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Membrane Stabilization, PhospholipaseA2, Albumin Denaturation, Protease Inhibition, as Viable Mechanisms for the Anti-Inflammatory Effects of Methanol Extract of *Rauvolfia vomitoria* Afzel Leaves

Ugwu Obiora Celestine [a,b*], Asogwa Felix Keneolisa [a], Ukpabi-Ugo Jacinta Chigozie [c], Asadu Chidimma Lilian [d], Ali Ibeabuchi Jude [e], Okonkwo Raymond Maduabuchi [a], Ugwoke Faith Ifeanyi [f], Offiah Raymond Ogbonna [g], Obasi Uchenna Emmanuel [a] and Anosike Chioma Assumpta [b]

[a] Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani, Nigeria.
[b] Department of Biochemistry, Faculty of Biological Science, University of Nigeria, Nsukka, Nigeria.
[c] Department of Biochemistry, College of Natural Science, Micheal Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[d] Department of Medical Biochemistry, College of Medicine, Enugu State University of Science and Technology, Parklane, Enugu, Nigeria.
[e] Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani, Nigeria.
[f] Uburu Bioresources Biotechnology Development Agency, Abuja, Nigeria.
[g] Department of Pharmacology and Therapeutics, Faculty of Clinical Medicine, Enugu State University of Science and Technology, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors UOC and ACA designed the study, performed the statistical analysis, wrote the protocol. Authors UOC, AIJ and UUJC wrote the first draft of the manuscript. Authors ORO and ORM review the final drafted manuscript. Authors AFK and ACL managed the analyses of the study. Authors UFI and ACL managed the literature searches. All authors read and approved the final manuscript.

**Corresponding author: E-mail: Obiora.ugwu@esut.edu.ng;*

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ABSTRACT

Background: The body uses inflammation as a defence mechanism to eliminate harmful stimuli like germs, damaged cells, or irritants and to initiate the healing process. However, the ongoing discovery of several medicinal plants and the testing of their bioactivity to produce information that will assist doctors and patients in making informed decisions prior to employing them have established.
Aims: The study valuate the phosphatelipase A2, membrane stabilization, albumin denaturation, protease inhibition, and platelete aggregation activities as viable mechanisms for the anti-inflammatory effects of the methanol extract of *Rauvolfia vomitoria* Afzel leaf (MERVL).
Methodology: The anti-inflammatory effect of (MERVL) was ascertained using the phosphate lipaseA2, membrane stabilization model, albumin denaturation, protease inhibitor, assay.
Place and Duration of the Study: Department of Pharmacology Lab, Enugu State of University of Science and Technology Agbani Nigeria, between March 2021 and August 2021.
Results: MERVL inhibited hypotonicity-induced haemolysis by 27.14, 41.10 and 65.70%, at the concentration of 0.4, 0.8 and, 1.0 mg/mL respectively.The highest percentage of inhibition (67.70%) was noticed at the highest concentration of the MERVL. These results were almost analogous to the standard drug (indometacin) used as it exhibited concentration dependent inhibition of albumin denaturation. Protease activity was significantly ($P < 0.05$) increased at all concentrations which follow the similar tendency as standard drug used. The results showed that MERVL has anti-inflammatory activities.

Keywords: Rauvolfia vomitoria; membrane stabilization; anti-inflammatory; methanol extract.

1. INTRODUCTION

"The body uses inflammation as a defence mechanism to eliminate harmful stimuli like germs, damaged cells, or irritants and to initiate the healing process" [1]. "The early phases of inflammation are characterised by the production of reactive oxygen species (ROS) and the recruitment of inflammatory mediators at the site of injury. When the production of ROS surpasses the capacity of antioxidants to reduce it, oxidative stress is unavoidable" [2]. "Oxidative stress and inflammatory processes are connected. The prolonged release of inflammatory mediators, which might result in oxidative stress, can lead to chronic inflammatory diseases. Anti-proteinases are oxidatively inactivated, whereas pro-inflammatory mediators are generated by gene activation in response to oxidative stress. Previous studies have shown that medicinal plants with anti-inflammatory characteristics can lower oxidative stress and boost immune function" [3]. "One of such plants with anti-inflammatory properties is *Rauwofia vomitera* (Apocyanaceae) leaf. It is a species of vomitoria in the family Apocyanaceae. It is also called serpent wood, snake root, and swizzle" [4]. "In local Nigerian languages, it is called *asofeyeje* in the western part of Nigeria" [5]. "Major phytochemical constituents of this plant include alkaloids, glycosides, polyphenols, and reducing sugars" [6]. "The active alkaloids of *R. vomitoria* include rauwolfine, reserpine, rescinnamine, serpentine, ajmaline serpentinine, steroid-serposterol and saponin" [7]. "*R. vomitoria* has been used over the years for the treatment of

hypertension and mental disorders and it is a common herb used traditionally for psychiatric management in Nigeria" [8]. The Rauwolfia vomitoria plant is used medicinally to treat inflammation because it contains certain phytochemicals. For instance, several studies have shown that alkaloids reduced the cytotoxicity of natural killer cells, the production of histamine by mast cells, the release of interleukin-1 by human monocytes, and the effect of platelet activating factor on platelets, among other things [9]. It has been demonstrated that alkaloids like tetrandine and its counterpart, berbamine, inhibit monocyte and neutrophil secretion of prostaglandin and leukotriene in humans. Berbarine's ability to reduce inflammation has demonstrated that alkaloids may play a significant role in chronic inflammation [10]. However, safer and more efficient medications have been made from medicinal plants as a result of the value of ethnomedicine [11]. Due to the lack of a safer and more potent anti-inflammatory medication, inflammation research continues to be of great interest. However, demand for natural products with anti-inflammatory properties and minimal side effects have increased.

2. MATERIALS AND METHODS

2.1 Plant Material

On March 13, 2021, when this study was being conducted, fresh leaves of Rawuofia vomitera were found on the Enugu State University of Science and Technology campus. Mr. A. O. Ozioko of the International Centre for Ethnomedicine and Drug production (InterCEED), Nsukka, Nigeria, verified the authenticity of the newly cut leaf. The plant with voucher specimen number INTERCEED/002 was placed at the InterCEED Herbarium.

2.2 Chemicals and Reagents

Chemicals used for this study were of the analytical grade and products of May and Baker England, British Drug House (BDH) England, Fluka Germany, Burgoyne, India, Harkin and Williams, England and Sigma Aldrich.

2.3 Preparation of Plant Material

The plant was gathered, cleaned, and dried in the shade. Using a mechanical grinder, the dried leaves were ground into a powder. Using a maceration flask, a weighed quantity (1000 g) was macerated in 2.5 L of absolute methanol. After being stirred frequently for 72 hours, it was filtered using a muslin cloth into a flask with a flat bottom. Whatman No. 1 filter paper was used to perform the filtration. To create the crude ethanol extract, the extract was concentrated using a rotary evaporator at a temperature of 45°C. The concentrated extract was kept in the refrigerator in a labelled sterile reagent bottle at a temperature of 2 to 40°C.

2.4 Anti Inflammatory Assays

Methanol Extract of *Rauwolfia vomitoria* leaves on Phospholipase A2 (PLA2) Activity: The effect of MERVL on PLA2 activity was carried out using method of Vane [12] with little adjustments by Enechi et al. [13]. Fresh blood samples were taken from healthy people and centrifuged for 10 minutes at 3000 rpm. The plasma collected in the supernatant was discarded. The red cells were reconstituted as a 40% (v/v) suspension with normal saline after being rinsed three times with an equal volume of normal saline. A preparation of fungal enzymes was made using an *Aspergillus nigerstrain* culture. A nutrient broth was used to cultivate *Aspergillus niger* for 72 hours at room temperature. The culture was put into test tubes with 3 ml of phosphate buffered saline and centrifuged for 10 minutes at 3000 rpm. The pellet was made up of fungus cells, and the supernatant was used to make a crude enzyme preparation. The following ingredients were incubated at 37°C for 1 hour: 0.2 ml of HRBC, 0.2 ml of CaCl2, 0.2 ml of crude enzyme preparation, and various concentrations of normal saline and the fraction (0.2-1.0 mg/ml).

HRBCs, CaCl2, and a crude enzyme preparation were present in the control tube. Separately, 0.2ml of boiling enzyme was applied to the blanks. The reaction mixture was centrifuged for 10 minutes at 3000 rpm. The absorbances of the solution were measured at 418 nm after a determined amount of the supernatant (1.5 ml) was diluted with 10 ml of normal saline. Prednisolone, an inhibitor of phospholipase A2, was employed as a standardard.

Methanol Extract of *Rauwolfia vomitoria* Leaves on Platelet Aggregation Inhibition: (Manaharan et al., Murugan, [14-15]: The platelet rich plasma was re-suspended in pH 7.4 Tris buffer together with 1.2 x 107 platelet cells for each experiment. Platelet aggregation was measured using a spectrophotometer, which recorded absorbance values. A variety of concentrations of the methanolic extract of Rawuofia vomitera (0.2, 0.4, 0.6, 0.8, and 1.0

g/mL) in isosaline were employed to assess the in vitro suppression of platelet aggregation. ADP was used to induce platelet aggregation at a concentration of 1mM. The typical drug used was indometacin. At 660 nm, the absorbance was taken after 5 minutes. Control was taken without the extract. The activity was calculated using the formula: (Control – Test)/Control x 100.

Effect of Methanol Extract of *Rauwolfia vomitoria Leaves* on Albumin Denaturation: Minor adjustments to the Mizushin and Kobyashi [16] approach were used. Test extract and 1% bovine albumin fraction in aqueous solution made up the reaction mixture, which had its pH adjusted at 37°C with a little amount of HCl. The extract sample was incubated for 20 minutes at 37 °C, followed by 20 minutes at 51°C, and then the samples were cooled before the turbidity was measured spectrophotometrically at 660 nm. The experiment was carried out three times. The percentage inhibition of protease inhibitor activity was calculated as: Percentage inhibition (%) = [{Abs control - Abs sample}/ Abs control] x 100.

Methanol Extract of *Rauwolfia vomitoria leaves on* HRBC membrane stabilization assay [17-19]: Freshly drawn blood was combined with an equal amount of Elsevier's solution. After that, it underwent a 15-minute, 3000 rpm centrifugation. Isosaline was used to wash the packed cells and to create a 10% solution. In isosaline, methanolic extracts of R. vomitera at various concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 g/mL) were produced. 1 mL phosphate buffer, 2 mL hyposaline, and 0.5 mL HRBC suspension were added to 0.5 mL of the extract and incubated for 30 minutes at 370C before being centrifuged for 20 minutes at 3000 rpm. At 560 nm, absorbance was measured. The extract-free control functioned as a negative control and indomethacin served as the standard.

2.5 Statistical Analysis

The data obtained were analysed using a one-way analysis of variance (ANOVA) in Statistical Product and Service Solution (SPSS) version 22.0 and presented as Mean ±SD. Mean values with $p < 0.05$ were considered significant.

3. RESULTS

The result in Fig. 1 depicts the influence of MERVL on phospholipase A2 activity. The MERVL significantly ($p < 0.05$) inhibited the PLA2 at various concentrations, with the highest percentage inhibition of the extract, 67.67%, being observed at the highest concentration of 1.0 mg/ml. The standard medication, indomethacin, is comparable to the plant extract.

The outcome in Fig. 2 illustrates how MERVL affects membrane stabilization. At 1.0 mg/ml, the extract significantly ($p < 0.05$) reduced the amount by which hyponicity-induced lysis of HRBC occurred as compared to other concentrations. However, the percentage inhibition also rises, peaking at 68.5% when the extract concentration increased by 1.0 mg/ml. This is similarly comparable to the widely used medication indomethacin, which has a percentage inhibition of 70.2% at doses of 1.0 mg/ml.

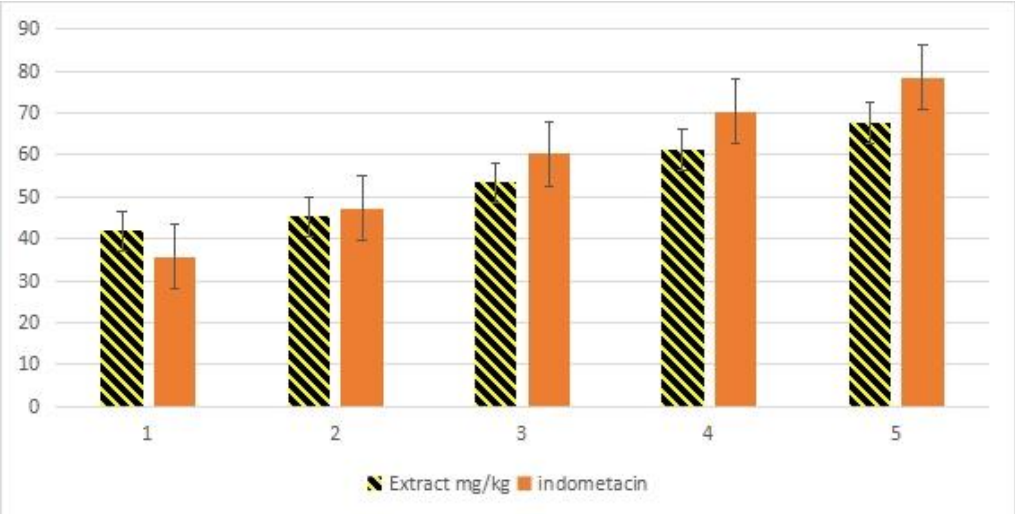

Fig. 1. Effect of methanol extract of *Rauwolfia vomitoria* on PLA2

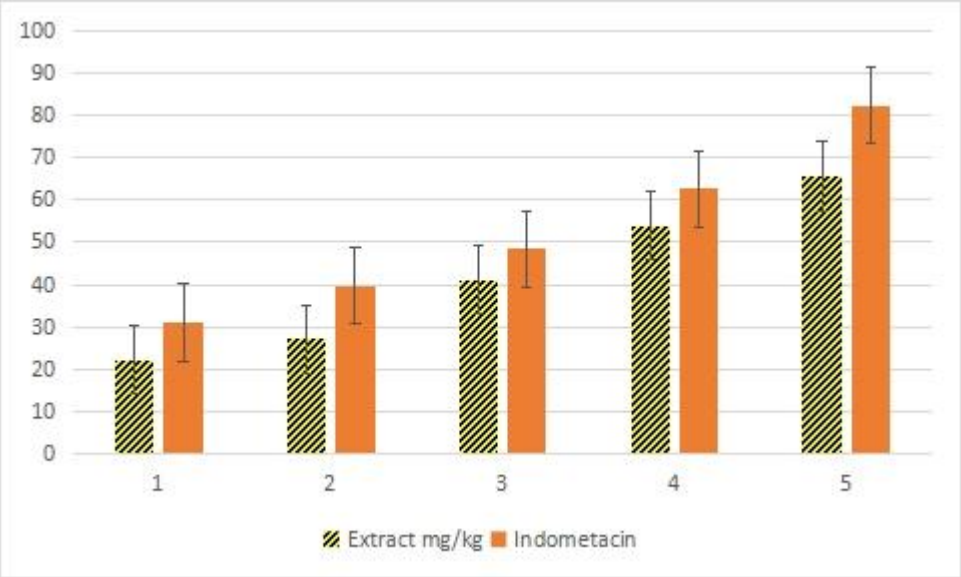

Fig. 2. Effect of methanol extract of *Rauwolfia vomitoria* on membrane stabilization

The result displayed in Fig. 3 demonstrated how MERVL affected albumin denaturation. The suppression of protein (albumin) denaturation by the MERVL was concentration-dependent. At various doses, the extract shown significant (($p < 0.05$)) percentage inhibition. At the highest MERVL concentration, the highest percentage of inhibition (62.06%) was observed. The standard drug (indomethacin), which demonstrated concentration dependent suppression of albumin denaturation, was measurable to these results.

Fig. 4 illustrates the impact of MERVL on protease activity. Protease activity was significantly (($p < 0.05$) more inhibited when the extract concentration was higher (1.0 mg/ml). When compared to the other doses, the greatest enzyme activity was found at 1.0 mg/ml, with a corresponding percentage inhibition of 53.98%. The proportion of enzyme activity rises with a matching rise in the percentage of inhibition as the concentration rises from 0.2 mg/ml to 1.0 mg/ml. This is comparable to the commonly used drug indomethacin.

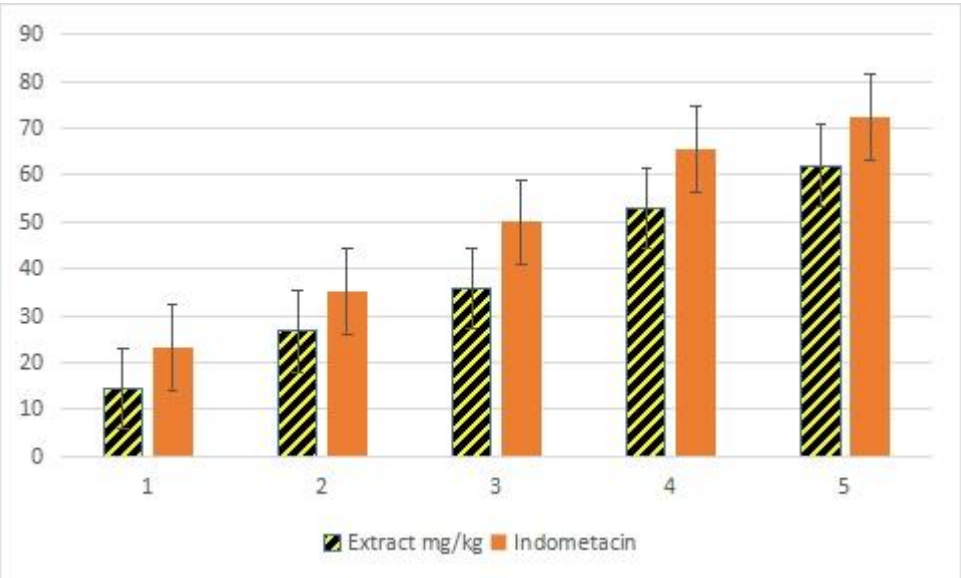

Fig. 3. Effect of methanol extract of *Rauwolfia vomitoria* on albumin denaturation

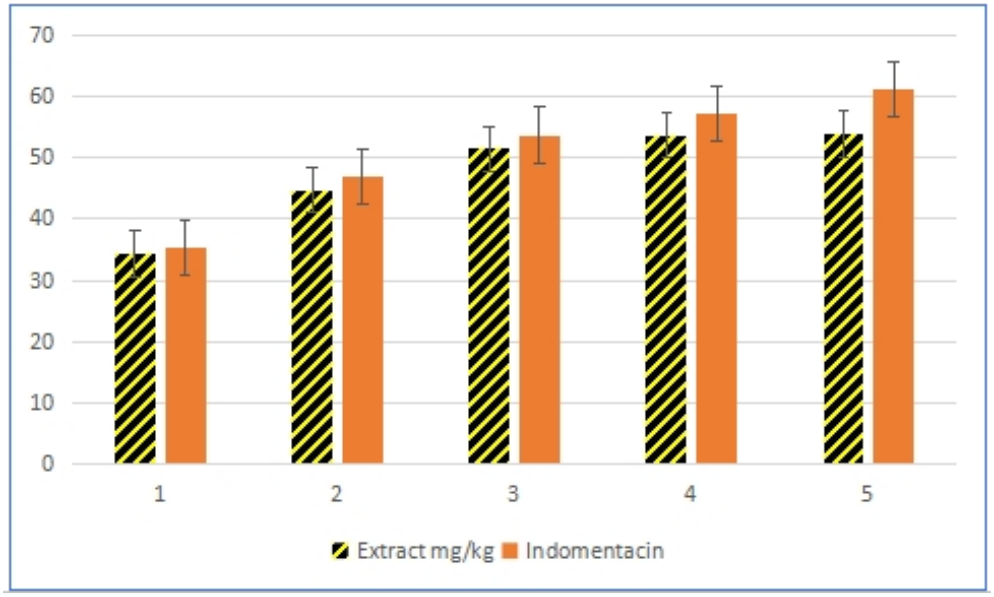

Fig. 4. Effect of methanol extract of *Rauwolfia vomitoria* on protease inhibition

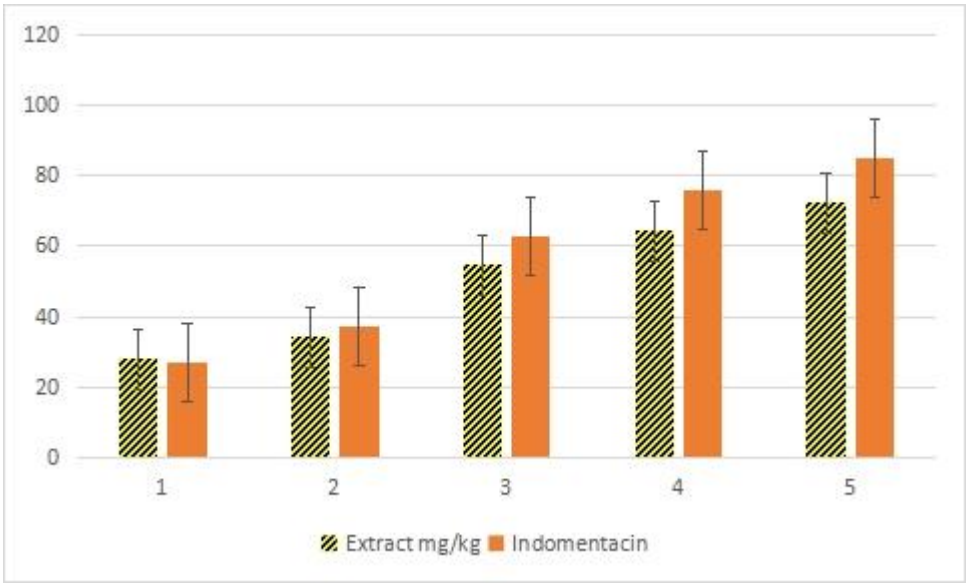

Fig. 5. Effect of methanol extract of *Rauwolfia vomitoria* on platelet aggregation

The result in Fig. 5 illustrates how MERVL affects platelet aggregation. The percentage inhibition on platelet aggregation increased significantly ((p < 0.05) with extract concentration (1.0 mg/ml). The greatest enzyme activity was seen at 1.0 mg/ml, which corresponded to a percentage inhibition of 75.93% when compared to the other doses. The proportion of enzyme activity rises with a matching rise in the percentage of inhibition as the concentration rises from 0.2 mg/ml to 1.0 mg/ml. However, comparing the extract to the standard drug (indometacin), a similar pattern was observed.

4. DISCUSSION

In contrast to the control, the methanol extract of Rauvolfia vomitoria Afzel leaf (MERVL) and indomethacin significantly (p < 0.05) prevented the lysis of the human erythrocyte membrane when administered in a concentration-dependent manner. The methanol extract prevented the lyses of the HRBC membrane brought on by a hypotonic solution. Due to the predominance of polyunsaturated fatty acids (PUFAs) in the RBC membrane, Divya [20] the cells are extremely vulnerable to oxidative damage, which causes

hemolysis, which releases hemoglobin and other internal cellular components. Heat, hypotonic solution, and other harmful substances can cause the RBC membrane to lyse. The MERV leaf at various concentrations significantly ($p < 0.05$) prevented the lysis of the HRBC membrane. The results indicated the capacity of MERVL to inhibit haemolysis. The inhibition of RBC membrane lyses is a measure of the anti-inflammatory activity. Divya, Zohra, [20-21] .The MERV leaf's ability to keep erythrocyte membranes stable suggests that it might also keep lysosomal membranes stable. By preventing the release of lysosomal components of activated leukocytes (such as bactericidal enzymes and proteases) that, upon extracellular release, cause further tissue inflammation and, ultimately, tissue damage, stabilising the lysosomal membrane is extremely important in regulating inflammatory responses. Cunha, Anosike, [22–23]. The finding supports membrane stabilisation as a plausible mechanism behind the anti-inflammatory effects of the Rawuofia vomitera leaf methanol extract. The impact of the Rawuofia vomitera leaf methanol extract on albumin denaturation is depicted in Fig. 2. The MERV proved successful in preventing denaturation of albumin. Albumin denaturation was considerably ($P < 0.05$) reduced by varying plant extract doses, and indometacin displayed a similar pattern. The extract inhibits differently depending on the concentration, with a 14.47% inhibition at 0.2 g/mL and a 62.06% inhibition at 1.0 g/mL. A marker for inflammatory and arthritic illnesses, protein denaturation is the process by which proteins lose their structure as a result of external stress or chemicals. [24] Berbert, Its anti-inflammatory properties are supported by the methanol extract of Rawuofia vomitera's capacity to prevent protein denaturation. Albumin denaturation was effectively inhibited by the methanol extract. The protease activity of the methanol extract of Rawuofia vomitera leaf is shown in Fig. 3. At various concentrations, the leaf strongly ($P < 0.05$) suppressed protease activity, and a standard medication exhibited a similar pattern. Similar outcomes were seen with the usual medication (Fig. 3). Protease inhibitors are crucial for a more accurate understanding of the fundamentals of protein interaction. Proteolytic enzymes such rutin, trypsin, chymotrypsin, pancreatin, papain, and bromelain are crucial modulators and regulators of inflammatory reactions. Serine protease is known to be abundant in neutrophils, which are found at lysosomes. Leukocyte protease has been implicated in the development of tissue damage during inflammatory reactions, and protease inhibitors have been shown to give a significant level of protection. Kajay, [25]. Different doses of the extract significantly ($p < 0.05$) reduced the protease activity. This might be due to the large amount of flavonoids present. Another example of its possible anti-inflammatory capabilities is provided by this experiment.

5. CONCLUSION

The results suggest that the plant can be a potential source of anti-inflammatory agents if exploited. Findings from the study show that the MERVL has outstanding anti-inflammatory activities, and this study also demonstrates that MERVL has modulatory effect on the vascular changes that come off during inflammation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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