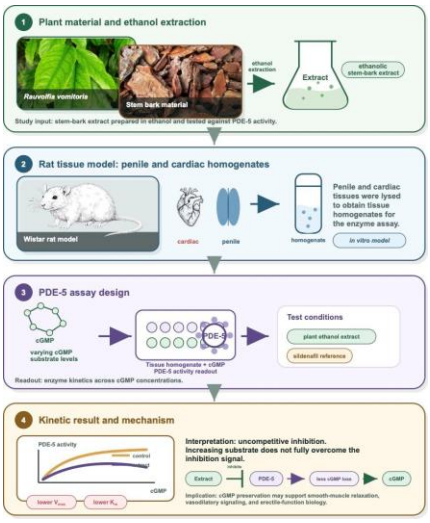
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In vitro Inhibition of Phosphodiesterase-5 Activities by Ethanol Extract of *Rauwolfia vomitoria* Stem Bark and its Therauptic Effect on Penile and Cardiac Tissue

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Abstract: *Rauwolfia vomitoria* (swizzle stick) is known to possess aphrodisiac potentials which inhibits or reduce the activities of phosphodiesterase-5 hence promoting erectile functions. Phosphodiesterase-5 (PDE-5) is a marker for erectile dysfunction and vasodilation of coronary and pulmonary arteries. This study assesses the impact of *Rauwolfia vomitoria* stem bark ethanol-based extracts on the activity of phoshodiesterase-5 in cardiac and penile tissue. The penile and cardiac tissues of 20 male Wistar rats were removed and homogenized after they were sacrificed by cervical dislocation. The phosphodiesterase-5 activity of the tissue homogenate were evaluated at varying concentrations of cyclic GMP (cGMP) and in the presence of standard drug (sildenafil) and ethanol extract of *Rauwolfia vomitoria.* Ethanol extract of *Rauwolfia vomitoria* was discovered to uncompetitively inhibit phosphodiesterase-5 activities in these tissues as a result of it reduced V_{max} and K_m obtained. This uncompetitive inhibition indicates that at high concentration of substrate, the inhibition of PDE-5 activity cannot be reversible. Hence, ethanol extract of *Rauwolfia vomitoria* may be effective in the inhibition of Phosphodiesterase-5 and consequently, erectile dysfunction. It may also improve vasodilation and may help in the treatment of cardiovascular diseases.



Keywords: Phosphodiesterase-5, *Rauwolfia vomitoria,* Penile, Cardiac, *in vitro*

1. Introduction

Although PDE-5 is highly expressed in penile tissue, it is also present in vascular smooth muscle cells and cardiac tissues. In the cardiovascular system, the NO–cGMP pathway regulates vascular tone, endothelial function, platelet aggregation, and myocardial contractility. PDE-5 modulates vascular resistance by controlling cGMP levels in smooth muscle cells. Inhibition of PDE-5 results in increased cGMP accumulation, leading to relaxation of vascular smooth muscle and vasodilation, thereby reducing systemic and pulmonary vascular resistance (Roy et al., 2023).

The dual involvement of PDE-5 in penile and cardiovascular systems underscores its clinical and pharmacological importance. Its inhibition has proven effective in: restoring erectile function in ED, managing pulmonary hypertension, and potentially improving cardiac performance and vascular health (Corinaldesi et al., 2015). The discovery of PDE-5 inhibitors was initially linked to cardiovascular research (e.g., angina treatment), but their profound effect on penile erection led to their widespread use in ED management Dhaliwal and Gupta, 2023).

Erectile dysfunction is a common form of male sexual dysfunction and is closely associated with impaired nitric oxide–cGMP signaling in penile smooth muscle. Semen parameters such as sperm concentration, morphology, and mobility are commonly employed as a stand-in for male fecundity in pregnancy risk assessments (Abah et al., 2023). Erectile dysfunction may occur as an adverse effect of selective serotonin reuptake inhibitors, including paroxetine. Phosphodiesterase-5 (PDE-5) and arginase are promising therapeutic targets for managing erectile dysfunction, while creatine kinase myocardial band (CK-MB) is a marker for coronary artery disease (Muritala et al.,

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2025). Medicinal plants have been investigated as potential sources of bioactive compounds for managing erectile dysfunction and related vascular disorders. *Rauwolfia vomitoria* has been used traditionally as an aphrodisiac, but its enzyme-inhibitory mechanisms remain incompletely defined.

Recent studies have reported that extracts of *Rauwolfia vomitoria* exhibits inhibitory activity against enzymes implicated in erectile dysfunction, including phosphodiesterase-5, arginase, and angiotensin-converting enzyme, while also exhibiting strong antioxidant properties. These effects may improve endothelial function and enhance nitric oxide bioavailability, thereby facilitating penile vasodilation and erection (Koloko *et al*., 2020). *In vitro* findings have further shown that the plant's phenolic constituents contribute to free radical scavenging and modulation of vascular enzymes involved in erectile physiology (Adebayo *et al*., 2023).

Previous research on animals also showed that the plant's ethanolic extracts improved sexual behavior metrics like mount frequency, intromission frequency, and ejaculation delay, confirming the plant's long-standing use as an aphrodisiac and fertility-boosting herb (Koloko *et al*.,2020).

The investigation of plant-based treatments with dual reproductive and cardioprotective advantages is highly relevant from a scientific and clinical standpoint given the rising incidence of erectile dysfunction and its strong correlation with cardiovascular illnesses. This study aimed to evaluate the effect of ethanol extract of R. vomitoria stem bark on apparent PDE-5-associated cGMP hydrolytic activity in rat penile and cardiac tissue fractions.

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2. Materials and Method

2.1 Materials

Fresh *Rauwolfia vomitoria* stem bark was obtained from Epe-Opin village, Ekiti Local Government Area, Kwara State, Nigeria. *Rauwolfia vomitoria Wennberg/Afzel., family Apocynaceae* was identified at the Plant Biology herbarium unit of the University of Ilorin's Faculty of Life Sciences. The voucher number, UILH/001/0402/2022, was provided.

Twenty male Wistar rats (*Rattus norvegicus*) weighing 180 ± 10 g and in good health were acquired from the University of Ilorin's Department of Biochemistry's animal holding unit. For two weeks, the experimental Wistar rats were kept in well-ventilated plastic cages and acclimated to 12-hour cycles of light and dark. Clean tap water and commercial rat pellets were used to feed the rats. The Wistar rats were sacrificed after being acclimatized for 14 days. Ethanol (analytical grade) (Lot No: 20210315), Dichloromethyl, Sildenafil citrate (Viagra) (Lot no.:A471719, a product of Pfizer, New York), cyclic guanosine monophosphate (Lot no.: BCBJ1515V), Trizma base (Lot no.: BCBJ8442V), Sigma Aldrich Chemical Company, located in St. Louis, USA, Crotalus adamanteus venom (SLBR1148V); Santa Cruz Biotechnology, located in Dallas, Texas, produced imidazole (Lot number: B0212). Reagent C was prepared when needed from 9% Ascorbic acid and Ammonium molybdate in ratio 4:1.

2.2 Preparation of ethanol extract of Rauwolfia vomitoria

Rauwolfia vomitoria stem bark was chopped into pieces then rinsed with distilled water to eliminate any remaining soil particles, oven-dried at 40 °C until a consistent weight was achieved,. In order to prepare for extraction, the dried plant pieces were ground up using an electric blender.

The extract preparation was carried out according to Yakubu and Nurudeen (2012). Powdered stem bark of *Rauwolfia vomitoria* (100 g) was macerated in 500 mL of ethanol for 48 hours, after which filtration with Whatman filter paper No. 1 (GH Healthcare Limited, United Kingdom) took place. The filtrate was concentrated under reduced pressure using a rotary evaporator. To stop fermentation, the final sample was kept in glass containers with labels and chilled at 4 °C.

2.3 Preparing homogenates of penis and heart homogenates (partially purified cGMP-hydrolyzing enzyme fraction)

The study was carried out using the procedure outlined by Muritala, (2018). In summary, twenty male rats were used in this study. The experimental animals were allowed to be acclimatized for 2 weeks in well-ventilated cages. They were fed with standard rat pellets (Grand Cereals Limited, Jos, subsidiary of UAC Nigeria Plc) and as a result, they were fed ad libitum. The rat were sacrificed by cervical dislocation once it had acclimated. Simultaneously, heart and penile tissues were excised immediately, cleaned with blotting paper to remove blood, weighed, and preserved in ice-cold 0.25 M sucrose-tris buffer (pH 7.4). After removing connective tissue and fat from the penile and cardiac tissue, they were cleaned with blotting paper, and preserved in 0.25 M sucrose and 10 mM Tris buffer. After that, the tissues were divided into pieces and homogenized on ice using mortar and pestle respectively. After that, the homogenate was centrifuged at 2000 g for 45 minutes to separate the cellular debris and to obtain a clear supernatants with temperature control in order to avoid degradation. Glass wool that had previously been cleaned in a 0.25 M sucrose-tris solution was used to decant the resulting supernatants.

2.4 Rat penile and cardiac phosphodiesterase-5 enzyme separation and partial purification

The homogenization was done mechanically using Teflon homogenizer. The homogenate was filtered using glass wool to remove the unbroken cells, the unbroken cells which were removed as residues was discarded then the filtrate

volume was measured and labeled as F1. The filtrate was brought to 50% ammonium sulfate saturation by adding an equal volume of saturated ammonium sulfate solution, adjusted to pH 7.0, and allowed to stand for 30 min on ice. After that, a cold centrifuge was used to separate the mixture for 40 minutes at 8000 g. The pellet was discarded while; the supernatant was dialyzed using 0.001 M Magnesium sulphate and imidazole solution as buffer for 2 hours. The essence of the dialysis is to remove ammonium sulphate from the sample. The buffer used for dialysis was cold and was constantly replaced as its temperature rises. The dialysate was then centrifuged at 8000 g for 40 minutes using cold centrifuge and the supernatant was discarded. The resulting pellet in each Eppendorf tube was dissolved with 0.1 mL of 0.001M Magnesium sulphate and imidazole and then labeled as F2.

For the cardiac tissue, after that, the dialysate was separated using a cold centrifuge at 8000 g for 40 minutes, and the resultant fluid was disposed of. The resulting pellet in each eppendorf tube was dissolved with 0.1 ml of 0.001 M Magnesium sulphate and imidazole and then labeled as F2.

2.5 Determination of phosphodiesterase-5-associated enzyme activity in the penile tissues

One unit of enzyme activity was defined as the amount of enzyme catalyzing the hydrolysis of 1 µmol of cGMP per minute under the assay conditions. Enzyme units per milligram of protein were used to express specific activity. Three test tubes were filled with 0.2 mL of Tris buffer using a pipette. Each test tube received 0.1 ml of the enzyme and 0.1 mL of MgSO4. This was incubated for 20 minutes at 37°C. Next, each test tube received 2.0 ml of cGMP. For twenty minutes, the test tubes were incubated at 37°C. Each test tube was filled with 100 µl of trichloroacetic acid. For ten minutes, the test tubes were centrifuged at 4000 g. Four fresh test tubes were assembled. Three test tubes were filled with 0.5 mL of the supernatant from the aforementioned solution, and each test tube—aside from the blank, which has 1.0 mL of distilled water—was filled with 0.5 ml of distilled water. The four test tubes were then filled with 2.0 mL of reagent C. The Versa Max 6 spectrometer was used to measure absorbance of the supernatant at 820 nm which is directly proportional to PDE5 enzymatic activity in the assay i.e the higher the absorbance the higher the enzymatic activity.

2.6 Determination of Phosphodiesterase-5 (PDE-5) associated enzyme activity in the heart tissues

0.2 ml of Tris buffer was pipetted into three test tubes. Each test tube contained 0.1 ml of the enzyme and 0.1 ml of MgSO4. This was incubated for 20 minutes at 37°C. Each test tube was then filled with 2.0 mL of cGMP for twenty minutes, the test tubes were incubated at 37°C. Each test tube was filled with 100 µl of trichloroacetic acid. For ten minutes, the test tubes were centrifuged at 4000 g. Four fresh test tubes were assembled. Three test tubes were filled with 0.5 ml of the supernatant from the aforementioned solution, and each test tube, aside from the blank, which has 1.0 ml of distilled water, was filled with 0.5 ml of distilled water. 2.0 ml of reagent C (a mixture of 9% ascorbic acid and ammonium molybdate solution in a of ratio 4:1, respectively) was then included to the four test tubes. Absorbance was read at 820 nm using Versa max 6 spectrometer.

2.7 Effect of sildenafil citrate on PDE-5 activity in the penilecardiac tissue

Aliquot amount (0.25 mL) of Tris buffer was pipetted into 10 test tubes labelled from 0 to 9 and varying volume of distilled water was included to each test tube. Each test tube received 0.05 ml of the enzyme and 0.05 ml of magnesium sulphate. The test tubes were shaken. Every test tube, with the exception of test tube zero, received varying amounts of sildenafil citrate. After adding 1.0 mL of cGMP to each test tube, 0.5 mL of crotalus atrox (contains a potent nucleotidase which further hydrolyse 3'5' GMP to 3' GMP) was added and the setup was incubated at 37°C for 20 minutes. 0.05 mL of Trichloroacetic acid (TCA) was added to each test tube after incubation. The test tubes were left on the bench to stand for 10 minutes. 1.0 mL of the supernatant obtained from each test tube was transferred into separate test tube. Another test tube was added and labelled blank in which 1.0 mL of distilled water was added. 2.0 mL of reagent C (9% ascorbic acid and Ammonium molybdate solution in ratio 4:1 respectively) was then added to all the test tubes including the blank. Absorbance was read at 820 nm wavelength using Versa max 6 spectrometer.

2.8 Determination of ethanol extract of Rauwolfia vomitoria stem bark on phosphodiesterase-5 activity in tissues

Aliquot amount (0.25 mL) of Tris buffer was pipetted into 10 test tubes labelled from 0 to 9 and varying volume of purified water was added to each test tube. Each test tube received 0.05 mL of the enzyme and 0.05 mL of magnesium sulfate. The test tubes were shaken. With the exception of test tube zero, different amounts of *Rauwolfia vomitoria* stem bark ethanol extract were also applied to each test tube. After adding 1.0 mL of cGMP to each test tube, the setup was incubated at 37°C for 20 minutes. Following incubation, 0.05 mL of trichloroacetic acid (TCA) was added to each test tube. The test tubes were left on the bench for ten minutes. 1.0 mL of the supernatant from each test tube was transferred into a separate test tube. Another test tube was added and labelled blank in which 1.0 mL of distilled water was added. 2.0 mL of reagent C was then included to

all the test tubes including the blank. Absorbance was read at 820nm wavelength using Versa max 6 spectrometer.

2.9 Rauwolfia vomitoria stem bark ethanol extract's impact on Cardiac tissue phosphodiesterase-5 activity

0.25 ml of Tris buffer was pipetted into 10 test tubes labelled from 0 to 9 and varying volume Purified water was included to each test tube. 0.05 ml of the enzyme was added, 0.05 ml of magnesium sulphate was also included to each test tube. The test tubes were shaken. With the exception of test tube zero, different amounts of *Rauwolfia vomitoria* stem bark ethanol extract were also applied to each test tube. After adding 1.0 mL of cGMP to each test tube, the mixture was incubated at 37°C for 20 minutes. Following incubation, 0.05 mL of trichloroacetic acid (TCA) was added to each test tube. The test tubes were left on the bench for ten minutes. 1.0 mL of the supernatant from each test tube mentioned above was transferred into a separate test tube. Another test tube was added and labelled blank in which 1.0 mL of distilled water was added. 2.0 mL of reagent C was then added to all the test tubes including the blank. Absorbance was read at 820 nm wavelength using Versa max 6 spectrometer.

2.10 *Mode of phosphodiesterase-5 inhibition*

The manner of inhibition of the enzyme was ascertained using 0.00033-0.0033 µg/mL
of each extract and their combination. In a series of test tubes, 10–100 µL of the extracts were pre-incubated with 20 µL of partly purified enzyme solutions for 10 minutes at 37ºC. Cyclic guanosine monophosphate (cGMP) at concentrations ranging from 1.38 to 41.36 µM was added to start the reaction. Using the equation derived from the calibration curve, the amount of inorganic phosphate released was measured spectrophotometrically and converted to reaction velocity (amount of inorganic phosphate released (Pi) divided by incubation time of 20 minutes) and enzyme activity. Reaction velocity (V) and substrate concentration (S) were shown in a double reciprocal plot (1/V versus 1/[S]). By employing Michaelis-Menten kinetics to analyze the double reciprocal (Lineweaver-Burk) plot, the manner of inhibition of the crude extract on PDE-5 activity was identified (Elmar, 1984).

The amount of enzyme that releases inorganic phosphate equal to 1 µM of substrate (cGMP) per minute at 20 ºC was defined as one unit of enzyme activity (Butcher and Sutherland 1962; Patil and Shastri, 1981).

The amount of enzyme required to hydrolyze 1 µmol of cGMP in 20 minutes at 37 °C was considered one unit. Enzyme units per milligram of protein were used to express specific activity (Eq. 1).

Phosphodiesterase 5 activity

$$P = \frac{V\Delta C}{V3} \qquad (1)$$

Where P is the enzyme activity, V is the reaction's total volume, ΔC is represented by Reaction velocity (Pi/20 minutes of incubation), V3 is the enzyme source's volume. However, specific enzyme activity (P') is calculated by dividing the total protein in the sample by the enzyme activity.

2.11 Statistical Analysis

The experiment's data was analyzed using analysis of variance (ANOVA) at $p<0.05$ and expressed as means of three determinations using the statistical package for social sciences (SPSS) version 13.0.

3. Results and Discussion

In this study, the enzyme phosphodiesterase-5 was isolated and then partially refined from rat penile and cardiac tissues. Result shows in Figure 2 and 3 indicating that a high level of activity in the homogenate was present in the precipitate after centrifugation. As the purification process proceeds there was an increased activity of the enzyme and hence, an increase in the purification yields. As shown in Table 1, the crude homogenate of penile PDE-5 (F1) had a purification fold of 1 and a purification yield of dialyzed fraction (F2) with approximately 2.1 folds, while the result of crude homogenate of cardiac PDE-5 with a purification yield of 1 fold and purification yield of dialyzed fraction with approximately 6.4 folds (Table 2). The greater purification fold observed in cardiac tissue suggests a higher degree of enzyme enrichment, possibly due to differences in tissue composition or enzyme distribution. Statistical analysis of enzyme activity and specific activity across purification steps revealed a significant increase ($p < 0.05$) in the dialyzed fractions compared to the crude homogenates, confirming the effectiveness of the purification process.

Phosphodiesterase-5 binds to and recognizes cyclic guanosine monophosphate, is a crucial regulator of cGMP signaling in the heart and other tissues (Ahmed *et al*., 2021). A soluble or receptor-based guanylate cyclase (GC) enzyme is typically ligand-mediated activated to begin the cGMP signal transduction pathway in a cell (Houghton et al., 2025). This increases the production of cGMP, which then exerts its effect by activating effectors like cGMP-dependent protein kinase G (PKG) and cGMP-regulated ion channels (Mangmool *et al*., 2023). Phosphodiesterase-5 (PDE-5) specifically targets cGMP by catalyzing the hydrolysis of the phosphodiester bond in cGMP through its catalytic domain, turning cGMP into the inactive 5'-GMP produced by nitric oxide (NO)- stimulated soluble guanylyl cyclase (Alqarni *et al*., 2025). Given its critical role in cGMP signaling, PDE-5 has been pharmacologically targeted for the treatment of a number of pathological conditions including erectile dysfunction and pulmonary arterial hypertension (Zong *et al.*, 2025).

The purpose of this study was to clarify the possible impacts of *R. vomitoria* ethanol extract on adult male rats' sexual

behavior and possible reproductive function in vitro. It was determined that the ethanol extract of *Rauwolfia vomitoria* stem bark significantly inhibits penile PDE-5 activity at substrate (cGMP) concentration compared to sildenafil (standard medication). The Lineweaver-Burk plot indicates that the extract inhibits PDE-5 uncompetitively, which suggests that the binding of the substrate at the enzyme's active site has no effect on the binding of the extract (*Rauwolfia vomitoria*), meaning it shares no structural similarity with the substrate. Its capacity to inhibit PDE-5 activity at high substrate concentrations is confirmed by its binding of the enzyme-substrate complex, which exposes the inhibitor bind site (ESI) and increasing the substrate concentration does not reverse the inhibitory effect of *Rauwolfia vomitoria* and hence, has a reduced Vmax and Km.

Table 1: Purification and yield of penile phosphodiesterase-5

Purification Step	TotalActivity (U/ml)	TotalProtein (mg/ml)	Specific Activity (U/ml/mg protein)	Purification yield (%)
Crude Homogenate	0.6612	4.11	0.1609	1
Dialysed Fraction	0.2700	0.81	0.3333	2.07

Enyme activity = Amount of enzyme that is required to catalyzed one molecule of substrate (cGMP) per minute

Table 2: Purification and yield of cardiac phosphodiesterase-5

Purification Step	TotalActivity (U/ml)	TotalProtein (mg/ml)	Specific Activity (U/ml/mg protein)	Purification yield (%)
Crude Homogenate	1.4916	10.88	0.1371	1
Dialysed Fraction	0.2725	0.312	0.8734	6.37

Enyme activity = Amount of enzyme that is required to catalyzed one molecule of substrate (cGMP) per minute

3.1 Effects of some inhibitors on substrate kinetics of penile PDE- 5 catalyzed hydrolysis of cGMP

This study examined the rates of cGMP hydrolysis in the dialyzed fraction of cardiac and penile PDE-5 in the presence of ethanol extract of *Rauwolfia vomitoria* and a conventional inhibitor (sildenafil). The enzyme activity increased as the substrate increased, and the curve in the absence of the standard drug and extract follows Michaelis-Menten kinetics, producing a hyperbolic curve; in the presence of sildenafil and ethanol extract of the stem bark of *Rauwolfia vomitoria*, the curve takes on a sigmoidal shape.

The result obtained in Figure 1 shows the effect of standard drug (sildenafil) on the hydrolysis of cGMP by penile phosphodiesterase-5 which inhibited the activity of penile phosphodiesterase-5 between the concentrations of 15 µmol – 30 µmol. Figure 1 shows highly significant inhibitory effect of the sidenafil citrate on the phosphodiesterase-5 activity on cGMP.

Result obtained in Figure 2 shows the effect of ethanol extract of *Rauwolfia vomitoria* in the presence of standard drug (sildenafil citrate) showing the highest inhibition of PDE-5 activity between 0.0017 µm/min – 0.0027 µm/min. It shows that the inhibitory effect of ethanol extract of *Rauwolfia vomitoria* on penile PDE-5 is higher when compared to the inhibitory effect of sildenafil citrate (standard drug) on penile PDE-5 activity.

The effect of the sildenafil and extract in the presence sildenafil on kinetic parameters (Vmax and Km) was obtained in Lineweaver-Burk plots where the double reciprocal plot shows the meeting point of the line graphs at the intercept of x and y. Figures 2 and 3 shows that sildenafil citrate and *Rauwolfia vomitoria* is an uncompetitive inhibitor of the PDE-5 activity in the penile, this is given by it reduced Vmax and Km (Michaelis constant). Values of kinetic parameters are displayed in Table 3.

3.3 Effect of Rauwolfia vomitoria ethanol extract and sildenafil citrate on the substrate kinetics of cardiac PDE-5-catalyzed hydrolysis of cyclic guanosine monophosphate

Figure 5 shows the rates of cGMP hydrolysis in the dialyzed portion of cardiac PDE-5 in the presence of ethanol extract of *Rauwolfia vomitoria* and standard inhibitor sildenafil. PDE-5's activity rate rises as concentrations increases. In the presence of standard drug and extract, PDE-5 activity was inhibited between 0.286µm/min – 0.2911µm/min in the presence of *Rauwolfia vomitoria* and 0.124 µm/min – 0.15 µm/min in the presence of standard drug (sildenafil). It shows that the inhibitory effect of ethanol extract of *Rauwolfia vomitoria* on cardiac PDE-5 is highly significant when compared with the inhibitory effect of standard drug (sildenafil) on cardiac PDE-5 activity.

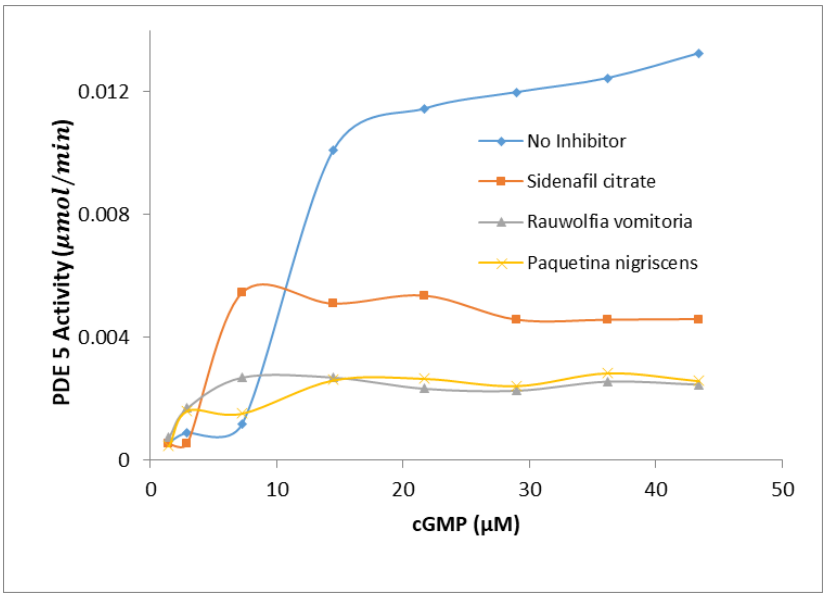


Figure 1: Effects of Sildenafil citrate and ethanol extract of Rauwolfia vomitoria on substrate kinetics of phosphodiesterase-5 catalysed hydrolysis of cyclic guanosine monophosphate

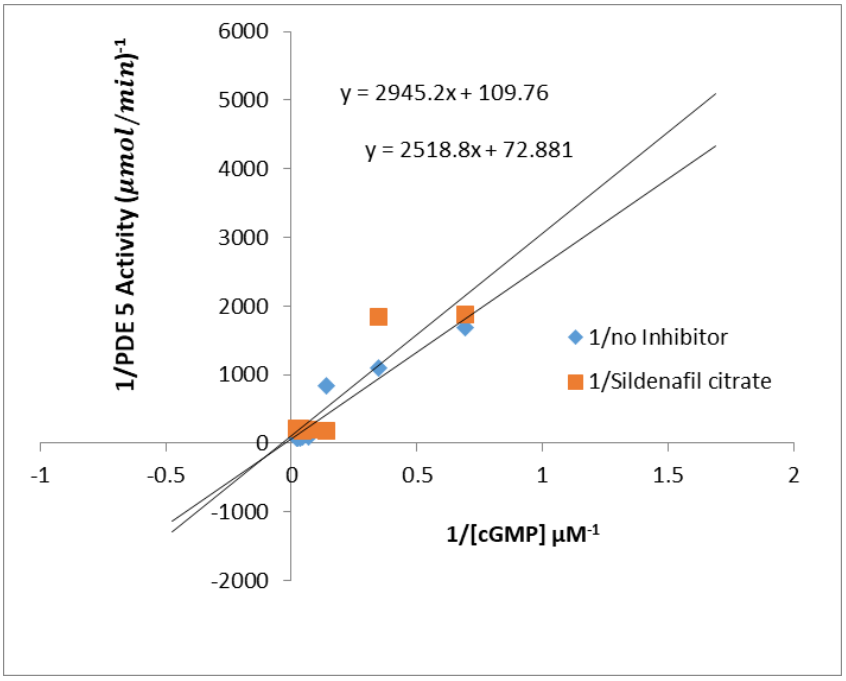


Figure 2: Lineweaver Burke plot showing sildenafil citrate's impact on the substrate kinetics of cyclic Guanosine MonoPhosphate hydrolysis catalyzed by penile PDE-5

The effect of the sildenafil and extract in the presence sildenafil on kinetic parameters (Vmax and Km) was obtained in Lineweaver-Burk plots where the double reciprocal plot shows the meeting point of the line graphs at the intercept of x and y. Figures 5 and 6 shows that sildenafil citrate is an uncompetitive inhibitor of the PDE-5 activity in the heart while ethanol extract of *Rauwolfia vomitoria* shows a mixed type of inhibition, this is given by the values of Vmax and Km (Michaelis constant). Values of kinetic parameters are displayed in Table 3.

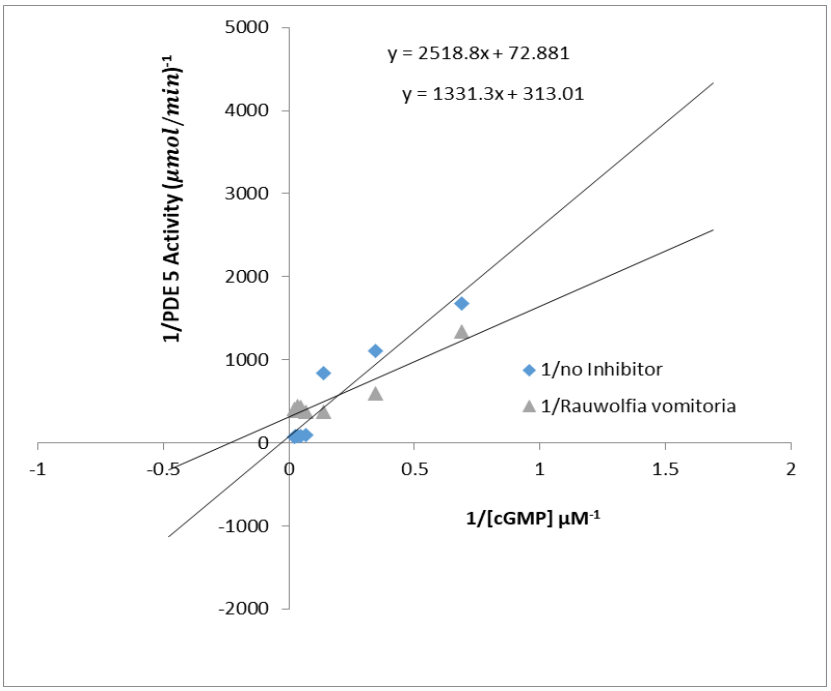


Figure 3: Lineweaver Burke plot Impact of ethanol extract of Rauwolfia vomitoria on penile phosphodiesterase-5 hydrolysis of cyclic guanosine monophosphate

Table 3: Effect of ethanol extract of R. vomitoria on the kinetic parameters of a cyclic GMP hydrolysis catalyzed by penile PDE-5

	V_{max}(µmol/min)	Km (µM)
Absence of Ligands	0.137	34.50
Sildenafil citrate	0.0091	26.86
R. vomitoria	0.0032	4.26

V_{max} is the maximal reaction rate of phosphodiesterase-5, K_m is the concentration of the substrate required for PDE-5 to reach half of its maximum reaction rate. It measures the affinity of the PDE-5 for cGMP

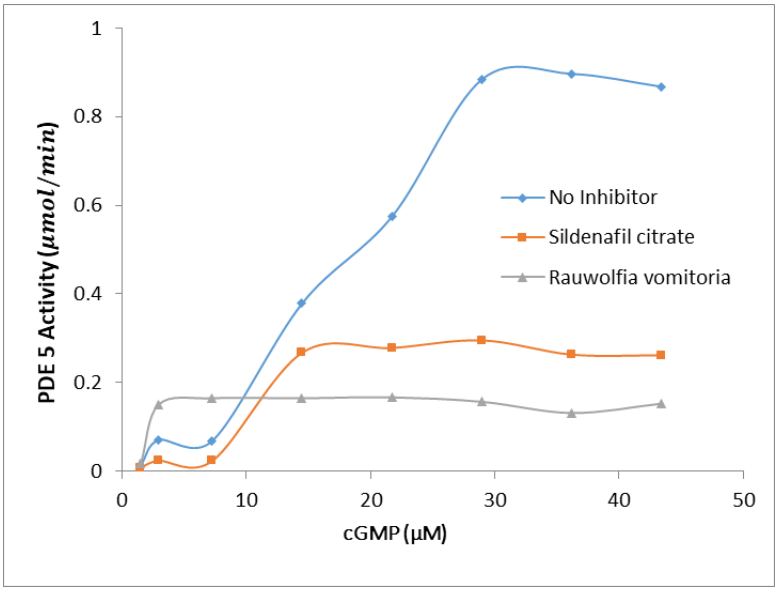


Figure 4: Effect of ethanol extract Rauwolfia vomitoria and sildenafil citrate on the substrate kinetics of cardiac PDE-5-catalyzed hydrolysis of cyclic guanosine monophosphate

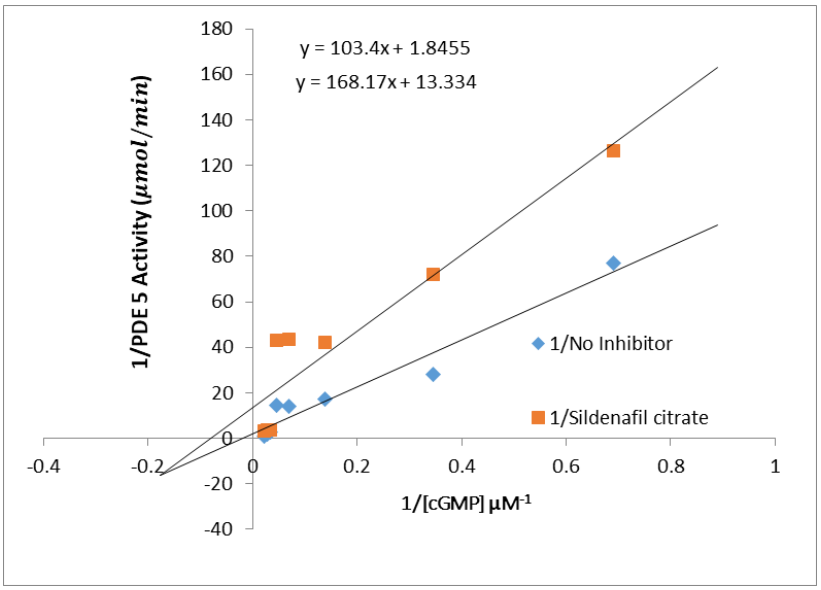


Figure 5: Lineweaver Burke plot Impact of sildenafil citrate on cardiac substrate kinetics of Phosphodiesterase-5 catalysed hydrolysis of cyclic guanosine monophosphate

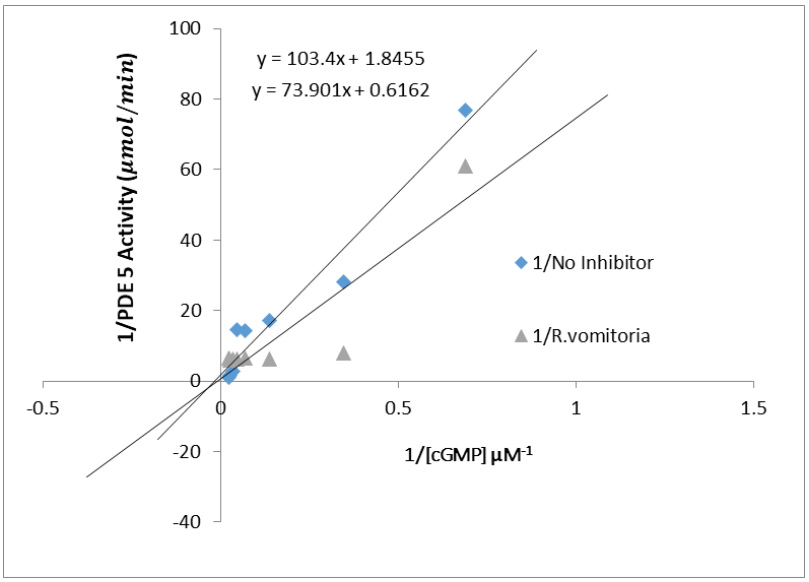


Figure 6: Lineweaver Burke plot Impact of ethanol extract of Rauwolfia vomitoria on cardiac substrate kinetics of Phosphodiesterase -5 catalysed hydrolysis of cyclic guanosine monophosphate

Table 4: Kinetic parameters of ethanol extract of R. vomitoria on cardiac phosphodiesterase-5 catalyzed hydrolysis of cyclic GMP

	V_{max} (µmol/min)	K_m (µM)
Absence of Ligands	0.542	56.04
Sildenafil citrate	0.075	12.61
R. vomitoria	1.623	120.61

V_{max} = the maximal reaction rate of phosphodiesterase-5, K_m = the concentration of the substrate required for PDE-5 to reach half of its maximum reaction rate. It measures the affinity of the PDE-5 for cGMP

In the cardiac tissue, activity of PDE-5 is inhibited significantly in the presence of ethanol extract of *Rauwolfia vomitoria* stem bark (EERVSB) more than standard drug (sildenafil). Inhibition of cardiac PDE-5 by sildenafil citrate may be as a result of the uncompetitive type of inhibition while EERVSB has a mixed type of inhibition of this enzyme. This result aligns with the report by Oyewusi *et al*. (2024) where the bacterial strain HOKA1 extract greatly reduced

the penile and cardiac cGMP with the highest penile and cardiac concentration between 0.013 and 0.183 µ (0.00–0.203 µ M/Min). Furthermore, the bacterial strain HOKA1extract reduced PDE-5 activities more effectively than sildenafil citrate, according to the kinetic parameters (V max M/Min as compared to the sildenafil citrate and Km) impacts.

Hence, ethanol extract of *Rauwolfia vomitoria* stem bark in these tissues inhibit PDE-5 activity, causing the smooth muscle around the penis to relax resulting in dilation and enhanced blood flow into penile tissue (Muritala *et al*., 2025). This blood is trapped in the penis leading to erection. However, more studies is needed to be carried out on the ethanol extract of *Rauwolfia vomitoria* stem bark in other to obtain more efficient functions and approval as a possible cure for erectile dysfunction and cardiovascular diseases.

4. Conclusion

Ethanol extract of *Rauwolfia vomitoria* stem bark brings about the reduction in the activity of the enzyme phosphodiesterase-5 which signals a potential in the possibility of enhancing penile erection, this is achieved by uncompetitively inhibiting PDE-5 activity in the penile and cardiac tissues. Additionally, it was concluded that cardiovascular disorders might be treated with the extract. However, the ethanol extract of *Rauwolfia vomitoria* stem bark inhibits PDE-5 more effectively in the penile than in the heart tissue. This study shown that while sildenafil citrate, the usual medication, has a greater PDE-5 inhibitory activity in the heart, ethanol extract of *Rauwolfia vomitoria* stem bark can be employed as an effective medication for treating erectile dysfunction in the penile tissue.

Authors' Contributions

M.H.F. designed the study, methodology, and supervision, O.S.P., S.L.B. and N.N.Y. conducted the experiments, O.S.P. and S.L.B. wrote first draft, B.M.M. and A.R.A. analyzed the data.

Declaration of Competing Interest

The authors declare no competing interests.

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