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EVALUATION OF THE HYPOGLYCEMIC AND ANTI-HYPERGLYCEMIC ACTIVITIES OF AQUEOUS AND HYDROETHANOLIC EXTRACTS OF *ERYTHROCOCCA ANOMALA* LEAVES IN RAT.

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ABSTRACT

Erythrococca anomala (Euphorbiaceae) is an annual medicinal plant, widely used in traditional medicine in Cote d'Ivoire and sub-Saharan Africa as an anti-inflammatory, laxative and purgative. In the present work, the aim was to evaluate the hypoglycemic and anti-hyperglycemic activity of the aqueous and hydroethanolic extracts of the leaves of this plant in the rat. The blood glucose was determined using a glucose reader Test strips). Administered orally at doses of 100 and 200 mg / kg body weight the extracts induce dose-dependent hypoglycemia in the rat. These aqueous and hydroethanolic extracts at a dose of 200 mg / kg body weight significantly reduced the hyperglycemia caused by administration by glibenclamide, the reference anti-hyperglycemic molecule (10 mg / kg body weight). Oral glucose at a dose of 5 mg / kg body weight to the rat.

KEYWORDS: *Erythrococca anomala*, Euphorbiaceae, Hypoglycemic, Anti-hyperglycemic, glibenclamide.

1. INTRODUCTION

Medicinal plants have long been used for the treatment of many human diseases in all regions of the world.[1] Plants are a potential source of hypoglycemic drugs and have been used in the treatment of diabete .While this disease can be controlled by nutrition and exercise[2], most patients require oral hypoglycemic agents to control their blood glucose levels. The diagnosis of diabetes is based on the measurement of blood sugar (blood sugar). Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia resulting from a defect in the secretion of insulin and / or the action of this hormone.[3]

However, the long-term use of hypoglycaemic drugs for the treatment of diabetes, such as sulfonylureas, metformin and others have a wide range of side effects[4,5], including a gradual decrease in their efficacy.

Erythrococca anomala is a medicinal plant of the family Euphorbiaceae, widely used in traditional medicine in Cote d'Ivoire and sub-Saharan Africa. Phytochemical studies have revealed the presence of phenols, flavonoids, alkaloids, sterols and saponins in leaves.[6] These various secondary metabolites hold the attention of playing an essential role in the treatment of diseases.[7]

Si=ilarly, studies have shown that the leaves have anti-inflammatory activity.[8]

In Côte d'Ivoire, the macerates of the leaves of this plant make it possible to fight against meningitis and malaria, while in Cameroon, decoctions and macerated leaves allow to treat dental pain; But used as laxatives and purgatives, make it possible to expel the worms; In Nigeria, bark is used against arthritis and rheumatism.[9]

However, there is no information on the hypoglycaemic and anti-hyperglycemic properties of the leaves of this plant. This study aims to evaluate the hypoglycaemic and anti-hyperglycemic activity of aqueous extracts and hydroethanolic leaves of *Erythrococca anomala* by a glucose reader (glucometer with reagent strips)

2. MATERIAL AND METHODS

2.1. Plant material

The plant material consists of leaves of Erythrococca anomala harvested (in the region of Yakssé-mé, Department of Adzopé), washed and dried at room temperature, protected from the sun. This plant has been identified at the National Floristic Center of Côte d'Ivoire where a sample (OAT -ErAn) is kept.

2.2. Animal equipment

Adult Wistar rats (128-130 g) were used for the evaluation of the antioxidant activity of the total extracts of Erythrococca anomala leaves intraperitoneally. These rats come from the animal house of the Ecole Normale Supérieure of Abidjan (ENS Cote d'Ivoire). All rats have access to water and are adequately fed with pellets.

2.3. Methods

2.3.1. Preparation of extracts

The aqueous extract is prepared from 100 grams of Erythrococca anomala leaf powder in one liter of boiling distilled water for fifteen minutes.

The solution thus obtained is filtered on hydrophilic cotton and then under vacuum with ordinary filter paper. The collected filtrate was placed in an oven at 40 ° C. A dark brown dry powder was obtained for the total crude aqueous extract of Erythrococca anomala.

The **Guédé-Guina** method[10] was used to obtain the 70% Erythrococca anomala hydroethanolic extract. A 70% hydroethanolic solution (ETOH / H_2O, 70: 30) was used for the preparation of Hydroethanolic extract of Erythrococca anomala in a vial. One liter of the hydroethanolic solution and 100 g of Erythrococca anomala powder were used for this purpose. The resulting mixture was homogenized with a magnetic stirrer for 24 hours. The homogenate is filtered on hydrophilic cotton and then under vacuum. The collected filtrate was concentrated in a rotary evaporator and then placed in an oven at 40 ° C.

For complete drying. The 70% hydroethanolic extract of Erythrococca anomala appears in the form of a dark green paste after drying.

2.3.2. Hypoglycemia test

The hypoglycaemicity is measured in order to determine the dose of the aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* capable of lowering the basal glucose of the rat.

The animal material consists of 42 rats distributed in 7 lots of 6 (all of mixed sex).

The rats were fasted for 16 hours before the experiment.

The various consignments received orally

-lot1 the solution of distilled water (control).

-lot 2 the aqueous extract of leaves of Erythrococca anomala 100 mg / kg.

-lot 3 the aqueous extract of leaves of Erythrococca anomala 200mg / kg.

-lot 4 the hydroethanolic extract of leaves of Erythrococca anomala 100 mg / kg.

-lot 5 the hydroethanolic extract of leaves of Erythrococca anomala 200 mg / kg.

The evolution of the blood glucose of the rats of the different batches is followed in the short term for 5 hours (T0, 1h, 2h, 3h, 5h) after the oral administration of the extracts. Blood is taken from the caudal vein and blood glucose is measured using a glucose reader (reagent strip glucose meter).

Percentage change in blood glucose (%) = (Gt-G0) x 100 / G0

G0: Basal blood glucose

T0: Time = 0min

Gt: glucose in time

2.3.3. Glucose Tolerance Test

2.3.3.1. Blood glucose measurement in pretreated rats with extracts of leaves of Erythrococca anomala.

Hyperglycemia is caused by oral administration of glucose to rats at a dose of 5 g / kg body weight. For this study, 30 rats were divided into 5 lots of 6 rats.

-lot1 the aqueous extract of leaves of *Erythrococca anomala* 200mg / kg + 5g / kg of glucose.

-lot 2 the hydroethanolic extract of leaves of *Erythrococca anomala* 200mg / kg + 5 g / kg of glucose.

-lot 3 glibenclamide 10mg / kg + 5 g / kg glucose.

-lot 4 distilled water + 5 g / kg glucose.

-lot 5 distilled water.

Blood glucose levels in the rats are measured just before the administration of the substances or distilled water and after 1h, 2h, 3h, 5h.

2.3.3.2. Measurement of blood glucose in post-treated rats with extracts of the leaves of *Erythrococca anomala*

The sample is the same as the pretreated rats. However, in this experiment the different batches of rats were dosed with 200 mg / kg PC extract or glibenclamide (10 mg / kg) 1 h after induction of hyperglycemia by oral administration of 5 g / kg PCR glucose. The glucose of the rats of each lots is measured just before the administration of glucose at T0, and thereafter 1h, 2h, 3h, 5h.

2.4. Statistical Analysis

The values expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using one2yj7 way ANOVA followed by Dunnett's test to significant, *p* values less than 0.05 were considered significant.

3. RESULTS

3.1. Hypoglycemia test

Fig 1 and Table 1 show variations and reduction in blood glucose in rats following oral administration of different doses of aqueous and hydroethanolic extracts of leaves of *Erythrococca anomala* or distilled water (control batch). The blood glucose in the control rats which received only distilled water did not vary throughout the duration of this study. It remains at 0.95 ± 0.05 g / L. The 100 mg / kg aqueous extract results in a significant decrease in blood glucose ($p < 0.05$); compared to distilled water but more significant at the 200 mg / kg ($p < 0.01$) dose of Ranging from 0.88 ± 0.10 g / L to 0.68 ± 0.18 g / L and from 0.88 ± 0.10 g / L to

0.58 ± 0.10 g / L respectively 19.82% and 34.09% reduction in basal blood glucose in rats. The hydroethanolic extract at a dose of 100 mg / kg bw ; Also significantly decreased blood glucose levels (*p <0.05)* in distilled water but more significant at the dose of 200 mg / kg (*p <0.01*) over the duration of the experiment which ranged from 0.86 ± 0.06 g / L to 0.79 ± 0, 10 g / L and 0.80 ± 0.06 g / L at 0.60 ± 0.10 g / L,19.76% and 30.5% reduction in basal glucose levels in rats. The aqueous and hydroethanolic extracts of leaves of *Erythrococca anomala* have a dose-dependent effect on blood glucose levels in rats.

3. 2. Glucose tolerance test

3. 2.1. Blood glucose measurement in pretreated rats with extracts of leaves of *Erythrococca anomala.*

Whether glibenclamide 10 mg / kg PC or aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* (200 mg / kg bw), 2 h later, significantly decreased (*p <0.05*) the glucose of pretreated animals. At present, administration of glucose at a dose of 5 g / kg bw results in significant increases *(p <0.05)* in the blood glucose of all pretreated animals or not. The peak of hyperglycemia, appearing at 2 h, this increase in blood glucose varies depending on whether the rats were pretreated or not with the various aqueous and hydroethanolic extracts or glibenclamide.

They are 1.2 ± 0.32 g / L and 1.4 ± 0.11 g / L respectively, an increase of 1.06% and 90.47% with the aqueous and hydroethanolic extracts. In rats receiving distilled water (positive control), the glucose administered results in an increase in blood glucose, with a peak of hyperglycemia which is of the order of 1.56 ± 0.28 g / L, an increase of 231.91%. In contrast, pretreated rats only with distilled water glucose from rats did not vary during the experiment **(Fig 2).**

Subsequently, hyperglycemia was progressively reduced respectively 10.95% and 1.58% with aqueous and hydroethanolic extracts with cases of hypoglycemia of 0.62 ± 0.15 g / L and 0.65 ± 0, 15 g / after 5 h of experiment (**Table 2**).

When rats were pretreated with glibenclamide at 10 mg / kg bw, hyperglycemia induced 2 h after glucose administration at 5 g / kg bw was 0.93 ± 0.10 g / L **(Fig 2),** An increase of 47.61%. After this, hyperglycemia is gradually reduced as a function of time until the initial blood glucose level is reached at 5 hours, a reduction of 71.42%, followed by a hypoglycemia of 0.18 ± 0.27 g / L. As a preventive measure, the extracts have a less pronounced anti-hyperglycemic effect than that of glibenclamide.

3.2.2. Measurement of blood glucose in post-treated rats with extracts of the leaves of *Erythrococca anomala*

Oral administration of glucose at a dose of 5 g / kg bw results in hyperglycaemia in the post-treated rats or not with the various aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala or glibenclamide* for 1 h. The peak is 1.58 ± 0.22 g / L and 1.74 ± 0.37 g / L respectively for aqueous and hydroethanolic extracts, an increase of 150.79% and 167.69%.

Subsequently, this hyperglycemia decreases progressively during the 5 hours of experiment with a return to the initial blood glucose. The reduction in hyperglycemia and the time taken to return to the initial value of blood glucose are variable depending on whether the animals were post-treated with extracts or glibenclamide. In rats receiving distilled water and glucose (5 g / kg bw), a maximum hyperglycemia of 2.30 ± 0.63 g / L appeared at 3 h, an increase of 228.57% . Subsequently, this hyperglycemia decreases progressively after 3 h. The glycaemia of rats given only distilled water does not change over time.

Glucose-induced hyperglycemia was significantly reduced (*p <0.05)* when glucose was administered, 1 h after, aqueous and hydroethanolic extracts at a dose of 200 mg / kg bw, With time **(Table 3 and Fig 3)** and the return to the initial blood glucose occurs at 5 hours, a reduction of 6.34% for the aqueous extract and 7.69% for the hydroethanolic extract. Glibenclamide (10 mg / kg bw), administered 1 h after administration of glucose, results in a reduced reduction in induced hyperglycemia over time and a return to the initial glucose level at 5 hours A reduction of 2%. Curatively, the extracts have a pronounced anti-hyperglycemic effect than that of glibenclamide.

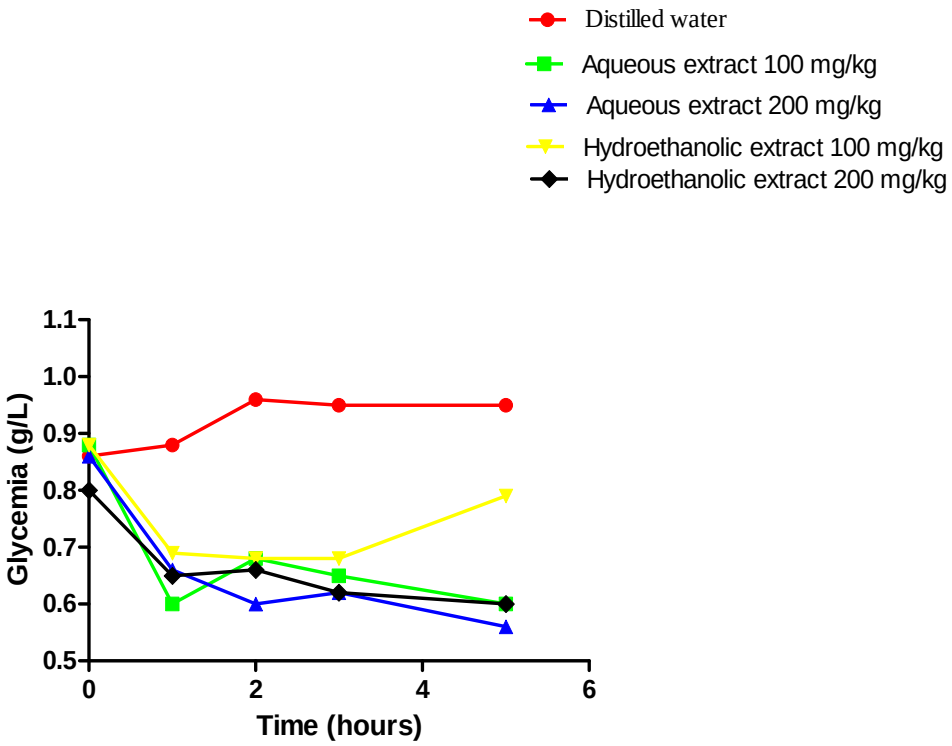

Figure 1: Dose-response effect of *Erythrococca anomala* leaves extracts on rat basal glucose.

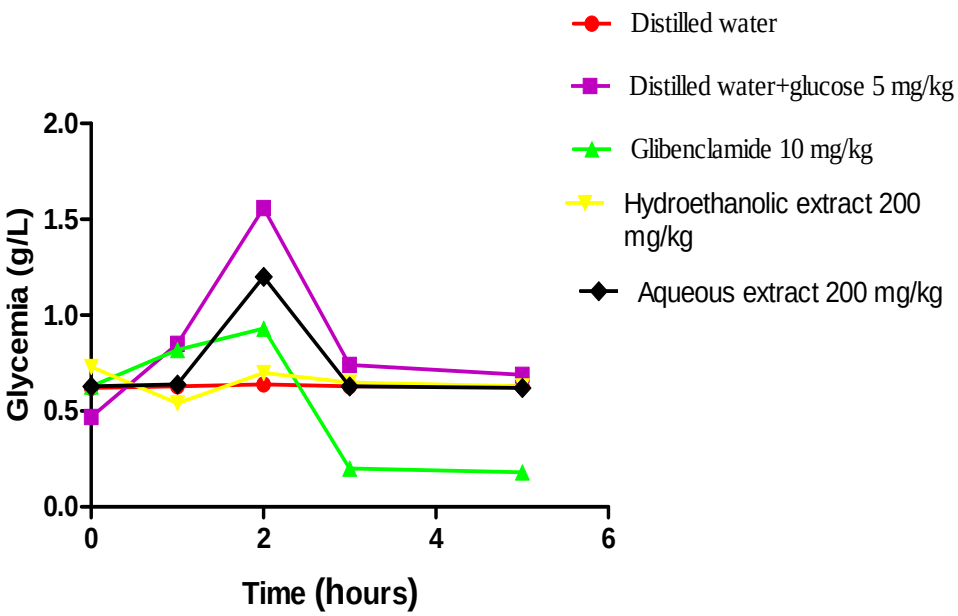

Figure 2: Effect of leaf extracts of *Erythrococca anomala* or glibenclamide on the glycaemia of normoglycemic rats.

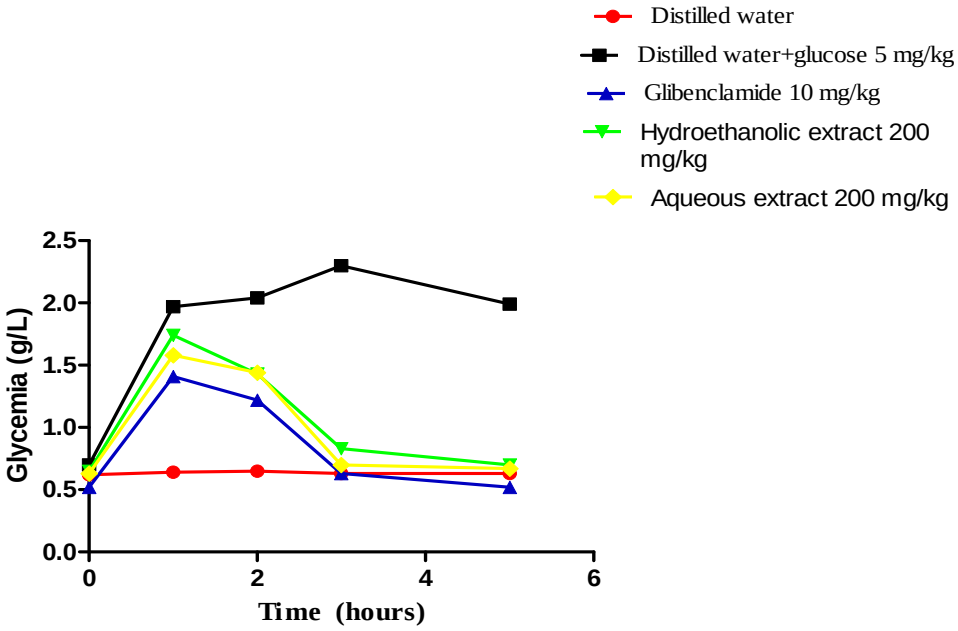

Figure 3: Effect of leaf extracts of *Erythrococca anomala* or glibenclamide on the glycemia of normoglycemic rats.

Table 1: Percentage of inhibition of blood glucose according to the doses of extracts.

Lots treated	1H	2H	3H	5H
Aqueous extracts 100 mg/kg	-21.59	-22.72	-22.72	-19.82*
Aqueous extracts 200 mg/kg	-31.81	-22.72	-22.72	-34.09**
Hydroethanolic extract 100 mg/kg	-23.25	-23.25	-22.09	-19.76*
Hydroethanolic extract 200 mg/kg	-18.75	-17.5	-22.5	-30.5**
Distilled water	-	-	-	-

The sign (-) = blood glucose reduction. The sign (-) = blood glucose reduction. The sign (+) = increase in blood glucose. Each value was the average ± SD, N = 6 rats, the data were analyzed by One Way ANOVA followed by the Dunnett test * $p < 0.05$, ** $p < 0.01$ where the extracts were compared with distilled water.

Table 2: Reduction of glucose-induced hyperglycemia in rats pretreated with leaves extracts of *Erythrococca anomala* or glibenclamide

Lots treated	1H	2H	3H	5H
Aqueous extracts 200 mg/kg	-25.02	+1.06	- 4.10	-10.95*
Hydroethanolic extract 200 mg/kg	+1.58	+ 90.47	+ 1,58	- 1.58
Glibenclamide	+ 30.15	+ 47.61	-68.25	-71.42**
Distilled water +Glucose	+ 80.85	+ 231.91	+ 57.44	+ 46.80
Distilled water	-	-	-	-

The sign (-) = blood glucose reduction. The sign (+) = increase in blood glucose. Each value was mean ± SD, N = 6 rats, data were analyzed by One Way ANOVA followed by Dunnett * $p < 0.05$, ** $p < 0.01$ where the Extracts and control were compared with Glibenclamide.

Table 3: Reduction of glucose-induced hyperglycemia in post-treated rats extracts from leaves of *Erythrococca anomala* or glibenclamide.

Lots treated	1H	2H	3H	5H
Aqueous extracts 200 mg/kg	+150.79	+128.57	+11.11	-7.69*
Hydroethanolic extract 200 mg/kg	+167.69	+120	+27.69	-6.34*
Glibenclamide	+171.15	+134.61	+21.15	-2.00
Distilled water +Glucose	+181.42	+191.42	+228.57	+184.28
Distilled water	-	-	-	-

The sign (-) = decrease of blood glucose. The sign (+) = increase of blood glucose. Each value was the mean ± SD, N = 6 rats, data were analyzed by One Way ANOVA followed by the Dunnett * $p < 0.05$ test, where the Extracts and control were compared with Glibenclamide.

4. DISCUSSION

The substances contained in the aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* are responsible for the pharmacological activities of these extracts. Indeed, substances such as polyphenols and flavonoids are generally recognized as having hypoglycaemic effects.[11] **Kambouché**[12] showed the anti-hyperglycemic effect of saponins. The study of the pharmacological effects of aqueous and hydroethanolic extracts showed that they induce a dose-dependent and progressive decrease in the glycemia of normoglycemic rats, as well as glibenclamide (the reference molecule). This study showed that extracts of leaves of *Erythrococca anomala* contain hypoglycemic substances because they act as glibenclamide, which is a hypoglycemic sulfonamide. The hypoglycemic sulfonamides bind to a specific receptor on the membrane of the pancreatic β cells in the vicinity of the dependent ATP potassium channel and cause the latter to close. This will lead to a membrane depolarization of the β-cells with opening of the voltage-dependent calcium channels and an influx of Ca^{2+} thus triggering by exocytosis the extrusion of insulin secretion granules.[13]

Indeed, it has been reported that sulfonylures induce hypoglycaemia in normoglycemic rats by stimulating the production of insulin by the beta cells of the pancreas, thus promoting the storage of glycogen in the liver.[14] The administration of glucose per os at a dose of 5 g / kg bw, results in a significant increase in blood glucose in rats followed by a gradual return to the initial glucose. Under the same experimental conditions, when the animals are pretreated or post-treated with the extracts at a dose of 200 mg / kg bw, glucose-induced hyperglycemia is significantly reduced and the return to the initial glycemia is much quicker. These effects were also observed with glibenclamide at 10 mg / kg bw. Thus, like glibenclamide, aqueous and hydroethanolic extracts of leaves of *Erythrococca anomala* at a dose of 200 mg / kg induce a significant decrease in glucose-induced hyperglycemia. These extracts therefore have hypoglycaemic effects and anti-hyperglycemic effects. The similar effects of the extracts with those of glibenclamide on blood glucose suggest that the extracts could act by the same mechanism as the reference anti-hyperglycemic substance used. Thus, hypoglycemia and reduction of hyperglycemia observed in rats treated with aqueous and hydro-ethanol extracts could be explained by stimulation of insulin secretion by the pancreas.[15]

CONCLUSION

This study showed that aqueous and hydroethanolic extracts of leaves of *Erythrococca anomala* induce dose-dependent hypoglycemia and 200 mg / kg body weight of anti-hyperglycemic effects.

The study of the polyphenolic composition of the various extracts elucidates the cause of the high hypoglycaemic and anti-hyperglycemic activity of the aqueous and hydroethanolic extracts.

By these tests, the extacts revealed a high hypoglycemic and anti-hyperglycemic power close to that of glibenclamide.
The aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* seem to present a real and potential interest by their activities hypoglycemic and anti-hyperglycemic, which could justify its use in the treatment of diabetes.

Ethical Approval

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Félix Houphouet-Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

REFERENCES

1. Palombo. E.A. Traditionalmedicinal plant extracts and naturalproducts with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evidenced-basedComplementary and Alternative Médicine 2011, Article ID 680354, p15. Doi : 10.1093/ecam/nep 067p.

2. Shaw C.S, Clark J, Wagenmakers J.M. Effect of exercise and nutrition on intramuscular fat metabolism and insulinsensitivity, Annual Review of Nutrition, 2010; 30: 3–34p.
3. Raccah D. Epidémiologie et physiopathologie des complications dégénératives du diabète sucré. EMC-Endocrinologie, 2004; 1(1): 29-42.
4. Nissen S.E, Wolski k. Effect of Rosiglitazone on the risk of myocardialinfarction and death from cardiovascular causes. New England Journal of Medicine, 2007; 356: 2457–2471.
5. Nissen S.E.Setting the record straight. Journal of American Medical Association, 2010; 303: 1194–1195.
6. Miezan BAP, Okpekon AT, Yapi HF, Bony FN, Gbassi G, Assi YJ. Chemical component and acute toxicity study of Erythrococca anomala (Euphorbiaceae). Asian Journal of Biomedical and Pharmaceutical Sciences, 2016; 6(57): 4-8. 5.
7. Oladele GM, Abatan MO, Olukynle JO, Okediran BS. Anti-inflammatory and analgesic effect of aqueous leafextracts of Gomphrenacelosioides and Momordicacharinda. J Nat. Sat. Sci. Engr. Tech, 2009; 8(2): 1-8.6.
8. Patrice M BA, Yapi HF, Opkekon AT, Bony FN, Kouakou-Siransy G, Kouakou YébouéKF. Evaluation of anti-inflammatory activities of *Erythrococca anomala*aqueous and ethanolic extracts from leaves in rat, 2016; 14(2): 1-7. IJBCRR. 28385.
9. Adjanohoun EJ, Ake-Assi L. Contribution au recensement des plantes médicinales de Côte d'Ivoire. Centre National de Floristique de l'Université Nationale de Côte d'Ivoire, Tome 1, 23-30. Lavoisier, Paris, 1979; 895.
10. Guédé FG. Extraction of mansoninfrom*Mansoniaaltissima*as cardiovascular agent (patent application) 1990. Ministère de la Recherche Scientifique, Côte d'Ivoire, 35.
11. Mangambu MJD., Mushagalusa KF., Kadima NJ.Contribution à l'étude phytochimique de quelques plantes médicinales antidiabetiques de la ville de Bukavu etses environs(sud-Kivu, R .D. Congo). *Journal of Applied Biosciences*, 2014; 75: 6211-6220.
12. Kambouche N., Merah B., Derdour A., Bellahouel S., Bouayed J., Dicko A., Younos C., 10-Karumi Y., Onyeyili Pa. Ogugbuaja Vo. Identification Of Active Principles Of M. Balsamina (Balsam Apple) Leaf Extract. J. Med. Sci, 2004; 4(3): 179-182.
13. Grimaldi A, Hartemann-Heurtier A, Jacqueminet S, Bosquet F, Masseboeuf N, Halbran M, Sachon C. (2009). Guide Du Diabète 4e Ed, Masson.
14. Gebreyohannis T. Shibeshi W. Asres K. Effects of Solvant Fractions of Cayluseaabyssinica (Fresen)Fisch. & Mey. On Blood Glucose Levels of Normoglycemic, Glucose Loaded and Streptozomicin-induced Diabetic Rodents 2014. *Journal of Natural Remedies*, 14: 67-75.
15. Jackson J E., Bressler R., Clinicalpharmacology of sulfonyllureahypoglycemic agents. Part I. DRUG, 1981; 212: 211-245.