *Ageratum conyzoides* L. Fractions in Normoglycemic and Diabetic Male Wistar Rats

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## ABSTRACT

*Ageratum conyzoides* L. is a plant used in traditional medicine against many human infections diseases, also in case of diabetes. In order to locate the actives fractions of this plant, which allows in the future to purifying and identifying its active ingredients, bioassay tests were performed in rats treated with glucose and in those with diabetes induced by streptozotocin (STZ). First, aqueous crude extract of the plant was fractionated by column chromatography into two fractions F1 and F2. Then, fraction F1 was subjected to another fractioning into three subfractions F1a, F1b and F1c. Results of this study revealed that, in both tests, the aqueous crude extract of *A. conyzoides* had an important antihyperglycaemic potential. However, in rats given glucose, the subfraction F1c was the more potent, while in rats with streptozotocin-induced diabetes, the most active subfractions were F1a and F1b. Consequently, it is suggested that *A. conyzoides* contains more than one antihyperglycaemic compound with different chemical characteristics and mechanisms of action.

**Keywords:** aqueous crude extract, diabetes, streptozotocin, treatment

## INTRODUCTION

*Ageratum conyzoides* L. (Billy goat weed; family Asteraceae) (Fig. 1) is commonly known in Cameroon as Nyada Elog (Tsabang *et al.* 2001), in Swahili as Kundambara, in Chagga as Matawana (Chhabra *et al.* 1989), in Brazil as "Mentrasto" and in Arabic as Korink, berquam or bergoman (Boulos 1983). It is found in the north and western regions of Africa, in Australia and in certain parts of Africa and South America. It is an annual herb, ramified and up to 1m tall. Its stem and leaves are covered with tiny white hairs. The flowers are purple and white and the fruits are black and easily dispersed (Gonzalez *et al.* 1991; Gargiullo *et al.* 2008).

*A. conyzoides* is easily cultured because of its easy adaptability and high potential of reproductively. Thus, it is considered by agriculturalist as a weed because it overshadows other plants with its growth and reduces their yield (Bansal 1988; Jha and Dhakal 1990; Kong *et al.* 1999; Singh *et al.* 2003; Batish *et al.* 2006; Nogueira *et al.* 2010).

Phytochemical studies have extracted many different compounds from *A. conyzoides*; these include kaempferol and glycoside (rhamnoside); quercetin, scutellarein, eupalentin, chromene, stigmast-7-en-3-ol,  $\beta$ -sitosterol, stigmasterol, fumaric acid, caffeic acid, saponin, some pyrrolizidine alkaloids, essential oils, ageratochromene derived, coumarin and alkane (Aalbersberg and Singh 1991; Gonzalez *et al.* 1991; Wiedenfeld and Roder 1991; Cambie and Ash 1994; Okunade 2002; Moura *et al.* 2005; Daniel 2006; Lans 2007; Kamboj and Saluja 2008). Pharmacological studies revealed antidiarrhoeal effect and its essential oils have antibacterial properties. There are also another pharmacological importance such as antiparasitic, anti-inflammatory, anticoagulant, myorelaxant, haemostatic, analgesic, antifungal and hypothermic properties (Lans 2007; Duke 2008).

*A. conyzoides* also has an insecticidal activity (in particular) against coleopters of flour (Durodola 1977; Sharma

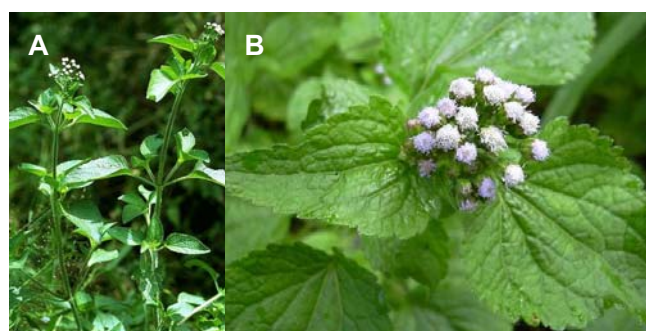


Fig. 1 *Ageratum conyzoides* plant (flowering stage). (A) Profile view. (B) Upper view.

*et al.* 1978; Aalbersberg and Singh 1991; Wiedenfeld and Roder 1991; Cambie and Ash 1994; Okunade 2002; Kamboj and Saluja 2008).

In traditional medicine, a decoction or infusion of *A. conyzoides* is used for the treatment of constipation, hepatitis, eczema, epilepsy, wounds, dizziness, diarrhoea, vomiting, fever, headaches, intestinal worms and filariasis (Burkill 1985; Okunade 2002; Mustafa *et al.* 2005; de Mendonça *et al.* 2006; Duke 2008). Fresh leaf aqueous extract is used in treating painful menstruation, itching of eye and against lice (Cambie and Ash 1994; Okunade 2002; Kamboj and Saluja 2008). Fresh leaves are chewed as an emetic, the leaves with the leaves of *Ocimum* and bush pepper are used as a cure for abdominal disorders (Ayensu 1978; Chah *et al.* 2006; Duke 2008). An ethnobotanical study reported that leaves or entire plant decoction is useful for the treatment of diabetes (Tsabang *et al.* 2001; Igoli *et al.* 2005; Soumyanath 2006). Our preliminary studies reported on the effect of the aqueous extract of *A. conyzoides* on hyperglycaemia in rats (Nyunai *et al.* 2006). The present research test the aqueous

crude extract of *A. conyzoides* and its different fractions on their antidiabetic activity.

## MATERIALS AND METHODS

*A. conyzoides* plants in flowering stage were harvested from around Yaoundé, centre region of Cameroon. The species was identified in the national herbarium, Yaoundé by comparison with the specimen N°19050/SFR/Cam stored in the herbarium. Thereafter, the leaves were air dried at the room temperature and then ground. The leaves were selected because of its traditional uses for the treatment of diabetes (Igoli *et al.* 2005; Soumyanath 2006). The powder obtained was boiled in water for about 30 min. Once obtained, the concoction was filtered twice and the filtrate dried for 3 days in an oven at 55°C. The yield of this extraction process was 29 % w/w of the dried powder.

### Fractionation of the aqueous crude extract

Twenty grams of the concoction was chromatographed using silica gel column with eluting solvent as butanol: acetic acid: water (6: 2: 2). With the aid of thin layer chromatography two principal fractions (F1 and F2) were obtained with percentage yields of 95 and 5%, respectively. The 2 fractions were further tested for their antidiabetic activities on rats using the glucose tolerance test (D-glucose dosage was 3 g/kg) since the 2 fractions were active. The F1 fraction (more abundant) was further fractionated in a column. At this time methanol was first used as eluent and then water (H<sub>2</sub>O) using the thin layer chromatography and 3 subfractions (F1a, F1b, F1c) were obtained.

### Experimental animals

Wistar albino rats raised in the pharmacology-toxicology unit, IAV Hassan II, Rabat, Morocco were used. The animals were fed standard rodent chow and had water *ad libitum*. Male rats were used for each study and divided into several groups in a homogenous manner taking into consideration the age and weight of the animals (180-220 g).

### Oral glucose tolerance test

This was carried out on normal rats with normal blood glucose level according the method of Schoenfelder *et al.* (2006). The animals were fasted for 16 h prior to the study. The plant material to be tested included the aqueous crude extract (ACE), F1 and F2 fractions and the F1a, F1b and F1c subfractions.

The different fractions were administered orally at a dosage determined in a manner equal to 200 mg/kg (most effective dose for aqueous crude extract) taking into consideration the extraction yield of each fractionated subfraction. A hypoglycaemic reference group, glibenclamide was administered to a group of rats as the positive control. Then eight groups with five animals each were constituted and the animals received a dose of 3 g/kg of glucose by oral route 30 min after having been treated with the extract or the different fractions of the plants as follows. Group I: distilled water (DW) (10 ml/kg, negative control), Group II: aqueous crude extract (200 mg/kg), Group III: F1 (190 mg/kg), Group IV: F2 (10 mg/kg), Group V: F1a (16 mg/kg), Group VI: F1b (16 mg/kg), Group VII: F1c (88 mg/kg) and Group VIII: glibenclamide (10 mg/kg).

Blood was collected from the animals before administration of the extract, or the different fractions and subfractions and then thereafter.

### Antihyperglycaemic effect of plant extracts on diabetic rats

Experimental diabetes was induced on rats by applying the protocol of Szkudelski (2001) and Schoenfelder (2005). This was obtained following an intravenous injection of a solution of streptozotocin (STZ) (60 mg/kg body weight) freshly prepared in an acidified saline solution. Non diabetic animals were kept as the control and they received acidified saline solution in place of STZ. After 72 hours when the diabetic state must have stabilised the

animals with blood glucose above 200 mg/dL were retained for the study (Nyunaï *et al.* 2006). The animals were divided into six groups with five rats each: a control group (Group A) and five groups of diabetic animals B, C, D, E and F). Group A rats received DW, those of group B, C, D, E, and F were treated with crude plant extract, subfractions of fraction 1 and glibenclamide (10 mg/kg). The doses of the different plant extracts administered were same as those used for the glucose tolerance test.

Blood sample was collected before the commencement of treatment with plant extracts then after.

### Blood collection and determination of blood glucose

The blood was collected from the tail after making a slight cut. Drop of blood was squeezed out and served for the determination of the blood glucose with the aid of a glucometer (Glucotrend®2, an Accucheck system of Roche Diagnostics, D-68298 GmbH Mannheim, Germany). The percentage of change in this parameter was calculated by applying the following formula proposed by Jiménez *et al.* (1986):

$$\% \text{ change of glucose} = (G_x - G_i / G_i) \times 100$$

where  $G_x$  is the glycaemia at time x and  $G_i$  is the glycaemia at initial time.

### Statistical analysis

All the values in the test are presented as mean  $\pm$  SEM (Standard Error of the Mean). Statistical differences between the means of the various groups were evaluated by one-way analysis of variance (ANOVA) using the SPSS program followed by Student's *t*-test.

## RESULTS

### Effect of different extracts of *A. conyzoides* on glucose tolerance

The means of blood glucose obtained from the different groups are presented in **Table 1**. It was observed that the crude aqueous crude extracts of *A. conyzoides* significantly reduced the increase in blood glucose induced 30 min after glucose administration when compared to the group that received distilled water.

The F1 fraction also presented an effective antihyperglycaemic importance. It did not only inhibit the hyperglycaemia induced by glucose but also reduced the initial glycaemia which decreased from 109.75  $\pm$  5.5 mg/dL in initial time to 102  $\pm$  3.16 mg/dL after 2 h 30 min. This fraction induced hypoglycaemia as from the second hour after administration of glucose. The F2 fraction had the same effect as F1 but was more effective. With the exception of blood samples obtained at an hour after glucose administration, all the other samples collected showed a decrease of the blood glucose with significant results after 2 h. After 2 h 30 min of glucose administration was observed a reduction of 36.7% in the blood glucose.

Though all the subfractions (F1a, F1b, and F1c) were active against increase in blood glucose, it was the F1c subfraction that had a significant effect. At first this subfraction prevented increase in blood glucose observed in the control, then continue to decrease and finally render the animals hypoglycaemic from the second hour after the beginning of the experiment with a reduction of blood glucose of approximately 17.5% ( $p \leq 0.05$ ).

Finally the glibenclamide reference hypoglycaemic drug used as a positive control was also very active by inducing a continuous reduction of blood glucose from 94.38 mg/dL ( $p \leq 0.05$ ) 30 min after glucose administration to 71.81  $\pm$  20.0 mg/dL ( $p \leq 0.005$ ) at the end of the experiment.

**Table 1** Blood glucose concentration (mean  $\pm$  SD) of the rats after receiving extract, fraction and subfractions of *Ageratum conyzoides* after glucose.

Group (n=5)	Glycaemia (mg/dL) at different times after treatment					
	0	30 min	60 min	90 min	120 min	150 min
DW	90 $\pm$ 9.44	88.39 $\pm$ 8.37 (-1.8%)	170.1 $\pm$ 19.77** (+89%)	157.8 $\pm$ 19.97** (+75.3%)	133.66 $\pm$ 12.76** (+48.5%)	110.7 $\pm$ 8.62 (+23%)
ACE	88.4 $\pm$ 5.60	88.2 $\pm$ 2.28 (-0.2%)	139.38 $\pm$ 21.18 (+57.7%)	123 $\pm$ 9.53** (+39.1%)	112.06 $\pm$ 8.44 (+26.8%)	96.9 $\pm$ 14.77 (+9.6%)
F1	109.75 $\pm$ 5.5	108.25 $\pm$ 6.29 (-1.4%)	115 $\pm$ 18.98 (+4.8%)	109.5 $\pm$ 19.05 (-0.2%)	106.75 $\pm$ 6.8 (-2.7%)	102 $\pm$ 3.16* (-7%)
F2	102.25 $\pm$ 2.99	92.5 $\pm$ 16.74 (-9.5%)	116.25 $\pm$ 12.04 (+13.7%)	94 $\pm$ 7.96 (-8%)	83.75 $\pm$ 8.66* (-18.1%)	64.75 $\pm$ 11.18** (-36.7%)
F1a	96.5 $\pm$ 5.07	96.75 $\pm$ 6.80 (+0.3%)	106.25 $\pm$ 14.97 (+10%)	93.5 $\pm$ 12.15 (-3.1%)	102 $\pm$ 13.54 (+5.7%)	88.75 $\pm$ 13.33 (-8%)
F1b	98.33 $\pm$ 5.80	105 $\pm$ 3.20 (+6.8%)	118.33 $\pm$ 2.45* (+20.3%)	113.33 $\pm$ 8.46* (+15.3%)	107.33 $\pm$ 6.95 (+9.2%)	87.33 $\pm$ 16.07 (-11.2%)
F1c	93 $\pm$ 2.16	91.5 $\pm$ 7.59 (-1.6%)	96.5 $\pm$ 11.90 (+3.8%)	98.25 $\pm$ 5.74 (+5.6%)	77.25 $\pm$ 8.22* (-16.9%)	76.75 $\pm$ 10.34* (-17.5%)
Glib	90.1 $\pm$ 7.10	67.58 $\pm$ 17.7** (-25%)	94.38 $\pm$ 12* (+4.8%)	85.32 $\pm$ 21** (-5.3%)	72.98 $\pm$ 23** (-19%)	71.81 $\pm$ 20.1** (-20.3%)

\* significant at  $p < 0.05$  is compared to initial glucose concentration of rats respectively of each group

\*\* significant at  $p < 0.005$  is compared to initial glucose concentration of rats respectively of each group

The values in parenthesis represent the percentage of reduction of the blood glucose in comparison with the initial value.

DW, distilled water; ACE, aqueous crude extract; F1, fraction 1 of ACE; F2, fraction 2 of ACE; F1a, subfraction a of fraction 1; F1b, subfraction b of fraction 1; F1c, subfraction c of fraction 1; Glib, glibenclamide; n, number of rats per group.

**Table 2** Blood glucose concentration (mean  $\pm$  sd) after the administration of *Ageratum conyzoides* L. (extract and its sub fractions) to the streptozotocin-induced diabetic rats.

Group (n=5)	Glycaemia (mg/dL) at different times after treatment				
	0 h	1 h 30 min	3 h	5 h	8 h
DW	372 $\pm$ 14.53	342.02 $\pm$ 24.11 (-8.05%)	361 $\pm$ 8.57 (-3%)	362.40 $\pm$ 9.86 (-2.6%)	363.44 $\pm$ 20.46 (-2.3%)
ACE	370 $\pm$ 13.55	348.73 $\pm$ 11.19** (-5.75%)	314.28 $\pm$ 22.80** (-15%)	312.43 $\pm$ 34.93* (-15.6%)	296 $\pm$ 34.64** (-20%)
F1a	341.5 $\pm$ 32.91	265.5 $\pm$ 71.01* (-22%)	302.5 $\pm$ 28.30 (-11.3%)	270.5 $\pm$ 34.06* (-20.5%)	261.5 $\pm$ 21.36** (-23.15%)
F1b	367 $\pm$ 31.18	411 $\pm$ 9.24* (+12%)	320 $\pm$ 1.15* (-12.81%)	290 $\pm$ 47.34* (-21%)	281 $\pm$ 34.64** (-23.43%)
F1c	275.25 $\pm$ 57.82	284 $\pm$ 45.03 (+3.2%)	271 $\pm$ 29.38 (-1.5%)	269.25 $\pm$ 13.94 (-2.2%)	268.75 $\pm$ 36.47 (-2.4%)
Glib	336 $\pm$ 30	356 $\pm$ 34.38* (+5.9%)	336 $\pm$ 20.06 (0%)	347 $\pm$ 35.32 (+3.3%)	284 $\pm$ 29.44** (-15.5%)

\* significant at  $p < 0.1$  is compared to initial glucose concentration of rats respectively of each group

\*\* significant at  $p < 0.01$  is compared to initial glucose concentration of rats respectively of each group

The values in parenthesis represent the percentage of reduction of the blood glucose in comparison with the initial value.

DW, distilled water; ACE, aqueous crude extract; F1a, subfraction a of fraction 1; F1b, subfraction b of fraction 1; F1c, subfraction c of fraction 1; Glib, glibenclamide; n, number of rats per group.

### Effect of different extracts of *A. conyzoides* on the blood glucose of diabetic rats

The results of this test are presented in **Table 2**. In spite of the changes on blood glucose based on the initial blood glucose (-8.05 to -2.30%) distilled water did not have any effect on the blood glucose of diabetics rats. On the contrary, the aqueous crude extract showed an important efficacy throughout the experiment by reducing the hyperglycaemia of -5.75% in the blood obtained at 1 h 30 min ( $p \leq 0.01$ ) to -20% in 8 h ( $p \leq 0.01$ ). In the same way, the F1a fraction was very effective in reducing the hyperglycaemia from -22% at 1 h 30 min ( $p \leq 0.1$ ) to -23.15% at 8 h ( $p \leq 0.01$ ).

With exception of blood sample collected at 1 h 30 min which remained hyperglycaemic throughout, the F1b reduced the blood glucose in the same way as the F1a: -12.81% in 3 h, -21% in 5 h and -23.43% in 8 h ( $p \leq 0.01$ ).

The effect of the F1c subfraction on blood glucose was comparable to that obtained in the control rats that received distilled water. The antihyperglycaemic activity of glibenclamide was moderate (-15.5%,  $p \leq 0.001$ ) with respect to the effect of aqueous crude extract (ACE) and the subfractions F1a and F1b.

### DISCUSSION

The objective of this study was in part to confirm the antihyperglycaemic effect of *A. conyzoides* and further deter-

mine the active fraction through bio guided fractionation. This allows in place the putting of the different steps of purification and identification of some active components of this plant.

The results of this study have clearly shown that *A. conyzoides* has an important hypoglycaemic activity, to this effect, thus, justifies its utilization for the treatment of diabetes in Cameroon, Nigeria and Reunion (Lavergne and Véra 1989; Tsbang *et al.* 2001; Igoli *et al.* 2005; Soumyanath 2006).

The glucose tolerance test showed that the F2 fraction was the most active. The fractionation into subfractions was carried out on fraction F1, also active and contained more extract (yield 95%). As opposed to distilled water (control group) and crude aqueous extract; this test equally showed that all the subfractions prevented increase in blood sugar, with a very pronounced effect of subfraction F1c. Glibenclamide showed an antihyperglycemic activity throughout which was generally similar to that of F1c.

The tests on the diabetic rats show that the aqueous crude extract and its subfractions F1a and F1b had an opposing effect on experimental hyperglycaemia while the subfraction F1c and the glibenclamide had moderate effect.

Taking into consideration its mechanism of action which is the stimulation of insulin liberation, glibenclamide is only effective in moderate diabetic condition and has little or no effect in a severe diabetic condition where the  $\beta$ -cells of the pancreas are destroyed (Ivorra *et al.* 1989; Suba

et al. 2004; Sokeng et al. 2005; Godwin et al. 2009).

Subfraction F1a, F1b and F1c have different thin layer chromatography profile, but all the fractions have manifested a certain antihyperglycaemic activity, though they are different based on the tests which suggested that the plant contains more active antidiabetic components with different chemical properties and mechanisms of action. We can thus admit that the subfraction F1a and F1b contain identical active substance but different from that of F1c. This hypothesis is supported by the fact that F1c is only active on the glucose tolerance test; meanwhile the F1a and F1b are very active in the hyperglycaemia induced by streptozotocin. In the two tests the F1c had a similar activity as the glibenclamide. This supports the assumption that this fraction acts in the same way resulting in hypoglycaemic by stimulating the liberation of insulin. Subfractions F1a and F1b, react complementing the pancreas; their effect can be attributed to the extra pancreatic mechanisms that influence the metabolism of glucose just like the stimulation of the uptake of the blood sugar by the peripheral tissues, inhibition of the production of endogenous glucose and/or activation of the glycolytic route by the stimulation of the activity of the glycogen synthetase (Burcelin et al. 1995; Mukherjee et al. 2006).

The phytochemical studies carried out on *A. conyzoides* showed that this plant contains many bioactive components and these include: flavonoids, alkaloids, coumarin, glucosides, chromenes, terpenoids and tannins (Okunade 2002). As reported by Oliver (1980), Mukherjee et al. (2006) glucosides, flavonoids, tannins, organic sulphurs components and alkaloids constituted the active metabolic part of hypoglycaemic plants; on the basis of Marles and Farnsworth (1995) and Yu et al. (2003) hypothesis which indicated that plants containing terpenoids and/or coumarins possess a hypoglycaemic activity, the hypoglycaemic effect of *A. conyzoides* would be in part due to the presence of the substances already isolated from this plant. These natural components can act separately or synergically to cause the global hypoglycaemic effect.

The study of the aqueous crude extract of the leaves of *A. conyzoides* and the subfractions indicated that *A. conyzoides* possess an important hypoglycaemic potential in streptozotocin-induced diabetic rats as well as in glucose loaded hyperglycaemic rats. The results of this study suggest that this plant may contain more than one antihyperglycemic component. It is then important for future research to identify these antihyperglycemic components and to understand their mechanisms of action.

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