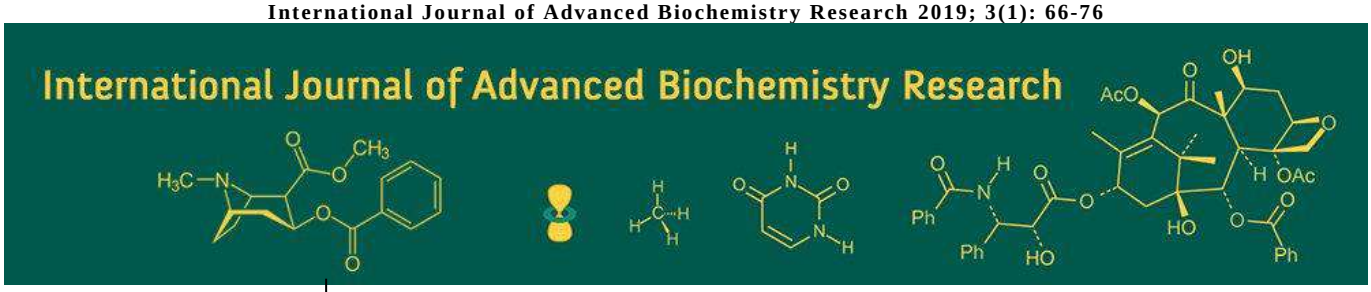

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Evaluation of *in vitro* anthelminthic, antiproliferative and antihypertensive potentials of *Rauwolfia vomitoria* Afzel. (Apocynaceae) leaf extract

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Abstract
Background: *Rauwolfia vomitoria* Afzel. (Apocynaceae) leaf is used to treat various diseases such as ascariasis, cancer, hypertension, other cardiovascular diseases, and various diseases in traditional medicine in Nigeria. This study evaluated the anthelminthic, antiproliferative and antihypertensive potentials of *R. vomitoria* leaf methanol extract in Wistar rats. In addition, the phytochemical, acute toxicity, total phenolic and flavonoid contents were also evaluated.
Results: Qualitative phytochemical screening revealed the presence of various metabolites with flavonoids and alkaloids the most predominant, with different amount of total phenolic and flavonoid contents per gallic acid and rutin equivalent respectively. NMR and GC-MS studies on the *R. vomitoria* showed the presence of methyl stearate ester a fatty acid ester. *In vitro* antioxidant activity by inhibition of DPPH and H_2O_2 scavenging radicals showed an IC_{50} value of 1.614 mg/mL and 0.101 mg/mL Acute toxicity investigation showed that extract is well tolerated at doses up to 5,000 mg/kg body weight RVE after two weeks. Anthelminthic and antiproliferative effects of the RVE showed dose-dependent activities in time of paralysis/death of earthworms and growth of guinea corn radicles respectively. The blood pressure reduced significantly in normotensive and RVE-treated hypertensive rats in dose-dependent fashion. There was significant dilation of the aortic blood vessel at high concentration of RVE with reduced heart rate and complete blockage of adrenaline and $CaCl_2$ stimulatory effects.
Conclusions: This study provided the justification that *R. vomitoria* leaf extract possessed anthelminthic, antiproliferative and antihypertensive potentials, hence, its use as an ethnomedicinal prescription for these conditions in traditional medicine. It further provides an avenue for new drug discovery.

Keywords: *Rauwolfia vomitoria*, anthelminthic, antiproliferative, antihypertensive

Introduction
Despite the recent advances in orthodox medicines globally, herbal medicines from traditional plants still occupy salient positions in a predominant population of the developing world aa well as among developed nations of the world. This is because, herbal medicines, in addition to being cheap in price and easily accessible, have been used for decades without having little or side effects when used in disease conditions. Similarly, the multitarget effects of medicinal plant-derived drugs (holistic approaches) are the basis of their utilization as has been used in traditional systems of medicine like Ayurveda, which is being practiced even in the western world's [1-3]. The knowledge on the use of medicinal plants has resulted in the discovery of lead active novel compounds by natural product researchers and pharmacognocists that have been used in the production of many orthodox drugs by pharmaceutical industries used as analgesics, anticancer, anti-inflammatory, antimalarial, anthelminthic, and antioxidant agents [1, 4].
For instance, helminths are pathogenic organisms which are of global concern owing to their parasitic activities in humans. It is estimated that over a billion people, mostly from developing nations, are reported to be infected with various class of helminths, resulting in colossal economic losses as well as various forms of diseases ranging from malnutrition to deaths. Despite the use of few numbers of conventional anthelmintic drugs to control helminths parasites, their efficacies have been reduced by the threat of development of resistance to these drugs by the parasites during treatment.

Hence, the need to seek for novel and alternative drugs from medicinal plants for the control of helminths are urgently required [5, 6].

Moreover, out of over the 500,000 plant species in the world, only less than 10% have been fully studied for their chemical and pharmacological potentials [7]. Very few of these plants were screened for their antiproliferative or growth inhibitory effects. Nowadays, the antitumor or anticancer agents have been one of immense benefits of plant-derived drugs, from where conventional drugs such as vincristine, vinblastine, taxol, as well as camptothecin were discovered for their cytotoxic effects on cancer or tumor cells. The need for continue search for improved anticancer or antitumor agents from medicinal plants is gaining global recognition due to sides effects from existing drugs used for cancer or tumor case [8, 9].

Similarly, medicinal plants have played crucial roles in the management of various cardiovascular diseases including high blood pressure (hypertension). Cardiovascular diseases have been reported as one of the leading causes of deaths worldwide, with hypertension being the most prevalence of them leading to some clinical pathologies like strokes, kidney disease, and myocardial infarction [10-12]. A report by the WHO [13], showed that about 600 million people suffer from high blood pressure (i.e., above 10% of the world's population), and have been on various types of synthetic cardiovascular drugs which have been beneficial but with several side effects. Plant-derived antihypertensive agents and many medicinal plants parts have little or no side effects when used high blood pressure case [14, 15], of which one of the plants is *Rauwolfia vomitoria* Afzel.

Rauwolfia vomitoria is a shrub or tree which grows to a height of over 10 m found in some West African countries like Southern Nigeria, Senegal, Cameroon, Ghana, and across other African countries like Egypt, Sudan, Uganda and Zaïre [16]. It is called '*'akanta''* in Igbo language in Nigeria. The plant has been used for various purposes in traditional medicine in southern and northern Nigeria as an abortifacient, antihypertensive, anthelminthic, antitumor, antirheumatic, antidote as well as treatment of fevers. Various types of metabolites have been isolated from the leaf, stembark and root using different types of chromatographic procedures. These metabolites include: alkaloid (e.g., reserpine), flavonoids, cardiac glycosides, and saponins [17-20].

Despite the vast usefulness of *R. vomitoria* in traditional medicine, there are no scientific data to justify the use of its leaf extract as anthelminthic, antiproliferative and antihypertensive agents. Therefore, the present study investigated these claims which may serve as a source towards new drug discovery.

Materials and Methods

Chemicals and reagents

All chemicals used in this study were of analytical grades, and includes the following: Albendazole (Afbend) and Lisinopril were purchased from Chanrai Healthcare Nigeria Ltd, methanol, gallic acid, rutin, DMSO, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, $AlCl_3$ and NaOH were purchased from JoeChem Nig. Ltd manufactured by Sigma Chemical Co. (St. Louis, MO), among others.

Collection of plant material and preparation of extract

Fresh leaves of *R. vomitoria* were collected in the morning hour from a forest at Eburu-mmiri, Nsukka, Enugu State, Nigeria during the month of September, 2021. It was identified and authenticated by Mr. C.A. Ukwubile of the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri. A voucher specimen number of *UMM/FPH/APN/003* was deposited for the plant at the herbarium. The dried powdered leaves weighing 1000 g were extracted by cold maceration technique in methanol (80% v/v). It was then filtered through Whatman no 1 filter paper into a clean 500 mL conical flask to obtain a clear filtrate. Finally, the filtrate was concentrated *in vacuo* using rotary evaporator to obtain a dark colored gel-like extract. The extract was then weighed and the% yield calculated as 6.28%. It was then stored in a refrigerator (temp. 40 ^{o}C) until when needed [21].

Qualitative preliminary phytochemical screening of extract

In an attempt to determine the presence of some metabolites such as alkaloids, flavonoids, triterpenes, saponins, tannins, cardiac glycosides, and anthraquinones in the extract, standard procedures were followed [22, 23].

In vitro study

Collection and identification of earthworms

Adult earthworms (*Pheretima posthuma*) measuring 4-8 cm long, were collected from water-logged soil in the evening hour at Fori-Maiduguri, Nigeria, and kept a beaker filled with moist soil from their habitat. They were identified by Mr. M.S. Bingari of the Department of Biological Sciences, Taraba State University, Jalingo, Nigeria.

Anthelminthic evaluation of extract

The Anthelmintic activity of the extract was evaluated following the procedures described by [24, 25], with slight modifications. Briefly, adult earthworms (*Pheretima posthuma*), were grouped into six groups of five worms of nearly same sizes. Group I served as the normal control, which received distilled water; Group II served the positive control, which received standard anthelminthic drug Albendazole (20 mg/mL), and the remaining groups received 1.25, 2.5, 5, 10 & 20 µg /mL concentrations of *R. vomitoria* extract (RVE) each. The earthworms were observed for the time of paralysis (i.e., when there are no pronounced movement) and deaths (i.e., when there is color change on their bodies) of each worm [26].

Antiproliferative evaluation of *R. vomitoria* extract

Preliminary antiproliferative effects of RVE was evaluated with slight moderations, using the methods previously described by [27]. Briefly, healthy seeds of guinea corn (*Sorghum bicolor*) were purchased from a local market in Maiduguri, and tested for viability by pouring them in a beaker filled with clean water. The viable seeds (i.e., those that do not float on the water) were selected and washed with ethanol (99.5% v/v) for 5 min in order to decontaminate the seeds prior to use. Six petri dishes, 9 cm in diameter were underlaid with cotton wool and filter papers, where each poured onto with 20 selected seeds of *S. bicolor*. Then, various concentrations of RVE (10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL, and 160 µg/mL) were poured into the petri dishes and the control petri dish contains 10 mL of distilled. The petri dishes were then incubated in dark cupboard for 96 h. The lengths of S. bicolor emerging radicles were then measured in 24, 48, 72, and 96 h.

Evaluation of DPPH free radical scavenging activity of RVE

The free radical scavenging activity of the RVE was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reagent following the methods described by [28]. Briefly, 5 g of RVE was dissolved in 100 mL methanol. Then, various RVE concentrations were added to 2.5 mL of DPPH solution and kept in the dark. The absorbance was measured after 30 min at 517 nm wavelength using the Multiskan SkyHigh Microplate UV/Vis spectrophotometer (Thermo Fisher Scientific Inc., MA, USA). The control was vitamin C. The% inhibition of DPPH free radical scavenging ability of the RVE was calculated with reference to the control.

Determination of total the flavonoid and phenolic contents of RVE

The total flavonoid and total phenolic contents of *R. vomitoria* leaf extract was determined following the methods described by [29, 30].

***In vitro* antihypertensive study of RVE**

Angiotensin converting enzymes (ACE) inhibitory assay

In an attempt to determine the ACE inhibitory effect of the RVE, the method previously described by GAO *et al*. [31], was used with slight modifications. Briefly, a reaction mixture containing 50 µL of 8 Mm hippuryl-L-histidyl-L-leucine (HHL) as a substrate, 100 µL of ACE solution and 50 µL of RVE was prepared. It was incubated at 37 ^{O}C for 1 h in an incubator. To terminate the reaction, 250 µL of 1 N HCl solution in order to form hippuric acid. The hippuric acid was then extracted with 3 mL ethyl acetate (Sigma Aldrich, St. Louis MO), and evaporated on a water bath to remove the ethyl acetate portion. The hippuric acid was then redissolved in a 20 mL beaker, and various concentrations of RVE 10, 20, 30, 40, and 50 mg/mL was each added [32]. Then the absorbance of each was measured at 228 nm wavelength using UV-vis spectrophotometer (Agilent technologies, UK). The ACE inhibitory effect of the RVE was evaluated using the formular below:

% Inhibitory activity of RVE = (AbsC-AbsS/AbsC-AbsB) 100, where;

AbsC = absorbance of control, AbsS = absorbance of sample (extract), and AbsB = absorbance of blank.

***In vivo* antihypertensive study of RVE**

Experimental animals

Thirty Sprague Dawley rats weighing 150-210 g of both sexes was used. They were kept in metal cages in standard laboratory conditions, with free access food and water *ad libitum*. The approval guidelines by the Animal Ethical Committee of PJ Rats Tailor, Jos, Nigeria. The experimental conditions are in compliance with the provisions of the National Institute for Health [33].

Evaluation of hypotensive activity of RVE in normotensive rats

The rats were grouped into three of five rats per group. The groups I, II, and III were administered 250, 500 and 1,000 mg/kg of RVE respectively. Blood pressures of the rats were taken at 0, 1, 3 and 6 hours using non-invasive blood pressure technique [34, 35]. The systolic blood pressure (SBP) and mean blood pressure (MBP) interpretations were made from pulse tracings, while, the diastolic blood pressure (DBP) was determined using the equation below:

DBP = (3MBP–SBP) ÷ 2

Evaluation of antihypertensive effect of RVE in glucose induced hypertensive rats

In evaluating this, the methods by [34, 35], were adopted with modifications. Briefly, the rats were further grouped into five groups of three rats. Group I was the control which was administered 10% Glucose-D solution (Nutra Healthcare, India) for three weeks, group II rats were administered 10 mg/mL Lisinosyn (Synermed Nig. Ltd.), while groups III, IV, and V were given 10% glucose solution plus 10, 20 and 40 mg/kg body weight (b.w.) RVE (p.o.) respectively, with free access to food and water. The blood pressures of and heart rate of the animals were determined at 0, 3, 6, 9, 12, 15, 18 and 21st days, using noninvasive procedure.

Evaluation of RVE effect on aortic blood vessel from rat's heart

Five Wistar rats of both sexes were injected with 1000 units heparin (i.p.) before dissection in order to prevent clothing of the blood [36], and partially anaesthetized using 0.1 mL diazepam (Biopharma, Nig. Ltd). The chest cavity was exposed using sterilized dissecting kits and the heart was carefully removed from the pericardial chamber. Then, the aorta a normal major blood vessel was immediately isolated and clamped to the cannulated ends of a micro dynamometer (model 7050). At intervals of 0, 2, 4,8 and 10 min, various concentration of RVE (0.1 µg/mL, 0.2 µg/mL, 0.4 µg/mL, 0.8 µg/mL and 1 µg/mL were applied in order to assess the heart rate and the rate of dilation of the aorta. Lisinopril was used the control drug. The mechanism of action of the RVE was studied as well by injecting various doses of adrenaline (Vixa Pharmaceutical Co. Ltd) and $CaCl_2$ (JoeChem, Nig., Ltd). Finally, the animals were chemically sacrificed under chloroform. The readings obtained from the changes in heart rate and dilation of the aortic blood vessel were expressed as means ± SD.

Statistical analysis

The results obtained were expressed as means ± SD. Data were expressed as $p< 0.05$ (one-way ANOVA) and considered statistically significant. Analysis of data was done using Microsoft excel version 2019 and SPSS version 23 software (SPSS Inc.).

Results

Phytochemical contents

The results on preliminary phytochemical evaluation showed the presence of various metabolites these are: carbohydrates, alkaloids, flavonoids, triterpenes, cardiac glycosides, and fixed oils (Table 1). From the results, carbohydrates, alkaloids, flavonoids and triterpenes were detected in large amount, whereas cardiac glycosides and fixed oils were the least.

Table 1: Phytochemical contents of *R. vomitoria* leaf methanol extract

Phytoconstituents	Test	Inference
Carbohydrates	Molisch's	++
	Fehling's	++
Alkaloids	Meyer's	++
	Dragendorff's	++
	Wagner's	++
	Tannic acid	++
Saponins	Frothing	-
	Hemolysis	-
Tannins	$FeCl_3$	-
Flavonoids	NaOH	++
	Shinoda's	++
	Lead acetate	++
Anthraquinones	Borntrager's	-
Cardiac glycosides	Keller Kiliani's	+
	Kede's	+
Triterpenes	Liebermann's	+
Fixed oils/fats	Spot	+
Note: (not detected), + (detected in small amount), ++ (detected in large amount).		

Anthelmintic evaluation of RVE

The *in vitro* anthelmintic activity of the methanol extract of *R. vomitoria* against the earthworm; *Pheretima posthuma*, is presented in Fig. 1 below. Earthworms in the control Petri dish survived throughout the periods of the study. The RVE induced a concentration-dependent time of paralysis (TOP) and time of deaths (TOD) in the treated groups within 120 min of the experiment. The standard drug albendazole showed the most potent activity against the worms with TOP value of 2.04 ± 0.01 min and TOD value of 3.16 ± 0.02 min. These values were not statistically significant from the extract ($p < 0.05$).

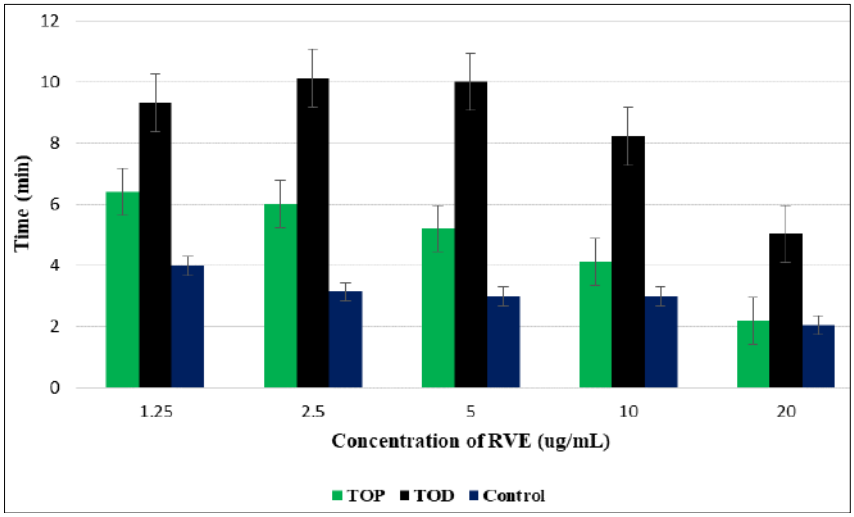


Fig 1: *In vitro* anthelminthic effects of RVE after 120 min against the earthworm (*Pheretima posthuma*). TOP; time of paralysis, TOD; time of death. Albendazole was used as the standard drug. Results are means ± SE (n = 3), +values of $p < 0.05$ are significant (one-way ANOVA).

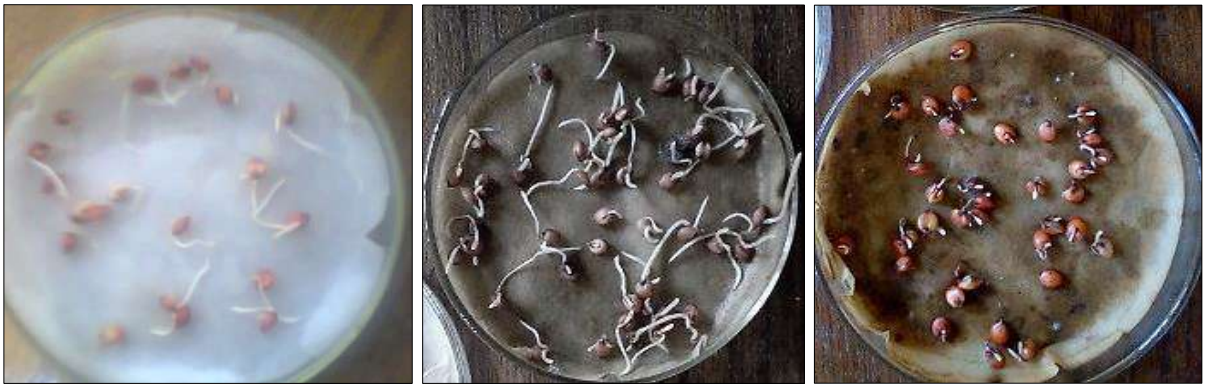
Antiproliferative effects of RVE on *S. bicolor* radicles

The RVE produced a concentration-dependent reductions in the lengths of *S. bicolor* radicles. The results revealed that the control petri dish had the radicle length of 1.54 ± 0.01 mm at highest concentration of 160 µg/mL after 120 h incubation, while the RVE produced growth inhibitory effect of 0.12 ± 0.01 mm under the same conditions. These reductions in the length of radicles were sustained in the treatment groups until 120 min with much reduction at 160 µg/mL RVE (Table 2; Fig. 2).

Table 2: Effects of RVE on guinea corn (*S. bicolor*) radicles after 120 min (n = 3)

Conc. (µg/mL)	Length of radicles (± SE mm)				
	24	48	72	96	120
D/H_2O	1.28 ± 0.01	1.38 ± 0.20	5.88 ± 0.01	8.82 ± 0.20	8.98 ± 1.12
10	0.12 ± 0.11*	2.10 ± 0.01*	3.23 ± 0.02*	4.12 ± 0.21*	4.86 ± 1.24*
20	1.10 ± 0.01*	1.24 ± 0.20	1.33 ± 0.01*	1.54 ± 0.20*	1.98 ± 0.01*
40	0.62 ± 0.20*	0.64 ± 0.01*	0.68 ± 0.14	0.88 ± 0.01*	1.01 ± 1.01*
80	0.42 ± 0.01	0.44 ± 0.20	0.47 ± 1.10*	0.52 ± 0.40*	0.58 ± 1.01*
160	0.10 ± 0.02*	0.12 ± 0.01*	0.16 ± 1.02*	0.20 ± 0.01*	0.18 ± 0.02*

Values are expressed as means ± SE; n = 3. * Values are significantly different at $p < 0.05$ (one-way ANOVA).



a) Control plate **b**) 40 µg/mL RVE **c**) 160 µg/mL RVE

Fig 2: Effects of *R. vomitoria* leaf extract of *S. bicolor* radicles at certain concentrations.

Antioxidant effects of RVE by DPPH scavenging and H_2O_2 reducing power

The results showed that there were increase in the% inhibition in DPPH free radical scavenging activities and H_2O_2 reducing power as the concentrations of extract increase (Fig 3). The study revealed that the RVE produced the highest antioxidant activity against DPPH with an IC_{50} value of 0.61 ± 0.01 µg/mL, while, it showed an IC_{50} value of 1.10 ± 0.20 µg/mL against H_2O_2.

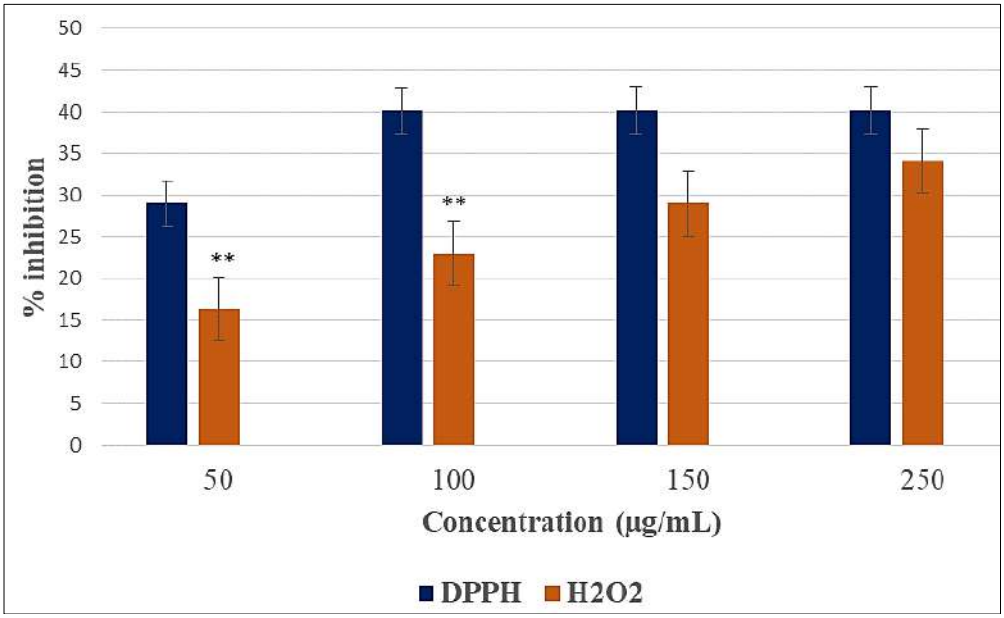


Fig 3: Effect of RVE on DPPH scavenging activity and H_2O_2 reducing power. Data are means ± SD for n = 3. ** Values of $p < 0.05$ versus ascorbic acid are statistically significant (one-way ANOVA). Ascorbic acid was used as the control.

Total flavonoid and phenolic contents

The evaluation of total flavonoid contents (TFC) by $AlCl_3$ colorimetric assay of RVE showed that the total flavonoid contents was 135.42 ± 0.22 mg rutin E/ g DW, whereas, the total phenolic contents (TPC) was found to be 208.82 ± 0.42 mg GAE/g DW.

ACE inhibitory effect of RVE

ACE is a central part of the renin-angiotensin system (RAS), which regulates body fluid volume thereby, controlling blood pressure. The result showed that the RVE inhibited ACE activity in concentration-dependent fashion. The highest percentage inhibition was obtained at 50 mg/mL of RVE, while the lowest was at 10 mg/mL of RVE (Fig. 4). This result was statistically significant versus the control ($p < 0.05$; one-way ANOVA).

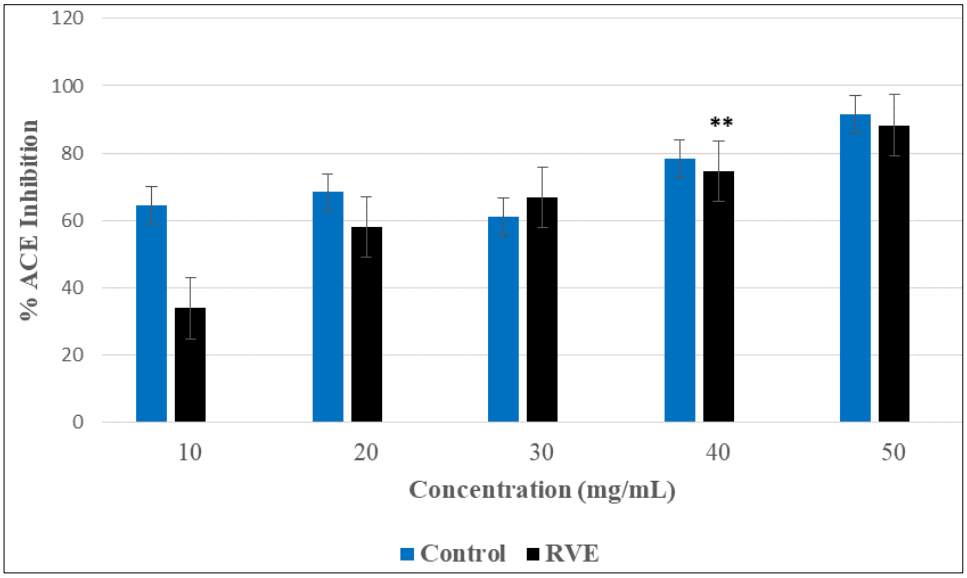


Fig 4: Effect of RVE on ACE at various concentrations. Results are means ± SD (n = 3), ** $p < 0.05$ (one-way ANOVA) versus control. The control and the RVE had an IC_{50} values of 5.47 ± 0.01 µg/mL and 12.01 ± 1.02 µg/mL respectively.

Evaluation of hypotensive effect RVE in normotensive rats

The results revealed that the extract showed a significant reduction in systolic blood pressure (SBP) at various concentrations and mean blood pressure (MBP) after one hour. There was also significant reduction in the diastolic blood pressure (DBP) within 1 and 3 hours. This hypotensive effect of the RVE reduced significantly at 3 to 6 h ($p < 0.05$; one-way ANOVA). These decreases were dose-dependent, with reductions in heart rate at the same time intervals (Fig. 5).

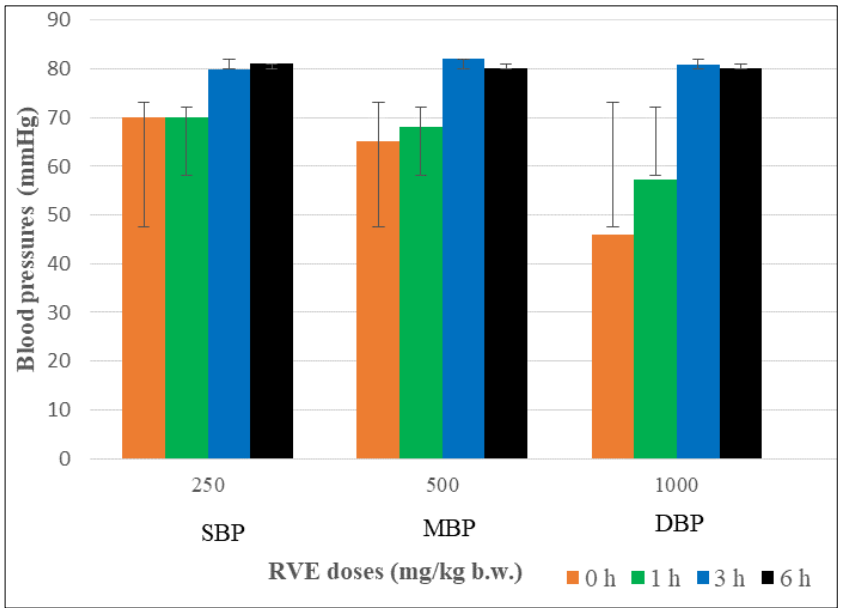


Fig 5: Effects of various doses of RVE on systolic blood pressure (SBP), mean blood pressure (MBP) and diastolic blood pressure (DBP) in normotensive rats after 6 hours. Values are means ± SD, $p < 0.05$ versus control (one-way ANOVA).

Evaluation of antihypertensive effect of RVE in rats

The results in Figs. 6 & 7 below, showed that the RVE at the highest dose of 40 mg/kg b.w. significantly prevented the increase in SBP, MBP, DBP as well as heart rate of the glucose-induced hypertensive Wistar rats especially at third week of the study when compared to the standard drug lisinopril.

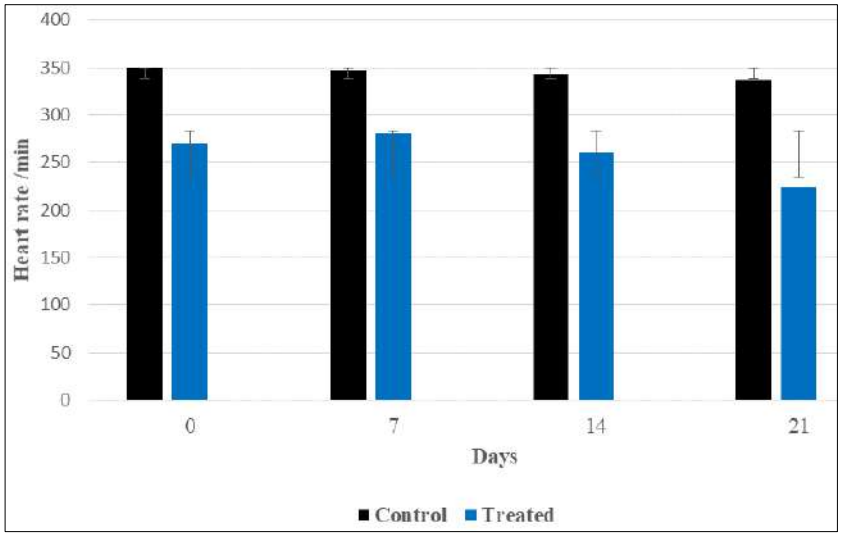


Fig 6: Effect of RVE on heart rate of normotensive rats after 21 days, [a] $p < 0.05$ versus control drug.

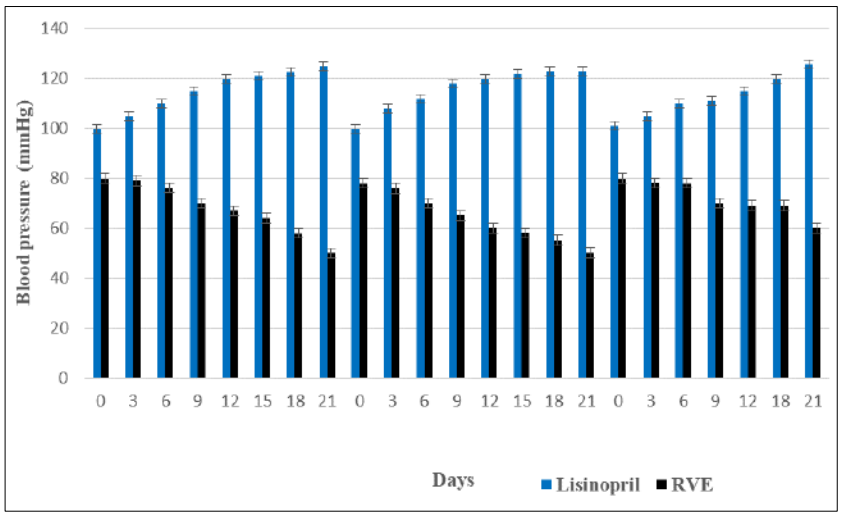
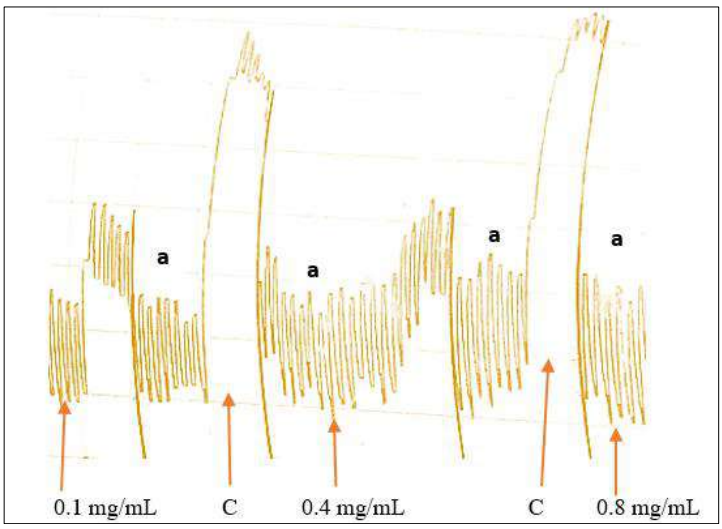


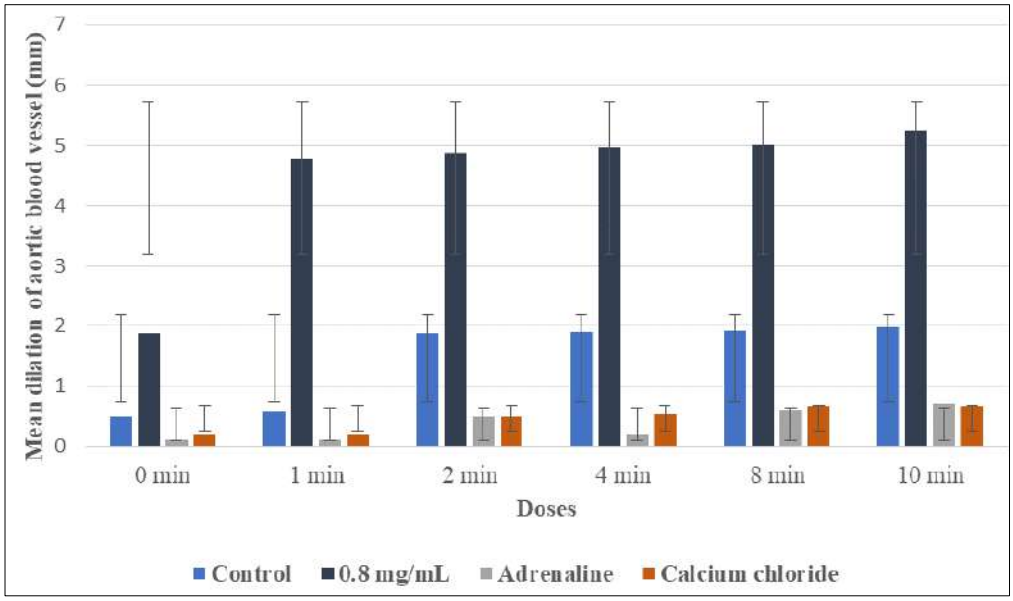
Fig 7: Effect of RVE on glucose-induced hypertensive rats; A: systolic blood pressure, B: mean blood pressure, C: diastolic blood pressure. Results are means ± SD, $p < 0.05$ is significant compared with the control.

Effects of RVE on rats' aortic blood vessel, adrenaline and $CaCl_2$

The results in Fig. 8 (a and b) revealed that RVE significantly increases the dilation of the aortic blood vessel in dose-dependent fashion from 0.1 – 0.8 mg/mL but decreases the rate of heart rate and contraction. The extract also exerts complete blockage on the effects of adrenaline (10^5 M) and calcium chloride (10^5 M) channels at 0.8 mg /mL b.w. in the rats, with reduced cardiac muscle contraction and heart rate in 10 min.



(a)



(b)

Fig 8: Effects of RVE on: (a) aortic blood vessel as recorded by micro dynamometer, (b) adrenaline and calcium chloride channels (results are means ± SD, while $p < 0.05$ is significant versus control), where a: aortic blood vessel dilations at various concentrations of RVE, C; control drug.

Discussion

Traditional medicines have been used since time immemorial in the Nigeria's healthcare system. These medicines from medicinal plants have been of immense benefits in all ramifications from health to economic points of view. This is because, natural medicines are cheap, easily accessible, and almost zero toxicity, which conferred some comparative advantages over orthodox medicines [37]. The quest for research on medicinal plants with potential pharmacological actions against diseases like helminthiasis, cancer, tumor and hypertension as well as other cardiovascular diseases has become very necessary in recent times, owing to the prevalence of these diseases globally.

The leaf of *Rauwolfia vomitoria* has been used for the treatment and managing several diseases from centuries in African traditional medicine ranging from fevers to more severe illness like helminthiasis, cancers and cardiovascular diseases [38]. Despite its uses, the potentials of the methanol leaf extract as anthelminthic, antiproliferative and antihypertensive agent has not been evaluated, hence, the purpose for this current study. Our study revealed that the *R. vomitoria* extract (RVE) contains various phytochemicals that facilitated the rapid time of paralysis and death of the earthworms (*P. posthuma*) in dose-dependent fashion (Table 1, Fig. 1). Although, the standard drug (Albendazole) showed lesser times of these parameters than the RVE, yet, the results were significantly different ($p < 0.05$; one-way ANOVA). It was observed from the anthelminthic study that the RVE firstly induced directionless rapid and restive movements in the worms before paralysis and death. It is possible the extract might have created burning sensation in the cuticle of the worms in order to penetrate the hemocoel, which resulted in the observed restive motions by the worms. For instance, carbohydrates found in this extract are readily metabolized, and the glycogen content of the helminths is high. This was also due to the presence of certain metabolite like alkaloids in the RVE which are usually associated with various degrees of sensations [39, 40].

On this current antiproliferative study, guineacorn (*S. bicolor*) seeds were used because of the ability of the seeds to germinate in an exponential rate within 24 hours under favorable conditions. This behavioral growth pattern likens the seeds of *S. bicolor* to cancer cells. Therefore, the ability of the RVE to slow down the growth of *S. bicolor* radicle in this study showed that it is capable of inhibiting the proliferative effect of cancer cells. The study further revealed that extract showed a concentration-dependent inhibition of the length of radicles within 120 h (Table 2, Fig. 2). Reports have shown that growth of most cancer cells are inhibited in the presence of chemotherapeutic agents in order to prevent metastasis [41]. This is likely the case of *S. bicolor* radicles in the current study.

In this current study, the reduction of the DPPH and hydrogen peroxide free radical scavenging activities by the extract showed that the plant is capable of reducing the negative effects of reactive oxygen species (ROS) in the body (Fig. 3). From our study, the amount of total flavonoid content of 135.42 ± 0.22 mg rutin E/ g DW and total phenolic content of 208.82 ± 0.42 mg GAE/g DW were obtained as determined by spectrophotometric method. There are good relationships between the total phenolic and flavonoid contents with antioxidant potentials of plant extract [42]. For instance, phenolic compounds can reduce free radicals scavenging activity, and they possess various biological functions as anticancer, anti-inflammatory, antioxidant, antihypertensive and hepatoprotective [43]. It is therefore, important to mention that *R. vomitoria* leaf methanol possessed similar functions. Similarly, cancer cells possess higher amount of ROS because of its higher rate of growth, which is different from the normal cells [44]. It has been reported that increased level of ROS is necessary for the growth of cancer cells, proliferate and invade other cells. It is possible that antiproliferative and antioxidant activities of the RVE in this current study was due to the presence of metabolites such as alkaloids and flavonoids in the plant that altered the redox balance necessary for the growth of *S.*

bicolor radicles and free radical scavenging activities of DPPH and H_2O_2.

In the current study, *in vitro* antihypertensive evaluation of *R. vomitoria* leaf methanol extract (RVE) was determined using the angiotensin converting enzymes (ACE) inhibitory assay model. It has been reported that ACE controls the blood pressure by converting angiotensin I to angiotensin II the active vasoconstrictor, thereby causing the blood vessels to narrow [45]. Our study showed an elevated percentage inhibition of ACE activity by RVE in concentration-dependent manner with an IC_{50} value of 12.01 ± 1.02 µg/mL (Fig. 4). It possible that the RVE inhibited the hypertensive activity of the ACE by preventing the conversion of the hormone angiotensin I to the active vasoconstrictor angiotensin II, thus, acting as an ACE inhibitor. Similarly, the potential of the extract to control hypertension was evaluated using glucose-induced hypertension model by dissolving 10% glucose-D in the drinking water of rats. From our study, the extract showed concentration-dependent antihypertensive effect. Much reductions were observed at 1000 mg/kg b.w. RVE. There were no significant changes in the systolic, mean and diastolic blood pressures (SBP, MBP and DBP) of the rats at 3 and 6 h (Fig. 5).

Also, there were reductions in heart rate of normotensive rats in dose-dependent fashion and decline in the values of SBP, MBP and DSB after 21 days administration of RVE (Fig. 6, Fig. 7). These observations could be attributed to the stimulating effect of RVE to release the neurotransmitter acetylcholine (ACh). For instance, acetylcholine has been reported to decrease the rate of heart beat thereby, decreasing the force of contraction of the heart. These chemical substance (ACh) lowers the blood pressure by stimulating the endothelium NO-dependent vasodilation in resistance arterioles [46, 47]. The RVE must have triggered the release of ACh due to the presence of metabolite like alkaloids. For example, reserpine a well-known alkaloid isolated from *Rauwolfia serpentina* was reported to work by slowing down the actions of the nervous system, thereby reducing the heart rate and relaxing the blood vessels [48]. The presence of alkaloids in large amount from *R. vomitoria* leaf methanol extract could be responsible for the result in the current study. Moreso, the dilation of aortic blood vessel in this current study was dose-dependent with maximum dilation witnessed at 0.4-0.8 mg/mL in 10 min as recorded by micro dynamometer while, the standard drug lisinopril does not record significant dilation of the blood as recorded by the micro dynamometer (Fig. 8 a).

In the current study, the beta-adrenergic blocking effect of the RVE was evaluated using adrenaline (10^5 M). Our result revealed that the extract blocked the hormonal-stimulating effect of adrenaline indicating the involvement of the beta-adrenergic receptor in its mechanism. The study further showed that the extract also blocked the calcium channel activity by blocking the stimulating effect of $CaCl_2$ (10^5 M), hence, the RVE interferes directly with the activity of this compound (Fig. 8b). It is also possible that the release of ACh effected the dilation of the aortic blood vessel through various mechanisms like activation of endothelial nitric oxide synthase and prostaglandin (PG) production [49]. Similarly, the reduction of heart rate in the current study could also be attributed to the fact that the vasodilation of blood vessel causes a reduction in systemic vascular resistance as well as an elevated blood flow, thereby lowering the blood pressure [49, 50]. The results obtained from this current study showed that *R. vomitoria* exhibited dose-dependent activities.

Conclusion

From our study, it is concluded that the pharmacological actions of the *R. vomitoria* evaluated in this current study such as anthelminthic, antiproliferative and antihypertensive activities may be due to the presence of various class of secondary metabolites like alkaloids, flavonoids and carbohydrates. The study showed that the extract exhibited its effects in dose-dependent fashion. The antihypertensive effect of the extract was achieved through the release of acetylcholine which facilitated the dilation of aortic blood vessel, lowers the heart rate, and inhibited the activities of angiotensin converting enzyme I. Our study finally revealed that the extract is an adrenergic and calcium channels blocker. Finally, the data obtained from this study, showed that *R. vomitoria* leaf extract may be serve as an ethnomedicinal prescription for helminthiasis, cellular proliferation and hypertension. There is the need to isolate and structurally elucidate these compounds responsible for the observed biological activities and determine their mode of actions.

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