

1 SYNERGISTIC EFFECT OF AQUEOUS EXTRACTS OF CROTON ZABENSICUS
2 AND VERNONIA AMYGDALINA LEAVES AS AN ANTIHYPERGLYCEMIC
3 AGENT IN AN ALLOXAN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Aims: This study was aimed at investigating the antihyperglycemic effect of combined extract of *Vernonia amygdalina* and *croton zabensicus* compare with a hypoglycemic drug, glibenclamide.

Methodology: Twenty 20 experimental animals were used (albino rats); the rats were divided equally into four groups of five rats each; namely A (control), B (glibenclamide 10mg/kg body weight), C (synergetic treatment 1000mg/kg body weight), D (synergetic treatment 500mg/kg of body weight). Diabetes was induced intraperitoneal using Alloxan Monohydrate to all the animals and their blood glucose rise above 200mg/dl.

Results: It was observed that group B and group C treated with glibenclamide (10mg/kg body weight) and synergetic aqueous extract (1000mg/kg body weight) show significant decrease in the blood glucose level from 451.75mg/dl to 64.50mg/dl and 339.50mg/dl to 182.50mg/dl respectively compared with group D with 278.25mg/dl to 194.75mg/dl. However, a change was also observed in the body weight of the groups; Group A (Normal control) showed continuous increase in the body weight, Group B, C and D were observed to have decrease in body weight from induction period, but a steady increase were observed as treatment commences.

Conclusion: Hence this combined extract can be used as antihyperglycemic; only that it is slower in remediation compared with the glibenclamide; but without side effect as may be in the case of most standard drug.

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Keyword: Synergistic, aqueous extract, *Croton zabensicus*, *Vernonia amygdalina* and hyperglycemic

24 **1. INTRODUCTION**

25

26 Diabetes mellitus is a metabolic disorder found in all nations of the world. It is one of the
27 most prevalent endemic diseases of the 21st century. Diabetes Mellitus is a syndrome of
28 impaired carbohydrate, fat and protein metabolism caused by either lack of insulin secretion
29 or decreased sensitivity of the tissues to insulin characterized by hyperglycemia

30 Type 1 diabetes typically occurs in children and young adults, though it can appear at any
31 age. In the past, type 1 diabetes was called juvenile diabetes or insulin-dependent diabetes
32 mellitus. Over time, high blood glucose damages nerves and blood vessels, leading to
33 complications such as heart disease, stroke, kidney disease, blindness, dental disease, and
34 amputations. No one is certain what starts the processes that cause diabetes, but scientists
35 believe genes and environmental factors interact to cause diabetes in most cases. Insulin
36 must be used in Type I, which must be injected; while diabetes mellitus Type 2 is a disease
37 of insulin resistance by cells. (1) has defined Diabetes mellitus based on laboratory findings
38 as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140 mg/dl) or
39 greater than 11.1 mmol/l (200 mg/dl) two hours after a carbohydrates meal or two hours after
40 an oral ingestion of the equivalent of 75 g glucose. The beginning of diabetes in rat is
41 judged as blood glucose being higher than the expanded normal upper level. The criteria for
42 rats are close to that for human. Alloxan is a toxic glucose analogue, which selectively
43 destroys insulin-producing cells in the pancreas when administered to rodents and many
44 other animal species (2). Diabetes mellitus is further characterized by an inability to reabsorb
45 water resulting in increased urination (polyuria), excessive thirst (polydipsia) and excessive
46 eating (Polyphagia). Herbal products are of interest to many patients and health care
47 practitioners since about 70% of population worldwide rely on herbal medicines for part of
48 their primary health care. In different regions and cultures, herbal products are used as
49 single herb, combination of herbs, or combination of herb(s) and drug(s) e.g China and India.
50 The most desirable interactions are those which can result in additional therapeutic benefit.
51 This is often the intended or expected outcome when using combination therapy. In African
52 traditional medicine, several plants include *Anacardium occidentale*, *Congronema latifolium*,
53 *Croton zabensis*, *Vernonia amygdalina* etc; have been used to lower hyperglycemia.
54 *Croton zabensis* is commonly known in Nigeria as *Kirobalcen maser* in Hausa and *Ajeobale*
55 or *Ajeofole* in Yoruba (3). The ethanolic extract of the leaf was reported to produce a
56 significant reduction in blood glucose level of diabetic rat (4). *Vernonia amygdalina* popularly
57 known as bitter leaf is a shrub of 2 – 5m tall. It is popularly called bitter leaf because of its
58 abundant bitter principles. It is cultivated in Nigeria mainly for its nutritional value.

59 Diabetes mellitus is a metabolic disorder found in both young and old, rich and poor. It can
60 be very expensive to manage; however, the use of conventional medical approach of simply
61 using insulin and oral drugs to control diabetes Mellitus is not only costly but inadequate,
62 boring and lack compliance and yet they are rarely available. This give rise to the increase in
63 counterfeit thus the patient's exposure to long term complication remains a risk. Nigeria
64 government has failed to recognize this as a challenge and proffer a lasting solution. This
65 research work intend to encourage the use of a synergistic herbal medicine as a competitive
66 antihyperglycemia for an effective management of type 1 diabetes which is readily available,
67 cheap and with no record of toxicity. The specific objectives of this work are to study the
68 synergistic effect of aqueous extract of *croton zabensicus* and *vernonia amygdalina* as an
69 antihyperglycemic agent in alloxan induced diabetic rats; the percentage yield of the
70 aqueous extraction of *croton zabensicus* and *vernonia amygdalina*; the phytochemical
71 analysis of aqueous extracts of *croton zabensicus* and *vernonia amygdalina* singly and in
72 combination and investigate the acute toxicity of aqueous extracts of *croton zabensicus* and
73 *vernonia amygdalina*.

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75 **2. MATERIAL AND METHODS**

76 **2.1 Materials**

77 The fresh leaves of *Vernonia amygdalina* and *Croton zabensicus* were got from Lagos state
78 polytechnic; Ikorodu bush at early hour of the morning at about 7a.m and identified by a
79 botanist in the Environmental Biology unit, Biological sciences department of Lagos state
80 polytechnic; Ikorodu With reference number: Ipbh: 016/001, 002

81 The animals used for this study include six albino mice of both sex (14 – 22g) and twenty
82 male albino rats (80g - 200 g) were bought from Lagos State Teaching Hospital LASUTH in
83 Ikeja. They were acclimatized for two weeks and fed with standard rat feed obtained from
84 ZM veterinary store at Odogunyan, Ikorodu; Lagos and given clean and sterile potable
85 water.

86 **2.2 Phytochemical Screening**

87 The phytochemical analysis was carried out using the method described by (5). The plant
88 extracts were screened for the presence of Tannins, Saponin, Flavonoid, Glycosides and
89 Phenol.

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91 **Test for Tannins: 5ml of extract with the addition of 0.1% FeCl₃ reagent solution was made.**

92 **The formation of greenish black precipitate was observed and regarded as positive for the**
93 **presence of alkaloids in all the extracts.**

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95 Test for Saponin: 2ml of each of the extracts was added into 5ml of distilled water and both
96 were vigorously shaken with the application of heat. It was observed that saponin was
97 present in all the extracts.

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99 Test for Flavonoid: 2ml of sample extract was diluted with sodium hydroxide and
100 hydrochloric acid solutions respectively and filtered. The filtrate, zinc dust and concentrated
101 hydrogen chloride were added together and red colour formation as observed, showing that
102 flavonoids was present in all the extracts.

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105 Test for Glycosides: 2ml of extract with the addition of hydrochloric acid solution (HCl) was
106 neutralized with sodium hydroxide (NaOH) solution, then few drops of ferric chloride solution
107 (FeCl₃) was added as well with 1ml of concentrated H₂SO₄ sulphuric acid underlaid. A
108 reddish brown ring at the interface was observed, indicating the presence of Cardiac
109 Glycosides in all the extracts.

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111 Test for Phenol: 2ml of extract was added to 5.0ml of 95% ethanol; they were boiled in
112 waterbath for five minutes and filtered hot. 5.0ml of distilled water was added and the
113 ethanol was evaporated at a reduced pressure in the waterbath. The resultant concentrate
114 with the addition of five drops of 1% of Ferric Chloride and 1% Potassium Ferric cyanide
115 solution were added. A violet, wine, red, purple colour was developed, indicating a positive
116 test for phenolic compounds.

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119 2.3 Preparation of the Extract

120 The *Croton zabensicus* and *Vernonia amygdalina* leaves were sorted out to obtain only fresh
121 leaves and washed with distilled water without squeezing to remove debris and dust particle.
122 They were air dried separately and ground into powder; 100g each of the powered leaves
123 were soaked with distilled water and ethanol respectively. The solutions were stirred at 1
124 hour interval for 72hours and filtered using a filter paper to obtain aqueous extract of *Croton*
125 *zabensicus* (A1), and aqueous extract *Vernonia amygdalina* (B1). The filtrates were
126 centrifuged at 1000 revolution per minute for 5minutes to obtain a pure and clear
127 supernatant; the extract was then concentrated using a rotary evaporator and weighed, the

128 weight was used to calculate the percentage yield of extract. The concentrated extracts were
129 kept in a refrigerator below 4⁰C for preservation till use.

130 Calculations

$$131 \quad \% \text{ yield of extract} = \frac{\text{weight of concentrated extracts} \times 100}{\text{Weight of powered extract}}$$

133

134 1g each of the extracts A1 and B1 was weight and mixed together with 0.5ml of tween 80
135 and stirred with glass rod until they dissolved. The mixture was making up to 10ml with
136 normal saline.

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138 **2.4 Acute Toxicity Studies**

139 The median lethal dose (LD50) of the aqueous extract was estimated using Lorke's modified
140 method. Six albino mice were weighed and divided into two groups H and L of three mice
141 each and followed by administration of different doses of the extracts to two groups of three
142 albino mice each; H and L . In this phase, each mouse in group L was administered with
143 different low dose (500, 1000 and 2000) mg/kg of the combined extracts respectively;
144 whereas group H was administered with high dose of 3000, 4000 and 5000mg/kg of the
145 combined extracts respectively. The animals were observed for signs of toxicity; such as
146 respiration, activeness and death within 21 days.

147 Calculation

$$148 \quad \text{i. volume of extracts administered} = \frac{\text{weight of mice (g)} \times \text{dosage (mg/kg)}}{1000 \times \text{concentration of extracts (mg/ml)}}$$

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152 **2.5 Induction of Alloxan (Diabetes)**

153 Twenty (20) albino rats were used. The rats were divided into four groups of five rats each; A
154 – D. At the first day before induction; all the rats in the groups were tested for diabetes and
155 they were all negative. A (control), B – D groups were induced with diabetes
156 intraperitoneally using Alloxan Monohydrate sigma Uk with a standard dose of 150 mg/kg
157 body weight and a concentration of 50mg/ml. 1g of alloxan monohydrate was suspended in
158 20ml of 0.9% normal saline everyday for four days; however; 48hours later; At sixth day
159 Diabetes was confirmed using digital glucometer . Animals with blood glucose level \geq 200
160 mg/dl were considered diabetic and included in the study. Body weights of all animals in
161 each group were monitored using a digital weighing balance throughout the period of the
162 experiment; so as to calculate the volume of extracts to be administered each day.

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164 **2.6 Combined Extract and Drug Administration**

165 At the end of the nine days, the animals that tested positive for diabetes were fasted for 12
166 hours, and then the blood glucose level was checked and recorded and administered the
167 following drug and extracts orally as treatment once daily in a 24 hour cycle at 8: am for nine
168 days; B received (glibenclamide 10mg/kg body weight); C received (combined extract
169 1000mg/kg) and D receives (combined extract 500mg/kg of body weight).

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171 **2.7 Statistical analysis**

172 Data collected were expressed as mean, standard error of mean (SEM). Statistical
173 significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version
174 17.0 with Duncan's Multiple Range Test (DMRT) option. A value of $P < .05$ was considered to
175 indicate significant difference between groups.

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178 **3. RESULTS**

179 **Table 3.1 percentage yield of extracts**

Plant	Weight of powdered plant	Weight of concentrated extracts	% yield of extracts
A1	100g	9.26g	9.26%
B1	100g	9.99g	9.99%

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182 **3.2 Phytochemical analysis**

Sample	Tannin	Phenol	Saponin	Flavonoid	Glycosides
A1	+	++	++	++	+
B1	+	+	+++	+	+
A1+B1	++	++	+++	++	+

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187 **3.3 Acute Toxicity Studies.**

Mice	Body weight	Dosage	Volume	Respiration	Activeness	Death
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	(g)	(mg/kg)	(ml)			
L1	14.05	500	0.035	Normal	Very active	No death
L2	16.02	1000	0.080	Normal	Very active	No death
L3	20.04	2000	0.200	Normal	Very active	No death
H1	21.70	3000	0.326	Fast	Active	No death
H2	15.49	4000	0.309	Very fast	Weak	No death
H3	18.89	5000	0.472	Rapid pulse	Very weak	No death

188 Key

189 L1 : low dose (500mg/kg)

H1: high dose (3000mg/kg)

190 H2: high dose (4000mg/kg)

L2; low dose (1000mg/kg)

191 H3: high dose (5000mg/kg)

L3: low dose (2000mg/kg)

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194 **Table 3.4 Effect of treatment blood glucose**

GROUP	FBG0 (mg/dl)	FBG1 (mg/dl)	FBGL5 (mg/dl)	FBGL7 (mg/dl)	FBGL9 (mg/dl)
A	118.00±19.79 ^a	120.50±19.33 ^a	119.50±2.24 ^a	117.00±19.44 ^a	117.00±20.28 ^a
B	128±5.88 ^a	451.75±54.43 ^c	191.25±8.95 ^a	106.00±16.63 ^a	64.50±11.90 ^a
C	124.75±13.53 ^a	339.50±124.73 ^{bc}	204.25±131.77 ^a	194.00±134.95 ^a	182.50±139.72 ^a
D	122.75±16.35 ^a	278.25±136.80 ^b	214.5±139.23 ^a	204.25±139.39 ^a	194.75±141.89 ^a

195 Significant difference (p< .05) appears between groups with different superscript while

196 significant difference (p< .05) does not appear between groups with the same superscript.

197 n = 4;

198 Key

199 FBG0; Blood Glucose before alloxan induction

200 FBG1; Blood Glucose 48 hours after alloxan induction

201 FBG5 ; Blood Glucose after 5days Treatment

202 FBG7: Blood Glucose after 7days Treatment

203 FBG9: Blood Glucose after 9days Treatment

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207 **Table 3.5: Effect of treatment on body weight**

GROUPS	BW0 (g)	BW1(g)	BW9 (g)	CBW(g)	%WG
A	109.50±12.81 ^a	112.00±12.67 ^b	135.25±12.20 ^b	25.75±1.25 ^b	23.80±3.21
B	147.75±19.98 ^b	144.00±5.35 ^c	159.50±6.24 ^c	12.25±0.95^{ab}	8.22±0.40
C	103.75±5.56 ^a	101.75±5.73 ^{ab}	118.00±16.91 ^{ab}	14.25±15.90^{ab}	13.65±15.15
D	97.75±5.73^a	95.25±6.55^a	104.50±14.27^a	6.75±13.76^a	6.97±14.39

208 Significant difference ($p < .05$) appear between groups with different superscript while
 209 significant difference ($p < .05$) does not appear between groups with the same superscript, n
 210 = 4.

211 Key

212 BW0: body weight before induction

213 BW1: body weight after 48hour of induction

214 BW9: body weight after nine days treatment

215 CBW: change in body weight (BW9-BW0)

216 %WG: percentage weight gain

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218 **DISCUSSION**

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220 The result confirms that synergistic treatment of *C. zabensis* and *V. amygdalina* produced
 221 more antihyperglycemia properties with the high dose of 1000mg/kg of body weight when
 222 compared with a lower dose of 500mg/kg of body weight while the conventional
 223 antihyperglycemic drug like glybenclamide produced a hypoglycemic condition after ninth
 224 day of treatment. The rats were considered treated when their fasting blood glucose level
 225 returned to almost their basal blood glucose levels. The increase in blood glucose level of
 226 diabetic rats was found to have reduced after oral administration of synergistic aqueous leaf
 227 extract of *C. zabensis* and *V. amygdalina* and glibenclamide respectively and shows
 228 significant ($p < .05$) decrease in the blood glucose level. It was also observed that the rats in
 229 group B and C got treated quickly as compared to group D probably because the rats in
 230 group B and C were administered with an antidiabetic drug of a higher dose of the combined
 231 extract (1000mg/kg) respectively as compared with the rats in group D with a low dose of
 232 500mg/kg. The efficacy of the extract on hyperglycemic rats corroborates the result of other
 233 researchers who had systematically demonstrated that the extract from the plant *C.*
 234 *zabensis* and *V. amygdalina* have antidiabetic properties (4). Polyphenolics such as
 235 tannins and Saponins from several plant extracts had been shown to reduce blood glucose
 236 levels through inhibition of α - amylase and sucrose from the intestine. Flavonoids were

237 reported to regenerate the damaged pancreatic β -cells in diabetic animal (6). Diabetes is
238 also characterized by weight loss, alloxan administration brought about marked reduction in
239 body weights of experimental rats. These reduced body weights were found to have
240 increased significantly after the nine days treatment in group B, C and D. The percentage
241 weigh gain in group C (13.65%) seems to be prominent when compared with group A, group
242 B (8.22) and D (6.77). After nine days of treatment, this is an indication that the healing
243 process was slow in the plant extracts and faster in the glibenclamide (standard drug). The
244 facts that hypoglycemic drugs have side effects which can further complicate the health of
245 patients; however, the natural herbs can serve as compliments to hypoglycemic drugs (7).

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248 **4. CONCLUSION**

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250 It can be concluded that synergetic treatment of aqueous extracts of *croton zabensicus* and
251 *vernonia amygdalina* leaves as an antihyperglycemic agent in an alloxan induced diabetic
252 albino rats produced more competitive result with a dose of 1000mg/kg body weight.

253 This investigation has demonstrated that the use of combination of aqueous extract of the
254 two leaves of *C. zabensicus* and *V. amygdalina* is safe, effective, cheap and more
255 comfortable for the management of diabetes mellitus. Further research needs to be carried
256 out to ascertain the appropriate dosage in relation with the duration of the extracts
257 administration for treatment of hyperglycemia in order to prevent hypoglycemia; which is also
258 a concern. Considering the efficacy of the synergistic treatment of combined extracts;
259 awareness should be created to promote the medicinal advantage of the plants over a
260 standard drugs and a careful selection should be made following a thorough toxicity
261 investigation; when combining two or more medicinal plants for treatment; so as to prevent
262 complications; bearing in mind that some could be contradicting or antagonizing each other.

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266

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269 this research

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271 **Ethical Approval:**

272 As per international standard or university standard written ethical approval has been
273 collected and preserved by the authors.

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