

Evaluation of Hypoglycaemic and Anti Hyperglycaemic Activity of the Aqueous Extract of the Roots of *Jatropha curcas* L. (Euphorbiacées)

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Abstract: The species *Jatropha curcas* of the family Euphorbiaceae, widespread in Central Africa, is a shrub 5 to 8 m high; widely used in traditional medicine. The interest of the present study is the evaluation of the hypoglycemic and antihyperglycemic activity of the aqueous extract of its roots and the formulation of capsules from the dry extract. After the harvest of *Jatropha curcas* in the region of the center Cameroon, roots underwent clearly a decoction in distilled water, maceration in ethanol 80 % and in pure methanol. Characterization of secondary metabolites families was performed by coloring and phytochemical precipitation tests. Subsequently, some of these metabolites were measured from standards. The animal material (male and female rats) was prepared for demonstration of hypoglycemic and antihyperglycaemic activity using as method the measurement of blood glucose every 30 minutes for 3 hours. The determination of the yields of the extracts showed a significant profitability, 8,14% for the aqueous extract. Phytochemical screening analysis and determination of phenolic compounds revealed that the aqueous extract had high levels of total phenols (24.037 mg Eq AG / g dried material), total flavonoids (9.493 mg Eq Q / g DM), flavanols (2,704 mg Eq R / g DM), flavones and flavonols (2,053 mg Eq Q / g DM), saponins (6,270%), tannins condensates (0,019 mg Eq C / g DM) and anthocyanins (2,625 mg / ml), relative to the methanolic extract and the ethanolic extract. In addition, the methanolic extract was found to be the most titrated in alkaloids (0.698%). Administration of the aqueous extract of root at a dosage of 500 mg/kg of body weight significantly decreased glycaemia ($p < 0.05$) compared to the group of rats which received distilled water at 10 ml/kg and showed a significant change in percentage of glucose reduction ($p = 0.003$), comparable to that of glibenclamide 10 mg / kg of body weight, the sulfonylurea hypoglycemic drug of reference. Our results show that the roots of *J. curcas* have an interesting hypoglycemic effect suggesting their use as extracts in hypoglycemic treatments.

Keywords: *Jatropha curcas*, extract, hypoglycaemic, antihyperglycaemic.

INTRODUCTION

The diabetes is one of the most endemic world chronic pathologies of the XXIth century. It is the chronic disease which arises when the body cannot produce enough insulin or cannot use it correctly and which is diagnosed by an hyperglycemia [1]. The chronic hyperglycemia, which characterizes the diabetes, is at the same time a sign and a factor aggravating disorders of the insulino-secretion and the insulino-resistance [2], and major and primary risk factor of the coronary diseases [3].

The diabetes is a problem of public health both in the industrialized countries and in the merged countries. According to the first world report of the World Health Organization (WHO) on the diabetes in 2017, 422 million people would live with the diabetes in the world. Prevalence of the diabetes almost doubled since 1980, passing from 4,7 % to 8,5 % in 2014 and this prevalence increased more quickly in countries with low or intermediate incomes. In Africa prevalence of the diabetes passed from 3,1 % to 7,1 % between 1980 and 2014 [3]. In Cameroon it is estimated between 6 % and 8 % in 2014 [3].

The type 2 diabetes is the most prevailing form and progressed with the sociocultural changes; we consider that 91 % of adults affected by the disease have type 2 diabetes. The diabetes and its complications are one of major causes of

mortality in most of the countries [3]. In 2012 about 3,7 million deaths were bound to the glycaemia [4]. To mitigate this problem, hygieno-dietary measures are involved, the injection of the insulin as well as the treatment by the oral antidiabetics, who are heavy of consequences because of their high cost and because of their unwanted effects? Regarding the treatment by the modern medicine which remains expensive and sometimes not accessible, and the various unwanted effects of the molecules of synthesis, a new interest is concerned the alternative or complementary medicines, in particular the treatments with medicinal plants. According to the WHO, 80 % of the African population resort to the traditional medicine for needs for primary health [5]. With the constant increase of the use of medicine with plants and the fast expansion of the world market, the safety and the quality of the plant material and the finished products with plants or improved traditional medicine became a major concern of the authorities of health, pharmaceutical industries and the public [6]. The development of medicine stemming from the traditional pharmacopoeia, in particular the improved traditional medicine, occupies an important place in research institutes in Africa [4].

In Cameroon, numerous research teams put a lot into the valuation of the local flora of therapeutic interest. Several plants are then used by the populations as possessing hypoglycaemic and antihyperglycaemic properties. Among these certain plants were the object of scientific works. It is the case of *Jatropha curcas* L, an Euphorbiaceae. Recipes of which made with roots are traditionally used in the treatment of several diseases such as the diabetes.

The present study aims at estimating the hypoglycaemic and antihyperglycaemic activity of the aqueous extract of the roots of *Jatropha curcas* L. to the rats of origin Wistar and to set up a medicine with extracts of plant complying with the specifications of improved traditional medicine [7].

The main objective of this work was to estimate the effect of the aqueous extract of the roots of *Jatropha curcas* on the fasting and post prandial blood glucose on rats. The specific objectives were: 1) to obtain the aqueous extract of the roots of *Jatropha curcas* and a yield on extraction; 2) to determine the great classes of compounds through a phytochemical screening and the quantitative analysis of the roots of *Jatropha curcas*; 3) to highlight the hypoglycaemic and antihyperglycaemic properties in vivo of the aqueous extract on the rats of origin Wistar.

MATERIALS AND METHODS

Material

Animal material

Rats (*Rattus norvegicus*) of origin Wistar. Old from 8 to 12 weeks.

Vegetal material

Roots of *Jatropha curcas* L. collected in the center region of Cameroon.

Methods

Extraction procedure

The roots of *Jatropha curcas* L. were sorted out, cleaned and dried at room temperature shielded from sunbeams. The well dried roots were crushed and sieved. The resultant powder was submitted to an aqueous extraction and to a methanolic extraction.

• Determination of the yield of aqueous extract

The yield on extraction (Rd) expressed in percentage was calculated by the formula below:

$$\text{Rd} = \frac{\text{Weight of the obtained extract}}{\text{Weight of initial powder}} \times 100$$

Phytochemical screening

It was realized according to the protocol of Bruneton [8].

The results are classified there:

- Very positive reaction: +++
- Reaction averagely positive: ++
- Doubtful Reaction: +
- Negative Reaction: -

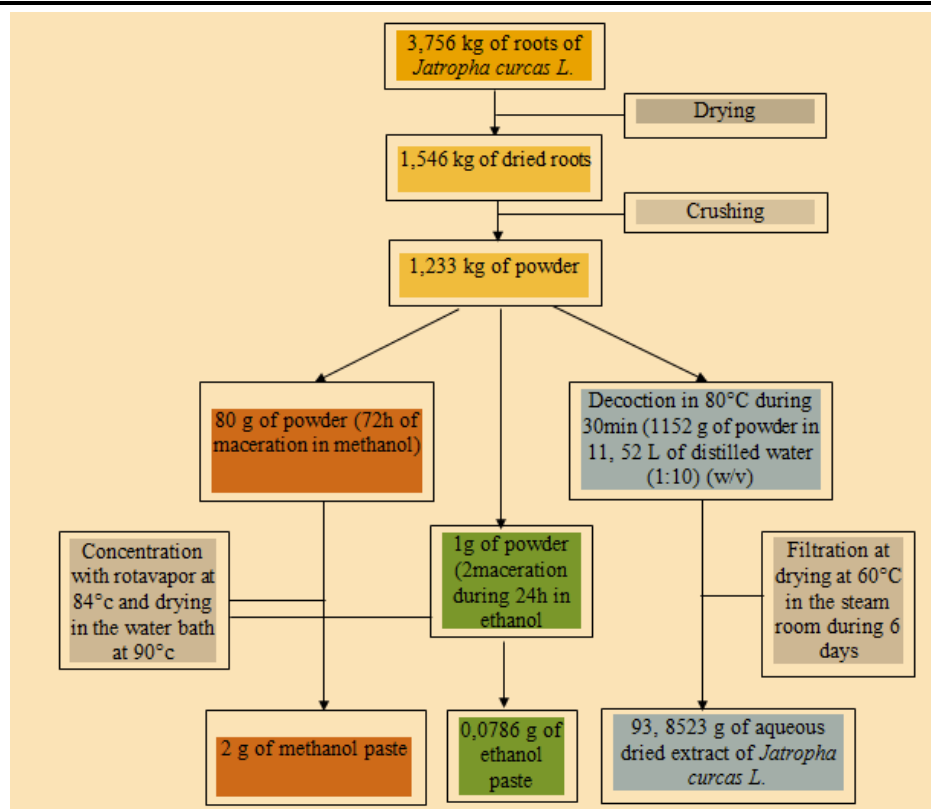


Fig-1: Procédure of extraction

The following tests were applied for the highlighting of the various components:

- highlighting of anthraquinones : Bornsträger test
- highlighting of saponins
- highlighting of alcaloïds : Mayer test
- highlighting of triterpenes and stérols : Libermann-Buchard test
- highlighting of flavonoïdes : Shinoda test
- highlighting of phenols
- highlighting of tanins

Quantitative analysis of extracts

Dosage of total phenols

The content in total phenols of the extracts was determined by an adapted method of Singleton and Ross with the reactive of Folin-Ciocalteu, whereas flavonoids are quantified by direct dosage by the aluminum trichloride [9, 10].

The concentrations of the total polyphenols contained in extracts are calculated by referring to the curve of calibration obtained by using the gallic acid as standard. The results are expressed mg equivalent in gallic acid / g dry material according to the following formula:

$$T = C \times \frac{V \times D}{P_s}$$

T: content in total phenol

C: concentration of polyphenols in equivalent in gallic acid by referring to the curve of calibration

V: volume of extract

D: factor of dilution

Ps: weight of the dried material

Dosage of total flavonoïdes

The method used for the estimation of rate of flavonoids is the one described by Ordonez *et al.* [11]. Interpretation: the concentrations of flavonoids contained in extracts were calculated by referring to the curve of calibration obtained by using the quercetin as standard. The results are expressed in mg equivalent in quercetin /g dry material.

Dosage of flavanols

The method used for the estimation of rate of flavanols is the one described by Kumaran and Karunakaran [12]. The concentration of flavanols contained in extracts was calculated by referring to the curve of calibration obtained by using the rutine as standard. The results are expressed in mg equivalent rutine / g dry material.

Dosage of flavones and flavonols

The method used for the estimation of rate of flavanols is the one described by Kosalec *et al.* [13]. The concentration of flavones and flavonols contained in extracts was calculated by referring to the curve of calibration obtained by using the quercetine as standard. The results are expressed in mg equivalent quercetine/ 100 g dry material.

Dosage of condensed tannins (proanthocyanidines)

The rate of proanthocyanidines in extracts was determined by using the Skerget *et al.* [14] method. The results were determined following the Beer-Lambert law, and the calculation was made with the following equation:

$$C = \left(\frac{A \times MW \times 1000}{\epsilon \times L} \right) \times 1000$$

MW: molecular weight of cyanidine (PM = 287,24 g/mol ; $\epsilon = 347001. \text{ mol}^{-1} \cdot \text{cm}^{-1}$; L=1cm).

The concentrations were expressed in μg equivalent cyanidine/ g dry material. ($\mu\text{g Eq C /g D.M}$)

Dosage of anthocyanins [15]

The concentration of anthocyanins can be approximately expressed by the following equation:

$$C \text{ (mg/ml)} = (\text{DOA-DOB}) \times 875$$

C: concentration of anthocyanin in mg/ml

DOA: optical density in cell A

DOB: optical density in cell B

875: Slope of the calibration line obtained with malvidine-3 glucoside.

Determination of the content in saponins

Procedure

The spectrophotometric method of Brunner [8] was used for the estimation of saponins in the plant sample. A portion (0,1 g) of crushed sample was weighed in a beaker of 25 ml and 10 ml of ethanol were added. The mixture was shaken in a vortex on a mechanical agitator during 5 hours to have a uniform mixture. Then, it was filtered through a filter paper Whatman N 1 in a beaker of 100 ml and 20 ml of a solution of magnesium carbonate (40 %) were added. The mixture obtained with the magnesium carbonate was again filtered to obtain a crystal clear and colorless solution. Then, 1 ml of the colorless solution were introduced in a 50-ml volumetric flask and 2 ml of solution of FeCl₃ (5 %) were added and completed to 50 ml with distilled water and let rest. The standard saponin (0-10 ppm) was prepared from a mother solution of saponins. The standard solutions were handled in a similar way with 2 ml of FeCl₃ (5 %). The absorbance of the sample as well as the solution of saponin standard were read after the development of the color on a spectrophotometer in a wavelength of 380 nm [16].

Interpretation

$$\% \text{ saponin} = \frac{\text{absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{weight of the sample}} \times 100$$

Determination of the content in alkaloids

Procedure

We let wallow during 24 hours 0,5 g of dry extract in 100 ml sulphuric acid (10 %), leaked out on paper and completed in 50ml with distilled water. We introduced 25ml of the filtrate into a separating funnel; then we added to 25 ml of NH₄ OH and 25 ml of CHCl₃. This mixture was shaken without emulsifying; then after settling the organic phase was taken away. We repeated this operation 3 times, and combined the organic phases. This solution was dried on anhydrous sodium sulfate, filtered and collected in a balloon beforehand weighed (T). Then it was concentrated until dryness in the rotavapor and the balloon was weighed with the residue, what gives (m): the obtained mass of alkaloids and the mass of the salt of ammonium formed (M) [17].

Interpretation

The percentage of alkaloids was calculated by the formula:

$$\% \text{ of alkaloids} = \frac{m - (T+M)}{PE} \times 100$$

avec PE = test sample

Evaluation of the biological activities of the aqueous extract of *Jatropha curcas* L

Evaluation of hypoglycaemic effect

Administration of the extract (dispatching of the batches)

To study the hypoglycaemic property of the aqueous extract of the roots of *Jatropha curcas* L we used 12 rats distributed at random in 4 batches of 3 animals each noted batch 1 to 4. Male rats and female in good health, chosen at random having 3 in 5 months of age were deprived of food, but no water 12 hours before the experience. After the fasting, every rat was weighed to determine the quantity of substance to administer to each. After the fast was administered glucose (2g/kg body weight (BW)). One hour and thirty times after, substances (the distilled water, the glibenclamide, the aqueous extract in the dose 250mg/kg and 500mg/kg) were orally administered with an oro-gastric tube following the distribution:

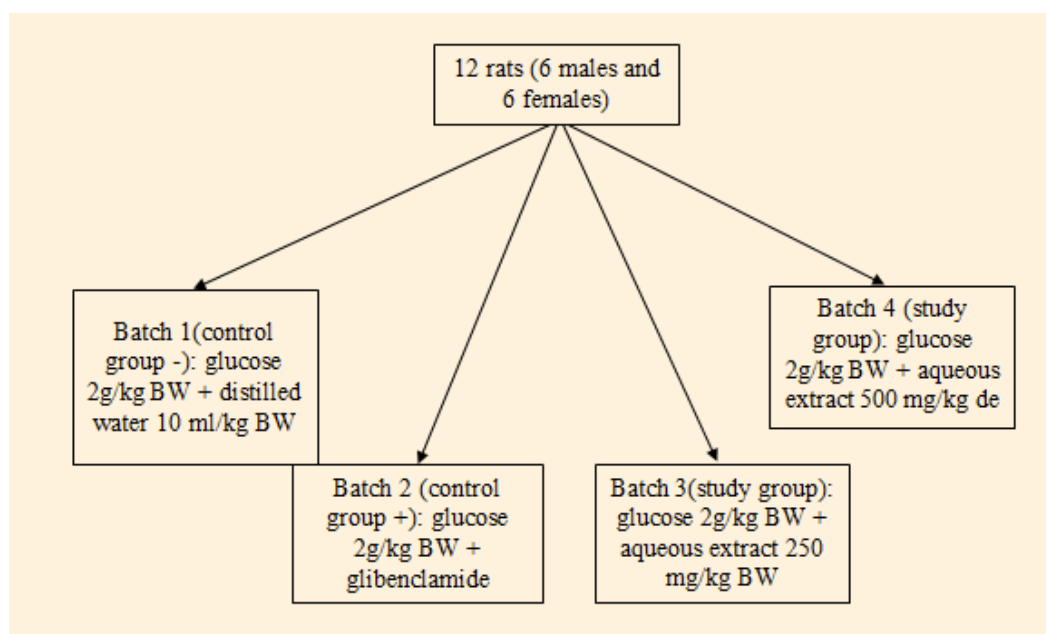


Fig-2: Evaluation of hypoglycaemic effect of *Jatropha curcas* L extracts: doses distribution

The glycaemia of all the rats was measured before the administration of the overload of the glucose to determine the basic glycaemia. These rats were then filled up by products to be tested and their glycemia was determined in 30th, 60th, 90th, 120th, 180th minutes by means of a glucometer. Six sampling of blood were collected by animal [18].

Evaluation of the antihyperglycaemic activity

Administration of the extract (dispatching of the batches)

To study the normoglycaemic property of the aqueous extract of the roots of *Jatropha curcas* L. on the hyperglycemia, their capacity to regulate an hyperglycemia caused by administration of glucose 2 g / kg body weight (BW) in solution in distilled water was estimated. The extract or the standard product were orally administered with an oro-gastric tube at the same time as overload of glucose, to better estimate the action of these on the hyperglycaemic activity of the overload of glucose by regulating its appearance:

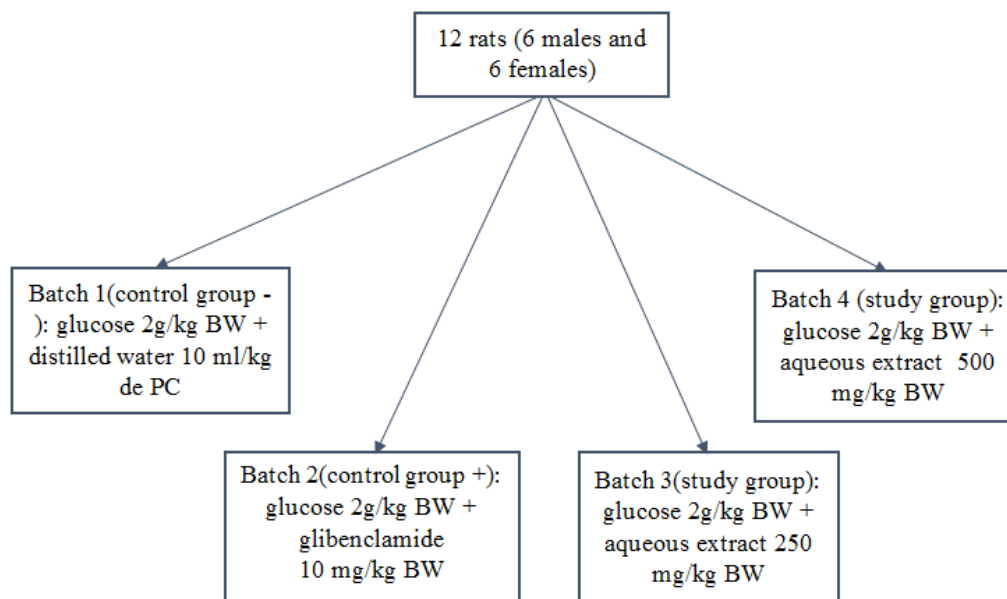


Fig-3: Evaluation of the antihyperglycaemic activity of *Jatropha curcas L* extracts: doses distribution

The glycaemia of rats was measured before the administration of the diverse products and in the 30th, 60th, 90th, 120th, 180th minutes after the administration of the diverse products (glucose, distilled water, glibenclamide and extract) [18].

RESULTS

II-1 Yield of extractions

According to the mass, the yield, the aspect and the color of extracts obtained from the powder of the roots of *Jatropha curcas L* are reported in the following table I:

Table-I: Results of yield, aspect and color of extracts of the roots of *Jatropha curcas L*

Extracts	Weight of powder(g)	Weight of extract (g)	Yield (%)	Color	Aspect
Aqueous	1152	93,85	8,14	Glossy brown	Crystal
Méthanolic	80	2	2,5	Yellow ochre	Sticky
Ethanolic	1	0,0786	7,84	Greyish	Pasty

We notice that the aqueous extract gave the higher yield with 8, 14 % followed by the ethanolic extract with 7,84 % while the methanolic extract presented the lowest yield with 2,5 %.

II-2 Results of the phytochemical screening

The results of the chemical reactions of characterization are presented in the table II.

The number of "+" is function of the intensity of the tint and/or of precipitates. The reactions were positive with alkaloids, anthocyanins, coumarines, flavonoid, cardiac glycosides, polyphenols, gallic tannins and catechic tannins for our three extracts (aqueous, methanolic and ethanolic).

- Anthraquinones are present in the aqueous extract and absent in the methanolic and ethanolic extract;
- Coumarines are absent in the aqueous extract and present in the methanolic and ethanolic extract;
- Saponosides are present in the aqueous extract and the methanolic extract and absent in the ethanolic extract;
- Steroids are present in the methanolic extract and absent in the aqueous extract and the ethanolic extract ;
- Terpenoides are absent in the three extracts.

Table-II: Results of the reactions in test tube realized on the extracts of roots of *Jatropha curcas* L

Search	coloration	Methanolic extract	Aqueous extract	Ethanolic extract
Alkaloids (Valser Mayer)	Yellowish white precipitate	+	+	+
Anthocyanins	Dark tint which turns to the purplish blue	+	+	+
Free anthraquinones	More or less red tint		+	-
Coumarins (UV 366nm)	Purple fluorescence	+	-	+
Flavonoids	Rose orangy color (flavones)	+	+++	++
Cardiac glycosides	Fluorescent particles	++	+	+
Polyphenols	blackish blue Color	+	+++	++
Saponosides	Presence of foam	+	++	-
Stéroïdes	Brownish red ring	+	-	-
Gallic tannins (FeCl3 à 2%)	Blackish blue	++	+	+
catechic tannins (HCl)	Soluble red precipitate	+	++	+
Terpenoïdes		-	-	-

II-3 Results of quantitative analysis of extracts

The determination of the contents of total phenol, flavonoid, flavanols, flavones and flavonols, the condensed tannins, the anthocyanins, saponins and the alkaloids in: aqueous, methanolic (MeOH) and ethanolic (EtOH) extracts of *Jatropha curcas* L was made by spectrophotometric dosages. This quantitative analysis was determined from the equations of the linear regression of every curve of calibration. The yield of the extraction of alkaloids and dosage of saponins in the various extracts was expressed in percentage (%). Every graph of the contents represents the average (n = 3).

Content in total phenols

The results of the dosage of total phenols revealed a variability of content of extracts. They showed that the aqueous extract has a strong content in total phenols (24,037 mg / g) compared with the methanolic extract (10,648 mg/g) and the ethanolic extract (6,510 mg/g).

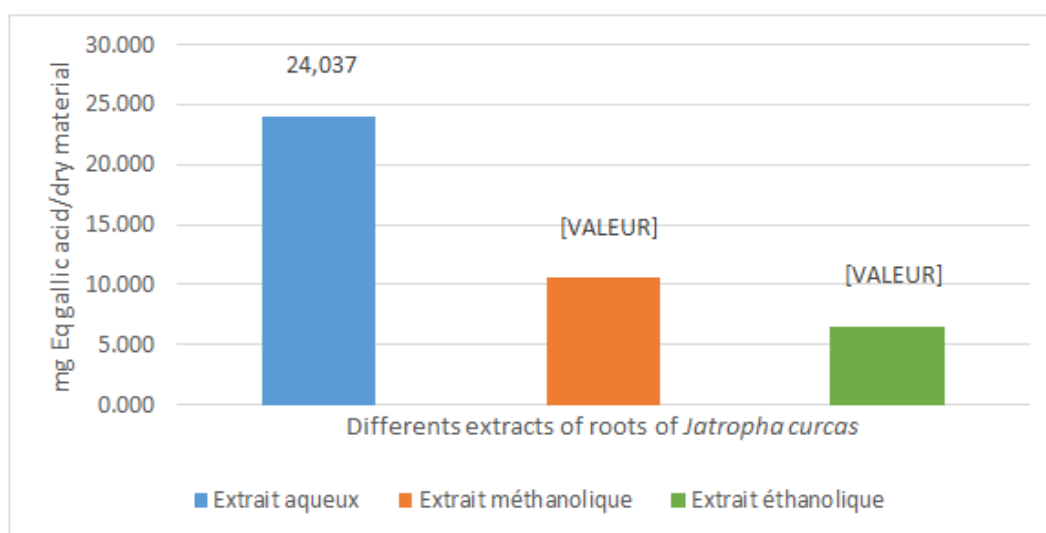


Fig-4: Content in total phenols of the extracts of roots of *Jatropha curcas* L

Content in total flavonoids

The results of the dosage of flavonoids revealed that the aqueous extract is in a strong content (9,493 mg/g) compared with the ethanolic extract (3.035 mg/g) and the methanolic extract (0.971 mg / g). Basing on these data, we can deduct that the latter represent 46,62 % of total phenols of the ethanolic extract, and about 34,49 % of total phenols of the aqueous extract. This rate does not exceed 9,11 % of total phenols of the methanolic extract.

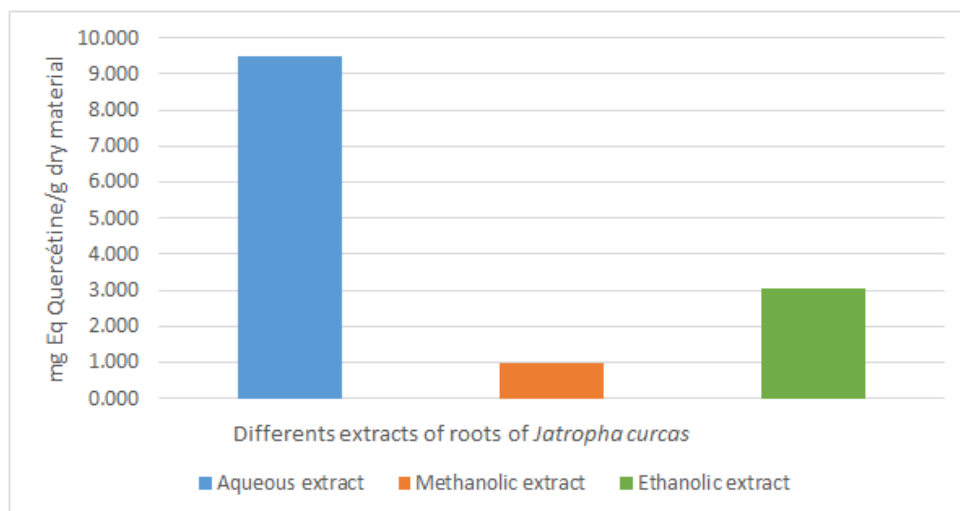


Fig-5: Content in total flavonoids of the extracts of roots of *Jatropha curcas L*

Content in flavanols

Concerning flavanols, the aqueous extract presented the strongest content (2,704 mg / g) compared with the ethanolic extract (1,870 mg / g) by representing 28,73 % of phenols of the ethanolic extract and 11,24 % of total phenols of the aqueous extract. The methanolic extract presented no content of flavanols.

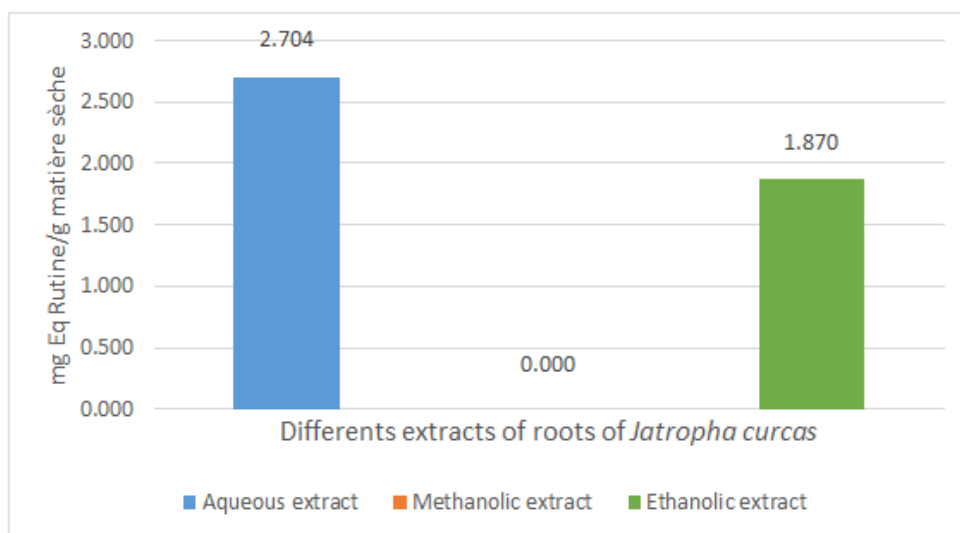


Fig-6: Content in flavanols of the extracts of roots of *Jatropha curcas L*

Contents in flavones and flavonols

The results of the dosage of flavones and flavonols reveal that the aqueous extract presented the strongest content (2,053 mg/g) compared with the methanolic extract (0,918mg/g) and the ethanolic extract (0,619 mg/g) by representing 9,37 % of phenols of the ethanolic extract, 8,55 % of total phenols of the methanolic extract and 8,53 % of total phenols of the aqueous extract.

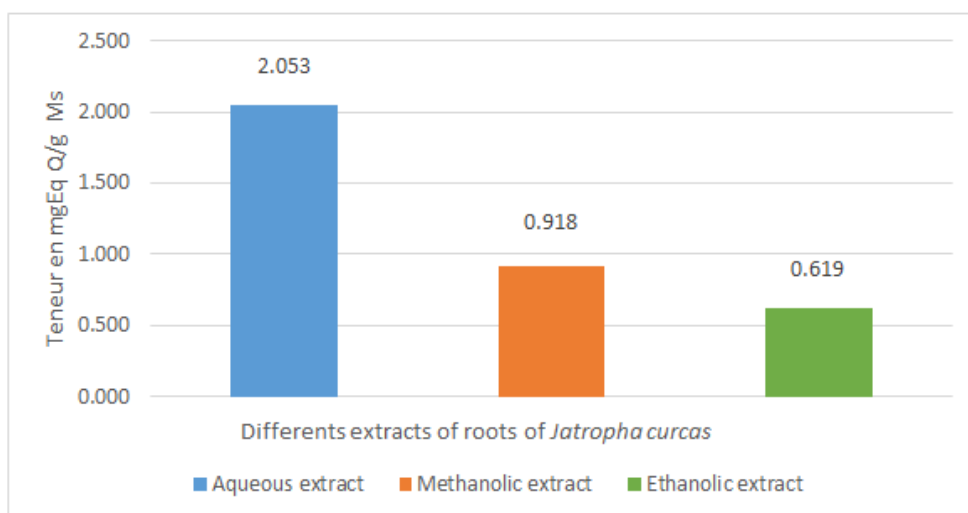


Fig-7: Contents in flavones and flavonols of extracts of roots of *Jatropha curcas L*

Content in condensed tannins

The results of the dosage of the condensed tannins reveal that the aqueous extract presented the strongest content (0,0192 mg / g) compared with the methanolic extract (0,0046mg / g) and the ethanolic extract (0,0012 mg / g) by representing 0,080 % of phenols of the aqueous extract, 0,043 % of total phenols of the methanolic extract and 0,018 % of total phenols of the ethanolic extract. The variability of content in condensed tannins by the analyzed extracts is presented below.

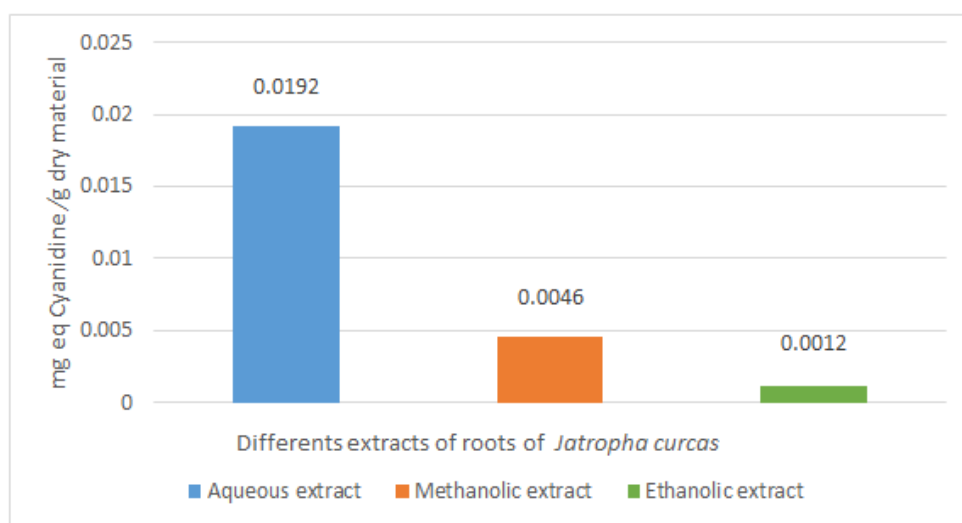


Fig-8: Content in condensed tannins in extracts of roots of *Jatropha curcas L*.

Content in anthocyanins

The results of the dosage of the condensed tannins reveal that the aqueous extract presented the strongest content (2,625 mg/ml) comparing with the methanolic extract (1,400 mg/ml) and the ethanolic extract (1,225 mg/ml).

A variability of content in anthocyanins of the analyzed extracts is presented in the figure 9 below:

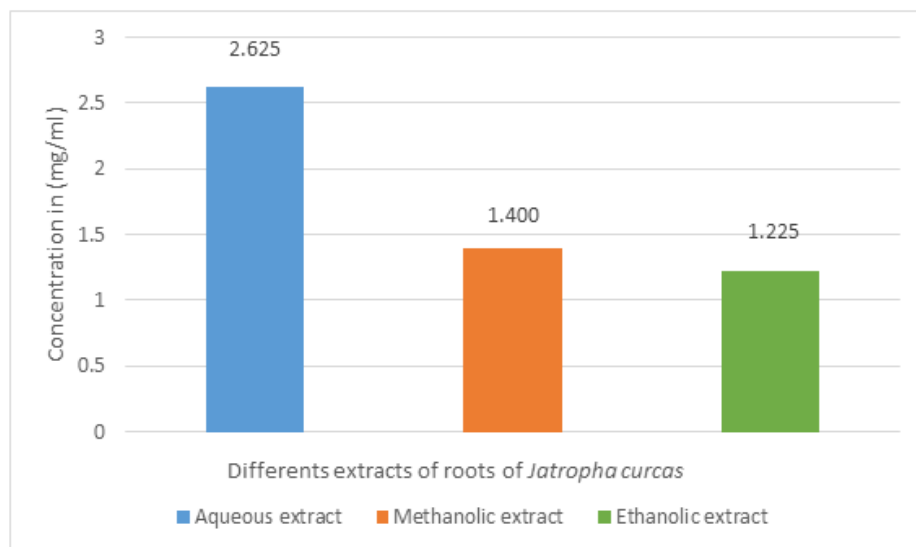


Fig-9: Content in anthocyanins in extracts of roots of *Jatropha curcas L.*

Content in saponins

The results of the dosage of saponins reveal that the aqueous extract presented the strongest content (6,270 %) compared with the methanolic extract (2,472 %) and the ethanolic extract (0,000 %). The rate of saponins in extracts is presented in the figure 10:

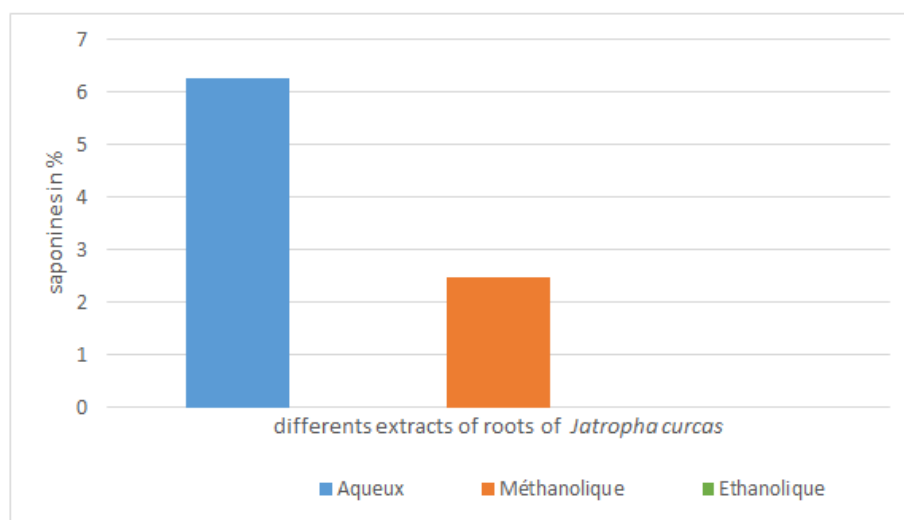


Fig-10: Content in saponins of extracts of roots of *Jatropha curcas L.*

Content in alkaloids

The results of the extraction of alkaloids reveal that the methanolic extract presented the highest yield (0,698 %) compared with the aqueous extract (0,614 %) and in the ethanolic extract (0,000 %). The content in alkaloids of extracts is presented in the figure 11 below.

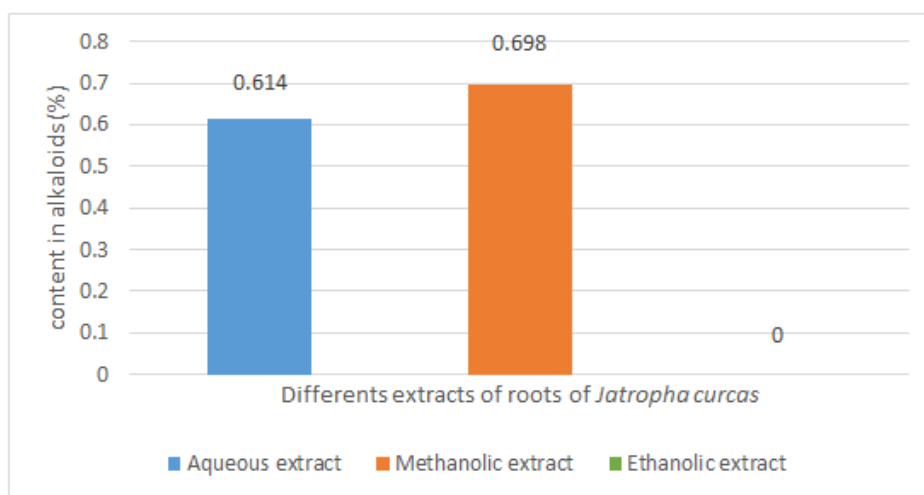


Fig-11: Content in alkaloids of extracts of roots of *Jatropha curcas L*

The results of the quantitative analysis of the extracts of the roots of *Jatropha curcas* were recapitulated in the table III below:

Table-III: results of quantitative analysis of extracts of the roots of *Jatropha curcas*

Parameters	Selected standard	Wavelength (nm)	Extracts (mg eq/g)		
			Aqueous	Methanolic	Ethanolic
Total phenol	Gallic acid	765	24,100	10,64	6,51
Flavonoids	Quercetine	430	9,490	0,97	3,03
Flavanols	Rutine	440	2,710	0	1,87
Flavones and flavonols	Quercetine	415	2,050	0,91	0,61
Condensed tannins	Cyanidine	530	0,019	0,0047	0,0013
			Content in mg/ml		
Anthocyanins	Malvidine-3 glucoside	520	2,625	1,4	1,225
			Content in percentage (%)		
Saponins	Saponin	380	6,27	2,471	0
Alkaloids	-----	-----	0,614	0,698	0

The values are the average of three assays

Table-IV: Results of the averages, the standard deviations and the percentages of reduction for the hypoglycaemia activity

Batches		Glycaemia (mg/dl)					
		0min	30 min	60 min	90 min	120min	180min
1. Negative control	10mL/kg	143,40	138,40	118,80	105,00	100,80	91,60
		±0,50	±0,51	±0,54	±0,43	±0,46	±0,25
2. Positive control	10 mg/kg	139,20	117,40	100,40	87,20	79,60	67,20
		±0,25	±0,47*	±0,40*	±0,52*	±0,24***	±0,67***
	% réduction	0	15,17	15,49	16,95	21,03	26,64
3. Extract	250 mg/kg	138,20	110,00	107,40	96,40	98,40	113,80
		±0,08	±0,11*	±0,10	±0,05*#	±0,02	±0,08
	% réduction	0	20,52	9,6	8,19	2,77	-24,23
4. Extract	500 mg/kg	137,60	106,80±	96,40	85,00	75,40±	64,20
		±0,41	0,51***#	±0,48***	±0,45***	0,29***#	±0,39***
	% réduction	0	22,83	18,86	19,05	25,2	29,91

II-4 Results of the evaluation of biological activities

Variation of the glycemia

The glycemia of rats was measured every 30 mn during 120 mn and 1 hour later in the 180th mn for the hypoglycaemia and antihyperglycaemia tests with a glucometer. Figures show the variations of the glycemia according to time after administration of every substance (water, glibenclamide, glucose, aqueous extract in 250 mg / kg and 500mg /

kg of body weight. The reference values of the glycaemia to the rats are: normoglycaemia: from 50 to 109 mg / dl on an empty stomach and when they are superior to 135 mg / dl there is hyperglycemia.

Hypoglycaemia activity

The basic glycaemia (78,1 ± 1,1 mg / dl) was determined.

RESULTS OF THE STATISTICAL ANALYSIS

The data are presented in the form of average ± standard deviation (DS). The test of Newman-Keuls was used to make the comparisons with the control. * Significant difference at p < 0,05 compared with the negative control, *** significant difference at p < 0,0001 compared with the negative control, # significant difference in p < 0,05 compared with the positive control, ### significant difference in p < 0,0001 compared with the positive control.

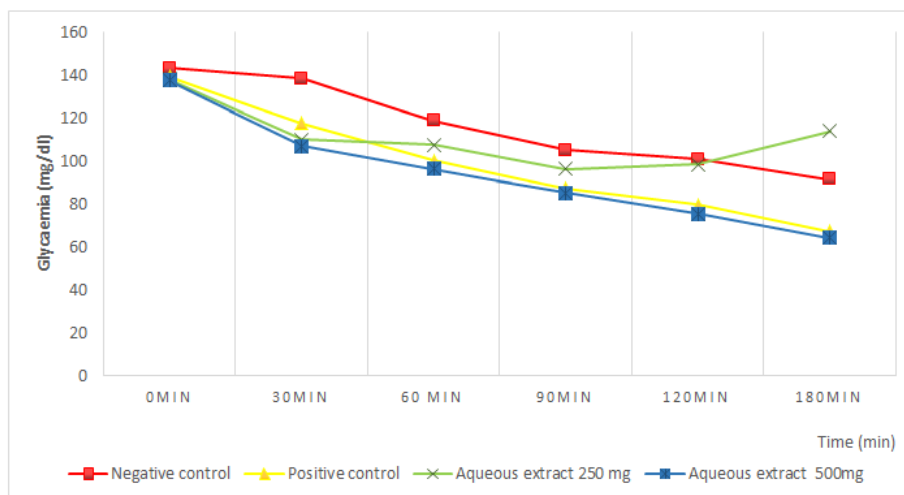


Fig-12: Variation of the glycaemia for the various batches in the test of the hypoglycaemia effect

Interpretation

The curve above shows that extracts in the dose 500 mg / kg of body weight leads to a significant reduction (p-value < 0,0001) 30 minutes after force-feeding of animals compared with the batch of the negative control until the 180th min. The reduction in the glycaemia at this dose is significant (p-value < 0,05) in the 30th and in the 120th minute compared with the batch of the positive control. A reduction in the glycaemia was observed, always in the same dose tested from the 60th, 90th and 180th min, but not being statistically significant (p-value > 0,05); no difference was recorded compared with the positive control.

Table-V: Results of the averages, the standard deviations and the percentages of reduction for the antihyperglycaemia activity

Batches		Glycaemia (mg/dl)					
		0 min	30 min	60 min	90 min	120 min	180 min
1. Negative control	10ml/kg	85,40 ±0,85	126,60 ±0,68	138,60 ±0,39	130,60 ±0,58	114,00 ±0,34	108,80 ±0,57
2. Positive control	10 mg/kg	80,00 ±0,04	130,80 ±0,10	109,20 ±0,06***	87,40 ±0,06***	79,80 ±0,04***	69,00 ±0,07***
	% réduction	0	-3,32	21,21	33,07	30	36,58
3. Extract	250 mg/kg	84,40 ±0,08	129,80 ±0,13*	114,00 ±0,10	81,20 ±0,11*	73,40 ±0,06*	98,00 ±0,09*#
	% réduction	0	-2,53	17,75	37,86	35,61	9,92
4. Extract	500 mg/kg	84,80 ±0,81	148,20 ±0,93***	101,40 ±0,38*	93,00 ±0,56***	96,00± 0,78***#	94,40 ±0,88***
	% réduction	0	-17,06	26,83	28,79	15,79	13,24

Antihyperglycaemia activity

The basic glycaemia was 80,4 ± 0,06 mg / dl. The results of the test of the antihyperglycaemia activity are recapitulated in the table V

RESULTS OF THE STATISTICAL ANALYSIS

The data are presented in the form of average \pm standard deviation (DS). The test of Newman-keuls was used to make the comparisons compared with the control. * significant difference with $p < 0,05$ comparing with the negative control, *** significant difference with $p < 0,0001$ compared with the negative control, # significant difference with $p < 0,05$ compared with the positive control, ### significant difference with $p < 0,0001$ compared with the positive control.

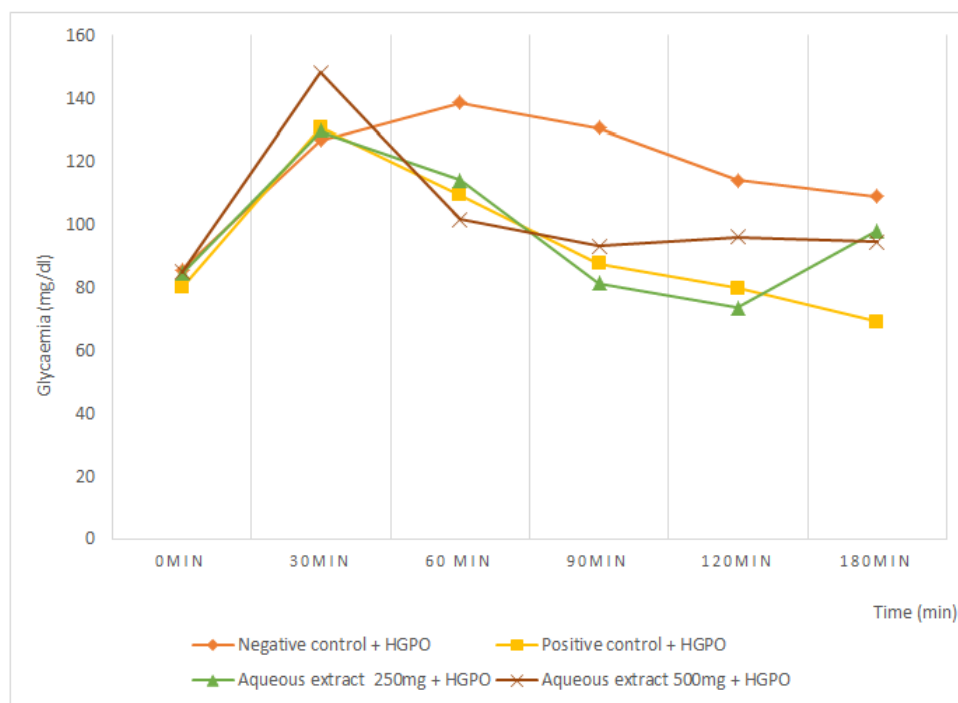


Fig-13: Variation of the glycaemia for the various batches in the test of the antihyperglycaemic effect

Interpretation

The curve above shows that the reference molecule (glibenclamide) led to a significant reduction (p -value $< 0,0001$) of the glycaemia 60 minutes after force-feeding of animals compared with the batch of negative control. This reduction was significant (p -value $< 0,05$) in the 120th and 180th minute compared with animals having received both doses. Furthermore we also note a reduction in the glycaemia to animals having received the extract in both doses, a statistically significant difference (p -value $< 0,05$) was recorded by the 60th to the 180th minute after the force-feeding of animals compared with the batch of the negative control, and no statistically significant difference (p -value $> 0,05$) was registered compared with the animals of the positive control.

II-5 Yield on extraction and phytochemical screening

The extraction of extracts from the roots of *Jatropha curcas* gave a 8,14 % yield for the aqueous extract, 7,84 % for the ethanolic extract and 2,5 % for the methanolic extract. These results are close to those of Namuli *et al.* in [36] which found 8,32 % for the aqueous extract, and those of Ousseynou [35] which found 6,46 % for the ethanolic extract and 4,87 % for the methanolic extract. The best yield was obtained with the aqueous extract.

The phytochemical study highlighted the wealth of the roots of *Jatropha curcas* in alkaloids, anthocyanins, coumarines, flavonoid, cardiac glycosides, polyphenols, saponines, gallic tannins, catechic tannins and triterpenes. It was reported by Owolabi *et al.* [19] that these classes (alkaloids, saponines, tannins and flavonoids) of compounds are known to have an antidiabetic activity. According to the works of Odiogenyi *et al.* [20], some of these secondary metabolites possess an hypoglycaemic and/or antihyperglycaemic activity by inhibiting the aldose reductase (phenols, flavonoids) and also antioxidant (tannins). These results are in correlation with those found by Aiyelaagbe *et al.* [21] for the aqueous extract and Uche *et al.* [22] for the methanolic and ethanolic extracts.

II-6 Contents in phenolic compounds

The aqueous extract allowed the obtaining of the highest total phenolics and flavonoid contents followed by methanolic and ethanolic extracts. This was predictable because the water is a solvent of higher polarity [23] that the other solvents and thus it is capable of extracting more phenolic and flavonoid compounds. The quantities in total phenols of the extracts of roots varied between 6.51 and 24,03 mg EAG / g dry material. Those results are clearly lower

than those of Wafa Ghnimi [24], who had found during his works 108,18 - 124,94 mg EAG / g DM in the roots of *Jatropha curcas*, This could be understandable by the geographical distribution and the process of extraction which were widely different.

The values of total flavonoids obtained went from 0,97 to 9,49 mg EQ/g DM. These results are similar to those of Osamuyimen *et al.* [25] Which had found 0,41-9,33 mg EQ/g in the seeds of *Jatropha curcas*. According to Chang *et al.* [26] the spectrophotometric analysis using the chloride of aluminum can underestimate the content in flavonoids because it is specific in flavones and in flavonols and not in flavanones, from where the hypothesis that the quantity of total flavonoids detected could be underestimated. The content in flavanols was raised in the aqueous extract followed by the ethanolic extract and nothing was detected in the methanolic extract.

The content in saponins was the highest in the aqueous extract (6,27 %) followed by the methanolic extract (2,47 %) and none was detected in ethanolic extracts. This is attributed to the fact that saponins is consisted of one or several acids. The glycosides functions are thus soluble in the water and some are soluble in the methanol, and none in the weakly polar solvents such as the ethanol [23]. These results are lower than those of Ehsan *et al.* [27] which had found 19 % of saponins in the methanolic extract of seeds and superior to those of Joy *et al.* [28] which had found 0,72 % of saponins in the aqueous extract of the fruit of *Jatropha curcas*. This could be understandable by the uneven distribution of the secondary metabolites in the various organs of the plant the abundance of which can vary according to the nutritive elements.

The content in alkaloids was the highest in the methanolic extract (0,69 %) followed by the aqueous extract (0,61 %) and none was detected in the ethanolic extract. These results are lower than those of Joy *et al.* [28] which had found 1,38 % in the aqueous extract of the fruit of *Jatropha curcas*. This could be understandable by the uneven distribution of the secondary metabolites in the various organs of the plant the abundance of which can vary according to the nutritive elements.

II-7 Hypoglycaemic activity

The glycaemia of the rats of the batch receiving glibenclamide varies significantly ($p < 0,0001$) from 30 min until 180 min after the force-feeding of the batch glibenclamide in the dose 10 mg/kg weighty physical comparing with the initial glycaemia (30 min before the force-feeding). It varies significantly ($p < 0,05$) comparing with the value of the glycaemia of the batch receiving distilled water (negative control batch). This significant variation ($p < 0,05$) of the glycaemia of the batch glibenclamide (positive control batch) comparing with the batch receiving distilled water (negative control batch) demonstrates that the glibenclamide (reference substance), is an hypoglycaemic substance. Which confirms the results of Venkateshwarlu *et al.* [29], Adebayo *et al.* [30] and of Arumugan *et al.* [31] which demonstrated the hypoglycaemic activity at the dose 10 mg/kg body weight to the rat.

The glycaemia of the rats of the batch 250 mg / kg BW does not vary significantly ($p > 0,05$) in the batch in the 60th, 120th and 180th minute compared with the value of the initial glycaemia (30 min before the force-feeding with the extract). This variation is not significant ($p > 0,05$) compared with the batch of distilled water (negative control). These results show that the aqueous extract of root of *Jatropha curcas* in the dose 250mg / kg BW do not lead to a considerable reduction in the glycaemia. In comparison with the results of Akouah *et al.* [32] showing that the ethanolic extract of *Rauwolfia vomitoria* 70 % in 500 mg / kg weighty did not possess hypoglycaemic activity because the difference of glycemic value was not significant ($p > 0,05$) compared with that of the batch distilled water.

The glycaemia of the rats of the batch 500mg / kg weighty varies significantly ($p < 0,05$), in the batch throughout the study compared with the initial value (30min before the force-feeding of the extract). This variation is also significant ($p < 0,05$) during the study compared with the batch distilled water (negative batch). It varies significantly ($p < 0,05$) in the 30th min and in 120th min compared with the batch glibenclamide (positive control batch). So showing that the aqueous extract of the roots of *Jatropha curcas* administered in 500 mg / kg weighty possess an hypoglycaemic activity and this activity is better pronounced than that of glibenclamide in the 30th and 120th minute after administration. In comparison with the results of Akouah *et al.* [32] and Shanti *et al.* [33] showing respectively that the ethanolic extract of *Rauwolfia vomitoria* 70 % in 500mg / kg weighty and the ethanolic extract 50 % of the leaves of *Jatropha curcas* in 500mg / kg weighty possessed an hypoglycaemiant activity because for these two extracts the difference of the glycemic value was significant ($p < 0,05$) compared with that of the batch distilled water, what will let consider that the chemical compounds responsible for the hypoglycaemic activity in the leaves of *Jatropha curcas* could be also in roots.

The various results obtained in our study let us to believe that the aqueous extract of the roots of *Jatropha curcas* L. has an activity dose dependent. The dose used for the glibenclamide is the one used in therapeutics. The glibenclamide, the sulphamide hypoglycaemic drug of 3th generation, appeared to be a good reference medicine. Let us

note that contrary to our product which is an extract, the glibenclamide is a pure molecule. We can thus say that the aqueous extract administered to 500 mg / kg weighty has a monophasic action which is a significant reduction ($p < 0,05$) in the glycaemia which is decreasing from the 30th minute. Its hypoglycaemic action is also as extended as that of the glibenclamide.

II-8 Antihyperglycaemic activity

The glycaemia of the rats of the batch glibenclamide (positive control batch) varies significantly ($p < 0,0001$) from 90 min after force-feeding with the glibenclamide in the dose 10 mg/kg weighty comparing with the initial glycaemia (at the time of the force-feeding with the glibenclamide and 30 min before the hyperglycaemia caused orally). It varies significantly ($p < 0,0001$) comparing with the value of the glycaemia of the negative control batch (batch distilled water + glucose in the dose 2g/kg weighty). This significant variation ($p < 0,0001$) of the glycaemia of the batch glibenclamide (positive control batch), comparing with the negative control batch demonstrates that the glibenclamide (reference substance), is a molecule responsible for antihyperglycaemic activity. This result confirms the results of Ndoumou et al. [34] which showed that the glibenclamide administered to rats in the dose 10 mg/kg weighty led to an antihyperglycaemic activity.

The glycaemia of the rats of the batch 250mg / kg weighty does not vary significantly ($p > 0,05$) in the batch during the study compared with the value of the initial glycaemia (at the time of the force-feeding of the extract in 250mg / kg and 30 min after the hyperglycemia caused by oral route). It varies significantly ($p < 0,05$) in the batch in the 90th and 180th min comparing with the value of the glycaemia of the negative control batch, but this variation is not significant ($p > 0,05$) compared with the positive control. So showing that the aqueous extract of the roots of *Jatropha curcas* administered to 250mg / kg weighty do not possess antihyperglycaemic activity. In comparison to the results of Shanti et al. [33] showing that the ethanolic extract 50 % of *Jatropha curcas* in 500 mg / kg weighty did not possess antihyperglycaemic activity. The variation of the glycaemia was not significant ($p > 0,05$) comparing with that of the negative batch control.

The glycaemia of the rats of the batch 500mg/kg weighty does not vary significantly ($p > 0,05$) in the batch during the study comparing with the value of the initial glycaemia (at the time of the force-feeding of the extract in 500mg/kg and 30min after hyperglycaemia caused orally). It varies significantly ($p < 0,05$) from the 30th min, comparing with the value of the glycaemia of the negative control batch and in the 120th min comparing with the value of the glycaemia of the positive control batch. These results show that the aqueous extract of the roots of *Jatropha curcas* administered to the dose 500mg/kg weighty cause a not significant reduction in the glycaemia after administration of the overload of glucose and thus does not possess antihyperglycaemic activity. In comparison to Shanti et al. [33] results, showing that the glycaemia of rats having received the ethanolic extract 50 % of the leaves of *Jatropha curcas* in the dose 500 mg/kg did not vary significantly ($p > 0,05$) comparing with that of the positive control.

Thus we can say that the aqueous extract in the dose of 250 mg/kg and 500 mg/kg has no antihyperglycaemic activity.

CONCLUSION

The objective of this work was the evaluation of an Euphorbiaceae: *Jatropha curcas* L. for the hypoglycaemic and/ or antihyperglycaemic effect of the aqueous extract of roots. The determination of the yields in extracts showed a significant level for the aqueous extract (8,14 %) and for the methanolic extract (7,84 %), while it was low in the ethanolic extract.

The phytochemical study of the extracts had concerned essentially the phenolic compounds; then we have highlighted hypoglycaemic and antihyperglycaemic activity of the aqueous extract of roots. The quantitative estimation of the phenolic compounds showed that the aqueous extract is richer in total, flavonoid polyphenols, condensed tannins, anthocyanins and saponosides than methanolic and ethanolic extracts. Regarding the hypoglycaemic and antihyperglycaemic activity, the results showed that the aqueous extract did not possess antihyperglycaemic activity in both studied doses. But in the dose of 500 mg possessed an hypoglycaemic effect.

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