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Antioxidant and anti-hyperglycaemic activity of *Euphorbia hirta* L. on Wistar rats

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ABSTRACT

The objective of this study was to investigate the antioxidant capacity and anti-hyperglycemic activity of aqueous macerate extracts of *Euphorbia hirta* L., in the treatment of hypertension associated with diabetes. The research focused on the aqueous maceration of the aerial part or totum. The search and evaluation of the antioxidant activity was done according to the method of Blois based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The search for antihyperglycaemic activity was performed on rats having received a glucose overdose. This activity was compared to those of glyburide and metformin. The totum showed a percentage inhibition of 63.66 ± 0.54% for 1 mg/mL of extract and an IC_{50} of 0.031 ± 0.001 µg/mL .A 200 mg/kg extract has shown a very good antihyperglycaemic activity; while on normal blood sugar, the activity was not important compared with glyburide and metformin. These results show the presence of antioxidant and normoglycaemic substances in aqueous macerated of *Euphorbia hirta* L.

Keywords: Anti-hyperglycaemic, antioxidant, *Euphorbia hirta*, glyburide, metformin.

INTRODUCTION

The use of medicinal plants for the treatment of metabolic diseases is gaining ground more and more in the world (Mamun-Or-Rashid et al., 2013). Diabetes and hypertension are frequently two associated pathologies in patients (Dembele et al., 2000). This association is a serious economic problem, lowers patient comfort and reduces the vital prognosis of the patient.

The World Health Organization has recommended and encouraged research and use of medicinal plants especially in countries where access to modern medicine and conventional treatment is difficult. Also, these drugs of modern medicine may have significant side effects. Thus, the search for medicinal plants, effective on diabetes as well as on hypertension, has been undertaken (N'Guessan, 2008; Tra Bi et al., 2008). Among others, they are: *Allium sativum* L.

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(Liliaceae), *Cassia occidentalis* L. (Caesalpiniaceae) *Chrysophyllum cainito* L. (Sapotaceae), *Clerodendrum inerme* (L.) Gaertn. (Verbenaceae), *Crescentia cujete* L. (Bignoniaceae), *Euphorbia hirta* (L) (Euphorbiaceae), L. *Jatropha curcas* (Euphorbiaceae) *Jatropha gossypiifolia* L. (Euphorbiaceae) *Mangifera indica* L. (Anacardiaceae) (N'Guessan, 2008; Tra Bi et al., 2008). *Euphorbia hirta* L. has been selected because of its anti-hypertensive activity already proven (Huang et al., 2012; Johnson et al., 1999). It is an annual plant, abundant on the waves along the roads and open grassland soils, found in India and tropical Australia and widespread in tropical and subtropical countries (Adjanohoun et al., 1986). Traditionally, this plant species is used in the treatment of gastrointestinal disorders, bronchial and respiratory diseases, kidney stones, diabetes, conjunctivitis (N'Guessan, 2008; Tra Bi et al., 2008). Many properties of the plant have already been demonstrated, among others, antipyretic, analgesic, bactericidal activity (Lanhers et al., 2005; Mamun-Or-Rashid, 2013).

This study aims at evaluating the antioxidant capacity and the anti-hyperglycemic activity of aqueous macerate extracts in the treatment of hypertension associated with diabetes.

MATERIALS AND METHODS

Chemicals and equipment

Glyburide and metformin were used as reference substances, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used for research and evaluation of antioxidant compounds. Blood sugar was measured using a glucometer (Accu-Chek® Active, Roche, Germany).

Animal material

Healthy albino mice (male and female) weighing 30-60 ages between 8 and 10 weeks and adult rats of both sexes, of the race Wistar aged from 4 to 12 weeks. The animals were acclimated for 2 weeks before experimentation. All animals were maintained under standard environmental conditions. The room temperature was 22±2 °C and relative humidity was 45-55%. Each cycle of light and darkness lasted 12 hours. The animals had access to feeds *ad libitum* to tap water and adapted feeds for rodents that is pellets.

Plant material

It consists of the aerial part (stem and leaves) of *Euphorbia hirta* L., collected in February 2012 in Adzopé, a locality in the Agnéby region north of Abidjan (Côte d'Ivoire). The identification was made in the Department of Botany at the University of Felix Houphouët Boigny, Abidjan. A specimen is deposited in the Herbarium of the National Centre of Floristic in the University under N° 01. FY February 2012. Plants harvested were washed with water and then dried in the shade on trays at room temperature.

Preparation of extracts

The medicinal plant was spray dried coarsely. 100 g were soaked in 1000 mL of distilled water for 24 hours without agitation. The filtrate, corresponding to the mode of use in the traditional medicine, was used at different doses for research of anti-hyperglycaemic activity. Two aliquots were taken. One was concentrated with a rotary evaporator and then lyophilized. The other with different solvents of increasing polarity successively (petroleum ether (EP), chloroform (C), ethyl acetate (AE) and butanol (B)). All extracts were dried, and used for chromatographic study and research of antioxidant activity.

Research and evaluation of the anti-oxidant activity by DPPH

The ability of the extract to scavenge DPPH radical was first sought by TLC as described for the first time by Blois (1958) using the DPPH (Takao et al., 1994). Then, this ability was assessed using the method of Velazquez et al. (2003). 200 µL of a freshly prepared methanolic solution of DPPH (20 mg L^{-1}) was mixed with 100 µl of extract solution (7 dilutions to ½ from 1 mg mL^{-1}). After 15 min of incubation in the dark at room temperature, absorbance was read at 517 nm against a negative control (100 µl of methanol and 200 µl of DPPH) and a blank sample (100 µl of extract and 200 µI of DPPH). All the determinations were performed in triplicate. DPPH radical inhibition percentage was calculated according to the formula of Miliauskas et al. (2004).

$$\text{Inhibition (\%)} = [(A_B - A_A) / A_B] * 100$$

Where A_B is the blank absorbance and A_A, the sample absorbance (test extract solution), IC_{50} value (50% inhibitory concentration of DPPH radical) was obtained by graphic determination. A Lower IC_{50} value indicates greater antioxidant activity.

Evaluation of the hypoglycemic and anti-hyperglycemic activity

The evaluation of the totum needs 66 adult rats of both sexes weighing between 100 and 200 grams. They were fasted for 12 hours before testing begins with free access to water. The rats were divided into 11 groups of 6, homogeneous weights. The Batches were made as follows:

Batch 1: normoglycaemic rats treated with water;
Batch 2: rats made hyperglycaemic treated with water;
Batch 3: rats made hyperglycaemic treated with Glyburide 5 mg/kg;
Batch 4: rats made hyperglycaemic treated with metformin 500 mg/kg;
Batch 5: rats made hyperglycaemic treated with the extract extemporaneous to 1000 mg/kg;
Batch 6: rats made hyperglycaemic treated with dry extract 100 mg/kg;
Batch 7: rats made hyperglycaemic treated with dry extract 200 mg/kg;
Batch 8: normoglycaemic rats treated with the extract extemporaneous 1 g/kg;
Batch 9: normoglycaemic rats treated with dry extract 100 mg/kg;
Batch 10: normoglycaemic rats treated with dry extract 200 mg/kg;
Batch 11: normoglycaemic rats treated with dry extract 1000 mg/kg.

Testing for anti-hyperglycaemic activity

An overload of glucose at 3g/kg of body weight was administered to rats in fasting state for 18 hours to cause hyperglycaemia (Kwashié et al., 1998; Gharras et al., 1999; Lukens et al., 1948). The rats that have hyperglycaemia (HG) greater than or equal to 0.5 g L^{-1} compared to the basic glucose level were selected. Immediately after, batches 2 to 7 were treated by gavage with water extract or reference antidiabetic. The administration volume is 10 mL kg^{-1} weight. Glucose (Gt) level of each animal was measured every 30 minutes (t) for 90 minutes from a drop of blood from the tail vein applied to an Accu-Chek® Active. Anti-hyperglycaemic activity was expressed as percentage of inhibition (Inh) of previously induced hyperglycemia compared to hyperglycemic control sample group (HG) and by following the formula:

Inh (%) = (HG-Gt) * 100) / HG.

Testing for hypoglycaemic activity

The rats in this study did not receive glucose overdose. After fasting, they received different doses of extract by gavage. Glucose

(Gt) was measured under the same conditions as above. The hypoglycaemic activity was expressed as a percentage decrease (D) of basic blood sugar (GB) over time compared to normoglycaemic control group according to the following formula:

D = (GB-Gt) * 100 / GB.

Statistical analysis

The data obtained for the study of anti-hyperglycemic and hypoglycemic activities are expressed as mean ± standard deviation. The comparison between different groups was made by analysis of variance (ANOVA), and the Student t-test was used to separate the means. A p value < 0.01 is considered highly significant and a p value < 0.05 is significant.

RESULTS

Research and evaluation of the antioxidant constituents

The yellow spots on purple background revealed by DPPH were abundant (Figure 1). The totum was well separated by the developing petroleum ether: ethyl acetate (1:2) and isolating pure compounds. Chromatogram 2 shows the presence of abundant antioxidants polar compounds, dragged along the length of migration. A small discontinuity is observed. This is due to the presence of the compound not having an antioxidant activity. The chromatogram 4 shows three spots of the ethyl acetate fraction of which is one dominant.

The aqueous maceration of the aerial part (stem and leaf) of *Euphorbia hirta* L. showed an ability to scavenge DPPH of 63.66 ± 0.54% at a concentration of 1 mg mL^{-1}. The evaluation of the antioxidant activity gave a mean inhibitory concentration IC_{50} = 0.031 ± 0.001 µg mL^{-1} (Figures 2-4).

Effect on hyperglycemia caused by oral glucose

Inhibition of hyperglycemia was significant ($p < 0.05$) at dose of 100 and 1000 mg/kg body weight and highly significant at 200 mg/kg ($p < 0.01$).

Comparing the inhibition dose at 100 to that of the 1000 mg/kg, the difference was highly significant at 30 min (-16.48% and -14.63%) and 60 min (-46.44% and -28.37%) ($p < 0.01$), but after 1 hr 30, inhibition of hyperglycemia was attenuated ($p < 0.05$) (-53.77% and -31.94%). This very significant difference was also observed between doses of 200 and 1000 mg/kg bw on the entire duration of the experiment (-28.46% -49.60% and -53.17% by contributing to -14 63% -28.37% -31.94%).

However, between doses of 100 and 200 mg/kg a significant difference was observed at 30 min after administration of totum. Thereafter, there was no significant difference between the effects exerted by the two doses.

The intensity of the decrease in blood glucose induced by the extract at 200 mg / kg was comparable to that induced by glyburide and metformin (Figure 5) without causing hypoglycaemia. Indeed, glucose level of the batch treated with the extract at 200 mg kg^{-1} is around the value of the basic glucose level and seems to evolve between 60 and 90 minutes (Figure 5) while glyburide and metformin exercise hypoglycemic effect.

Effect on normal blood sugar

On the basis of normal sugar level, extracts have not led to a significant decline. All changes remained around the basic sugar level. The percentage decreases were less than 10%. Indeed, they were for the samples at 60 and 90 min, 1% and -6% for the 100 mg/kg -6% and -8% for the dose of 200 mg/kg and -2%and -7% for the dose of 1000 mg kg^{-1} (Figure 6).

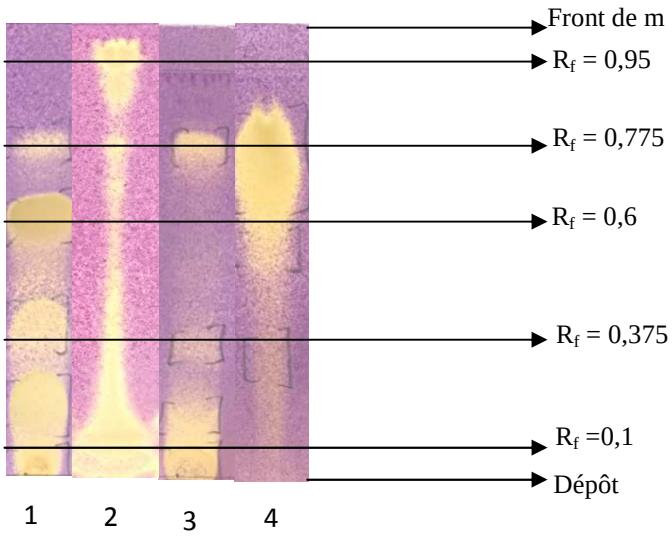

Figure 1: Chromatograms of the antioxidant activity of *Euphorbia hirta*.

Legend of Figure 1 :

Chromatograms	**1**	**2**	**3**	**4**
Extracts		Totum		F AE
Developers	EP-AE (1-2)	AE-MEC-AF-Water (5-3-1-1)		BAW (65-15-25)
Revelator		DPPH		

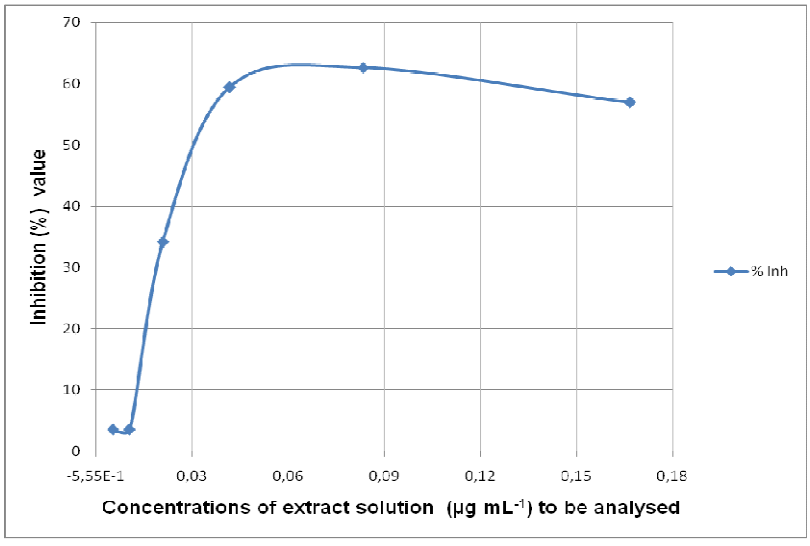

Figure 2: Inhibition curve of DPPH test 1 totum of *Euphorbia hirta*.

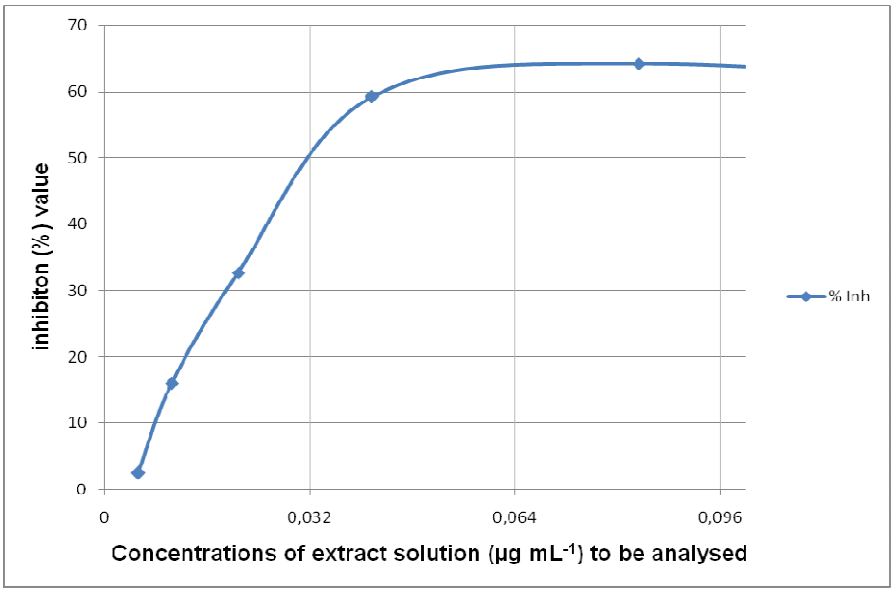

Figure 3: Inhibition curve of DPPH test 2 totum of *Euphorbia hirta.*

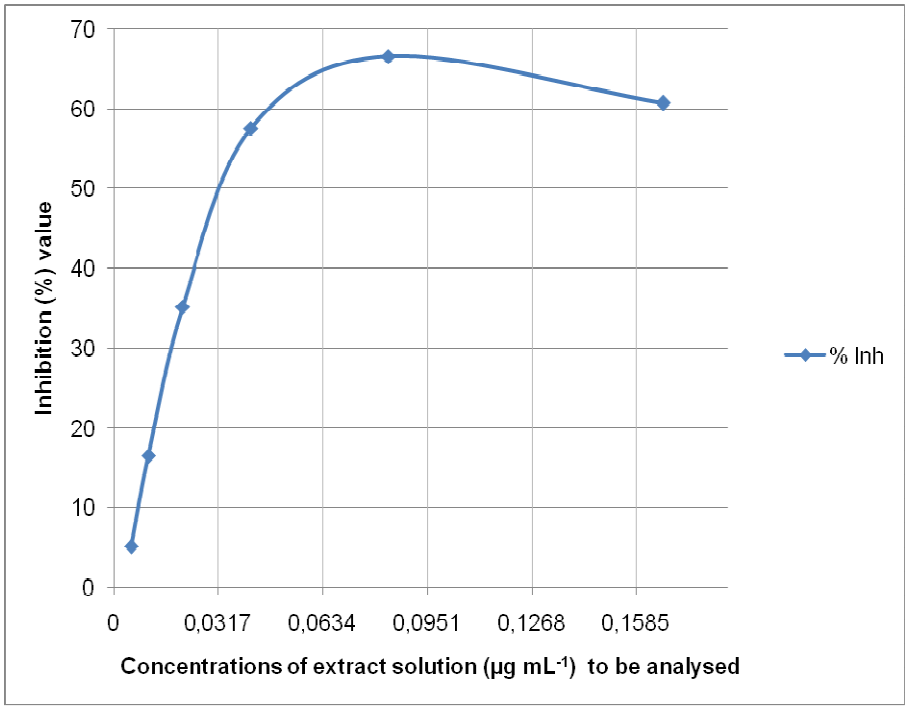

Figure 4: Inhibition curve of DPPH test 3 totum of *Euphorbia hirta.*

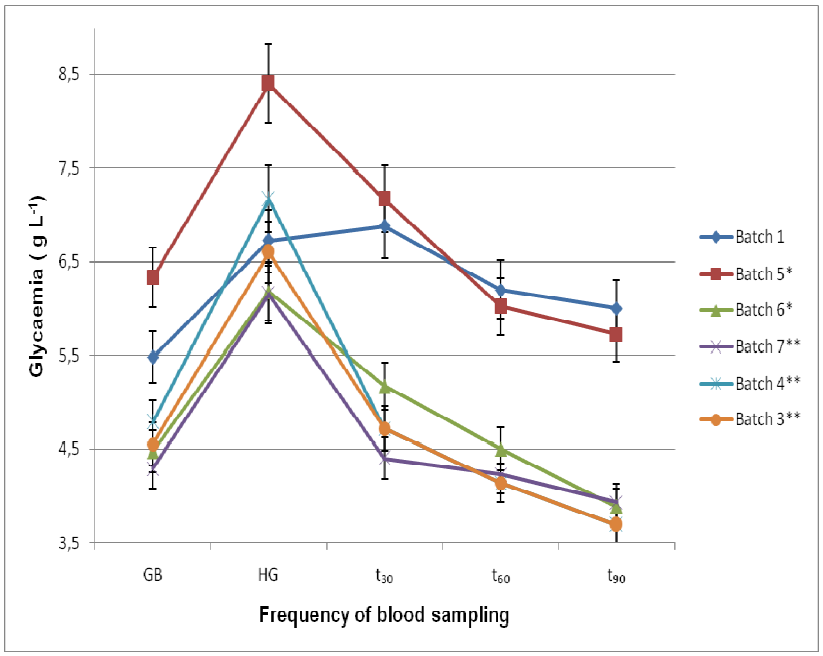

Figure 5: Glycaemia variation curve for hyperglycaemic rats treated with herbal medicine at 100 mg kg^{-1}, 200 mg kg^{-1} 1000 mg kg^{-1}, Metformin 500mg kg^{-1} and Glyburide 5mg kg^{-1}.
Mean ± SEM, n =6, p* < 0.05; p**<0,01.
Batch 1: hyperglycaemic rats treated with water; Batch 3: hyperglycaemic rats treated with Glyburide 5 mg/kg;
Batch 4: hyperglycaemic rats treated with metformin 500 mg/kg; Batch 5: hyperglycaemic rats treated with extract extemporaneous at 1000 mg/kg; Batch 6: hyperglycaemic rats treated with dry extract 100 mg/kg; Batch 7: hyperglycaemic rats treated with dry extract 200 mg/kg;

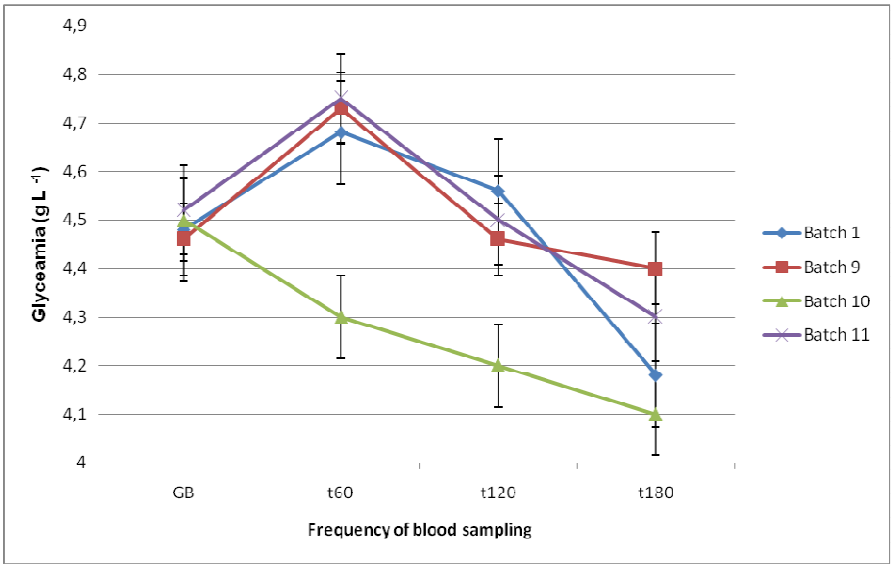

Figure 6: Glycaemia variation curve for normal glycaemic rats treated with herbal medicine at 100 mg kg^{-1}, 200 mg kg^{-1} and 1000 mg kg^{-1}.
Batch 1: normoglycaemic rats treated with water; Batch 9: normoglycaemic rats treated with dry extract 100 mg kg^{-1};
Batch 10: normoglycaemic rats treated with dry extract 200 mg kg^{-1}; Batch 10: normoglycaemic rats treated with dry extract 1000 mg kg^{-1}.

DISCUSSION

This study showed the effect of aqueous macerated *Euphorbia hirta* L. collected in Côte d'Ivoire on induced hyperglycemia and normal blood sugar of non-diabetic Wistar rats. Diabetes was treated with different herbs over a long period. The extracts of these plants have improved diabetes control and reduced side effects associated with drugs or synthetic hemi-synthesis (Bolkent et al., 2000; Aybar et al., 2001). Therefore, the search for more effective medicinal plants has become an important issue. The antioxidant compounds are known for their biological activities including multidirectional anti-diabetic (Brahmachari, 2008; Brahmachari et al., 2009) efficiency. Thus, a study of the aqueous extract of *E. hirta* L. on oral glucose tolerance in rats has shown a good reduction without causing hypoglycemia. Our results are in agreement with that of Kumar et al. (2010) who also found a highly significant anti-diabetic activity at doses of 250 and 500 mg/ kg body weight in rats made diabetic. This activity could have plenty of supporting antioxidant compounds present in the aqueous totum (Singh et al., 2013). Reduction in its antioxidant capacity was identified by thin-layer chromatography with a yellow color on a purple background (Onawumi et al., 2012). The antioxidant activity may be related to the presence of polar compounds with polyphenols (N'Guessan et al., 2011). The presence in the totum was remarkable compared to the ethyl acetate extract. However, the ethyl acetate extract is a majority antioxidant compounds with better reducing capacity (Tibiri et al., 2010). The anti-oxidant activity would be increased by less polar and non-polar substances (Jagadeesan et al., 2011; Tiwari et al., 2010). Measuring the absorbance of DPPH at 517 nm in the presence of aqueous extract of *E. hirta* L. showed a greater reduction of DPPH due to the important work done by trapping the extracts (Bakasso et al., 2008). Inhibiting concentration IC_{50}, which is the amount of compound required to decrease antioxidant initial DPPH concentration of 50% was determined graphically. The importance of antioxidant activity results in a low value of the IC_{50}. The aqueous extract of *E hirta* shows a low value of the IC_{50}. This activity confirms that of Basma et al. (2011). All this time, IC_{50} obtained with the aqueous extract is lower compared to the methanol extract of Basma et al. (2011). This difference could be due to the presence of compound extracted by water, such as mucilage, which precipitates in the presence of alcohol (Moussaid et al., 2012).

Conclusion

These results support the traditional use of the aqueous extract of *E. hirta* L. The totum may decrease hyperglycemia without risk of hypoglycemia. Thus, it can provide abundant antioxidant compound to treat or mitigate any disease related to a metabolic disorder which itself is linked to oxidative stress responsible for various metabolic diseases including hypertension with which it is often associated.

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