

Invitro Study of the Antioxidant and Anti-Inflammatory Potential of Rauvolfia Vomitoria Afzel Leaf Crude Extract

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Abstract- Oxidative stress is a physiological term that describes an imbalance between reactive oxygen species (ROS) generation and the body's ability to detoxify them or repair the resulting damage. Scientific validation of traditional medicinal plants is essential for their integration into modern healthcare systems and pharmaceutical developments. This study aimed to fill the existing knowledge gap by examining the antioxidant potential of *R. vomitoria* crude ex3tract using multiple assay methods and comparing the results with standard ascorbic acid. *Rauvolfia vomitoria* leaves were powdered and soaked in 100% methanol for 48 hours at room temperature with occasional agitations.. The extract demonstrated notable inhibitions of protein denaturation, trypsin activity, and DNA oxidation to fight inflammation. The study provided compelling evidence of anti-inflammatory potential of *Rauvolfia vomitoria*.

Keywords: Inflammation, ascorbic acid, trypsin, denaturation, inhibition, standard, pharmaceutical, medicinal

I. INTRODUCTION

Oxidative stress is a physiological state that arises when the body's defense mechanism against oxidation is overwhelmed by the radical atoms or group of atoms (Valko et al., 2007). These free radicals and the likes are generally referred to as reactive species (which reactive oxygen species, reactive nitrogen species and reactive chlorine species). Non-radical species like hydrogen peroxide (H₂O₂) generated during normal cellular process and probably exposure to environmental factors, aids oxidative stress (Pham-Huy et al., 2008). Despite the cell's signaling and immune defensive roles of reactive species, they can damage biomolecules (lipids, nucleic acids and proteins). In fact, diseases such as diabetes, cancers and few clinical disorders

have been linked to oxidative stress (Lobo et al., 2010).

The antioxidants of the first line of defense maintain redox homeostasis mobbing ROS (Halliwell and Gutteridge, 2015). However, issues of synthetic antioxidants (for example, propyl gallate tert-butyl hydroquinone) causing toxicities are already in the public domains (Kahl and Kappus, 1993). They had been indicated to increase shelf life of food and pharmaceutical products (Lobo et al., 2010). Bioactives freely existing in plants include flavonoids, alkaloids, tannins, saponins, and polyphenols, terpenes (Cowan, 1999).

Rauvolfia vomitoria Afzel, *Rauvolfia vomitoria* Afzel, is one of the tropical shrubs consisting of prominent antioxidants that its use is endemic in West and Central Africa to treat diseases (Odugbemi, 2008). The conglomerate chemicals (indole alkaloids-reserpine, ajmaline, serpentine, and yohimbine) are etiological attributes for the plant's medicinal richness (Okolie et al., 2011).

Besides alkaloids, flavonoids, tannins, saponins and phenolic compounds found in *R. vomitoria* enhance its therapeutic activities ranging from free radical scavenger to enzyme control (Sonibare and Akpan, 2017). In spite of its medicinal folklores and pharmacological histories, there are unresolved in the anti-inflammatory activity of *R. vomitoria* (Akinmoladun et al., 2010).

Day to day validations of medicinal plants is requisite to incorporate them into healthcare systems and drug discoveries (Liu, 2004).

Sources of Reactive Oxygen Species

ROS are produced both endogenously and exogenously. Endogenous sources include mitochondrial respiration, enzymatic reactions involving NADPH oxidase, xanthine oxidase, and cytochrome P450 enzymes. During oxidative phosphorylation, the electron transport chain in mitochondria can leak electrons, which react with oxygen to form superoxide radicals (Murphy, 2009). Exogenous sources of ROS include environmental pollutants, tobacco smoke, ionizing radiation, heavy metals, and certain drugs (Valko et al., 2007).

The dual nature of ROS—as both signaling molecules and agents of damage; makes them critical targets for therapeutic interventions. While low levels of ROS are necessary for physiological functions, excessive levels can overwhelm cellular defenses and trigger pathological processes.

How Oxidative Damage occurs

In lipid peroxidation, ROS disrupt cell membranes by attacking the polyunsaturated fatty acids, cause the formation of lipid radicals and aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE). The reactive adducts of the peroxidation products cause aberrant cytotoxicity, aberrant cells and inflammation from continued attacks on DNA, phospholipids and proteins. These products can further propagate oxidative damage and disrupt membrane integrity (Ayala et al., 2014).

Proteins are affected by several such oxidative modifications as carbonylation, nitration, and disulfide bond formation. The changes by which protein conformations are altered leading to impaired functions (Stadtman and Levine, 2003).

DNA damage by ROS are strand breaks, base modifications and cross-linking which result in mutations and genomic instability (Cooke et al., 2003).

Mitochondrial-derived ROS in inflammation

Mitochondria produce high-energy phosphate bonds in ATP by utilizing an electrochemical proton gradient. This gradient is created by the transfer of electrons through a series of electron carriers located

in the mitochondrial membrane. The electron transport chain consists of four complexes, spatially organized in order of increasing redox potential: Complex I (NADH-ubiquinone oxidoreductase), Complex II (succinate-ubiquinone oxidoreductase), Complex III (ubiquinol-cytochrome c reductase), and Complex IV (cytochrome c oxidase). The transfer of electrons to molecular oxygen is a tightly regulated process, with only a small percentage (1-2%) of electrons leaking out and reacting with oxygen to form superoxide ($O_2^{\bullet-}$). (Handy and Loscalzo, 2012) The primary sites of superoxide production in the electron transport chain are Complexes I and III, with Complex I being more prominent in skeletal muscle and neural cells, and Complex III being more prominent in endothelial cells (O'Malley and Fink, 2006). Mitochondrial superoxide reacts with manganese superoxide dismutase (MnSOD) in the mitochondrial matrix to form hydrogen peroxide (H_2O_2), which can cross the mitochondrial outer membrane and access cytosolic targets. This can lead to various functional outcomes, including the activation of redox-sensitive transcription factors (such as HIF-1 α and NF- κ B) (60), the activation of pro-inflammatory cytokines, and the activation of inflammasomes (Naik et al., 2011)

Handy DE and Loscalzo J. Redox regulation of mitochondrial function. *Antioxid Redox Signal* 16: 1323–1367, 2012.

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Medicinal plants as sources of antioxidants

Medicinal plants have been used for centuries in traditional healing systems across cultures, including traditional Indian medicine (Ayurveda), Traditional Chinese Medicine, and African ethno medicine. Their therapeutic potential is largely attributed to the presence of bioactive compounds known as phytochemicals (Cowan, 1999). Among their pharmacological properties, anti-inflammatory activity emerged as one of the most significant in

oxidative stress-connected cases like cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions (Lobo et al., 2010).

Oxidative Stress and the Need for Antioxidants

Oxidative stress is the consequential imbalance of the production of reactive oxygen species and the defense mechanism. The out-of-control release of ROS is the hallmark of oxidative stress. It damages molecular components (lipids, proteins, and DNA) which are scare heads in cells' integrity. However, mobilization of fighter agents to counter free radicals leads to inflammation (Valko et al., 2007). Under pathological conditions, body's defenses may be insufficient; necessitating externally sourced antioxidants from plants (Pham-Huy et al., 2008).

Phyto-Antioxidants

These are naturally occurring bioactive compounds of plant origins. All of these that show varied features in terms of colors, flavors and resistance to pathogens. Phytochemicals have garnered significant attentions for their health-related cases. Some plant extracts are chief sources of metabolites capable of mitigating effects of reactive species (RS), chelating metal ions and intervening in cellular antioxidant defenses; with aims at protecting against stress-related diseases (cancers, cardiovascular disorders, diabetes and neurological conditions (Liu, 2004; Lobo et al., 2010).

Phytochemicals are broadly classified into such classes as flavonoids, phenolic acids, alkaloids, tannins, saponins, and terpenoids that exhibit distinct chemical structures and antioxidant action.

Antioxidant-Rich Medicinal Plants

Green tea leaves are rich in catechins exhibit strong antioxidant activity. Polyphenols scavenge free radicals to inhibit lipid peroxidation, and modulate antioxidant enzymes. Regular consumption of green tea is linked to reduced disease risks (Sultana et al., 2023).

Turmeric is rich in polyphenolic curcumin with antioxidant and anti-inflammatory properties. Curcumin contains proteins like superoxide dismutase (SOD) and glutathione peroxidase

(GPx).mobs ROS, chelates metal ions, and improves intrinsic antioxidant (Nwozo et al., 2023).

Rauvolfia vomitoria is traditionally has been involved traditional medicines to treat a number of both severe and mild conitions. This Asian and West African plant had provided natural treatments for hypertension, mental disorders, and infections. The plant parts like roots and leaves are major embodiments of alkaloids (reserpine, ajmaline), flavonoids, and phenolic compounds. Indeed, these compounds have been undoubtedly demonstrating antioxidant and anti-inflammatory potency. Extracts of *R. vomitoria*, even in the crude, have shown to inhibit lipid peroxidation and scavenge DPPH radicals (Aliyu et al., 2019).

Mode of actions of Antioxidants

Some natural antioxidants are multifaceted :

- **Free Radical Scavenging:** Many compounds donate electrons to neutralize free radicals, preventing chain reactions that damage cells (Lobo et al., 2010).
- **Metal Ion Chelation:** Phenolics bind to transition metals like iron and copper to reduce their ability to catalyze ROS formation (Pietta, 2000).
- **Enzyme Modulation:** Some phytochemicals upregulate endogenous antioxidant enzymes such as SOD, CAT, and GPx via activation of transcription factors like Nrf2 (Ma, 2013).
- **Inhibition of Pro-Oxidant Enzymes:** Flavonoids can inhibit enzymes like xanthine oxidase and NADPH oxidase, which culminate in the reduction of ROS production (Middleton et al., 2000).

Taxonomic Profile of *Rauvolfia vomitoria*

Rauvolfia vomitoria Afzel belongs to the family Apocynaceae, as one the flowering plants comprises active compounds. It is a small green tree that is around three meters tall of glossy leaves (Odugbemi, 2008). The plant produces small white or pinkish flowers and bright red berries. (Oliver-Bever, 1986). The genus *Rauvolfia* comprises over 60 species of which some are used in traditional medicine. The species name "vomitoria" is derived from its historical use as an emeti (Odugbemi, 2008).

The scientific classification of *Rauvolfia vomitoria* is as follows (Adam, 1818):

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Order: Gentianales
Family: Apocynaceae
Genus: *Rauvolfia*

Species: *Rauvolfia vomitoria*



Rauvolfia vomitoria (Photograph: Arowolo A.S)

Rauvolfia vomitoria has made history in folklore medicines:

Traditional doctors employ various plant parts (a root, bark, leaves and seeds) for the treatment of a wide range of problems. Hypertension, mental disorders, epilepsy, fever, gastrointestinal disturbances, malaria, and infectious diseases are a few examples (Okoli et al., 2007). Preparations made from the root or bark are commonly administered orally or topically, depending on the type of the case.. In Nigeria, edative and tranquilizer have been made from *R. vomitoria* root extract. In Ghana, it is used as psychotic for the management of disorders and high blood pressure (Odugbemi, 2008). The plant vastly affordable practices (Oliver-Bever, 1986).

Flavonoids and phenolic compounds innately present in the plant contribute to its activities of scavenging free radicals, chelating metal ions, and modulating enzyme activity (Rice-Evans et al., 1997). Among other therapeutic roles making *R. vomitoria* a promising source for pharmaceutical developments .are anti-inflammatory, antimicrobial, and anticancer properties (Okolie et al., 2011).

Despite these previous findings, the antioxidant potential of *R. vomitoria* remains underexplored, particularly in standardized in vitro settings. Most studies focused on isolated compounds or use non-research methods limit the generalizability of results. Comprehensive evaluations of assays such as DPPH, ABTS, and FRAP are needed to validate its antioxidant efficacy and its therapeutic integration.

II. RESEARCH ELABORATIONS

Statement of the problem

Despite the widespread traditional use of *Rauvolfia vomitoria* in African ethnomedicine, its antioxidant potential has not been thoroughly validated through standardized scientific methods. Most existing studies are either anecdotal or limited in scope, lacking rigorous in vitro evaluations using established antioxidant assays. This gap in knowledge presents a challenge for integrating *R. vomitoria* into evidence-based therapeutic applications. Furthermore, the absence of comparative data with known antioxidant standards such as ascorbic acid or gallic acid limits our understanding of its efficacy and pharmacological relevance. Without comprehensive in vitro investigations, the therapeutic claims surrounding *R. vomitoria* remain speculative, and its potential as a natural antioxidant source remains underutilized.

Aim and objectives of the study

This research work is aimed at investigating the in-vitro antioxidant and anti-inflammatory potential of *Rauvolfia vomitoria* crude extract using standard antioxidant assays.

Specific Objectives

- To evaluate the in vitro antioxidant activity of the extract.
- To evaluate the in vitro inflammatory activity of *Rauvolfia vomitoria* crude extract.
- To compare the antioxidant and anti-inflammatory activity of *R. vomitoria* with standard antioxidants such as ascorbic acid.

Significance of the study

This study is significant for several reasons. First, it provides scientific validation for the traditional use of

R. vomitoria in treating diseases associated with oxidative stress. Second, it contributes to the growing body of research on plant-based antioxidants, offering insights into alternative therapies that are safer and more sustainable than synthetic compounds. Third, the study may inform pharmaceutical development by identifying bioactive compounds with therapeutic potential. Lastly, it supports biodiversity conservation by highlighting the medicinal value of indigenous plant species.

Oxidative stress is a physiological condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to detoxify the reactive intermediates (Valko et al., 2007). Chemically reactive molecules are superoxide anion (O_2^-), hydroxyl radical ($OH^\cdot$) and hydrogen peroxide (H_2O_2). These molecules are products of aerobic metabolism and xenobiotics, ionizing radiations (Pham-Huy et al., 2008).

Under normal physiological conditions, ROS are cellular messengers that mediate growth, immunity and maturity, gene expressions (Halliwell and Gutteridge, 2015). While excessive amount of the oxygen molecules becomes toxic and cause oxidative Stress; hallmark damaged cellular lipids, proteins, and nucleic acids. At the same time, they are indicative of various diseases like cancer, cardiovascular infraction, diabetes mellitus and Alzheimer's and Parkinson's diseases (Lobo et al., 2010).

Sample preparation

Rauvofia vomitoria leaves were collected, cleaned, and air dried to preserve its phytochemicals, after which they were ground into fine powder to increase the surface area for solvent penetration. The powdered sample was then soaked in 100% methanol in seal jars for 48 hours at room temperature with occasional agitation to enhance extraction efficiency; the mixture was filtered to separate the methanol solution containing dissolved bioactive compounds from the solid residue; finally, the filtrate was rotary-evaporated to concentrate the methanolic crude extract.

III. METHODOLOGY

In vitro antioxidant assessment Rauvofia vomitoria crude extract

DPPH (2,2-diphenyl-1-picrylhydrazyl) ASSAY: DPPH working solution was added to each cuvette extract and ascorbic acid in separate tubes. After incubation, absorbance of each reaction mixture was measured at 520nm on uv-visible spectrophotometer (Blois, 1958).

FRAP (Ferric Reducing Antioxidant Power): FRAP reagent was added to extract, ascorbic acid in test tubes. The absorbance of the incubated mixture was read at 590 nm on uv-visible spectrophotometer, recorded and compared to a standard curve prepared using known concentrations of standard ascorbic acid (Benzie and Strain, 1996).

ORAC-FL (Oxygen Radical Absorbance Capacity – Fluorescein) (Guohuan et al., 1993): Trolox, extract was added to the microtiter plate in separate plates followed by the working fluorescein probe solution. 15 μ L of the Free AAPH solution was added into the incubated wells. Readings of sample and standard wells were made with a fluorescent microplate reader at 37°C at wavelength of 480 nm.

In vitro inflammatory assay of Rauvofia vomitoria crude extract Protein denaturation assay (Chandra et al., 2012).: 2mL portion of extract and standard (diclofenac sodium) at varying concentrations, 3mL phosphate buffered saline (pH 7.4) was mixed with egg albumin to form a reaction mixture. The absorbance of the incubated mixture was measured at 280 nm by a UV/Vis spectrophotometer using tri-distilled water as the blank Trypsin inhibition assay: The absorbance of the reaction mixtures containing trypsin + N-alpha-benzoyl-DL-arginine—nitroanilide hydrochloride (BAPNA), extract was made and one without extract as soybean trypsin inhibitor (STI) was made. Proteolytic activity was measured by UV Spectrophotometer (Shimadzu UV-VIS spectrophotometer) at 410 nm for 5 minutes.

Quantitative determination of DNA oxidation by in vitro assay via High performance liquid

chromatography-Electrochemical detection, HPLC-ECD (Floyd et al., 1986)
Supercoiled plasmid DNA (pBR322) were incubated with extract and doxorubicin in separate tubes. Each mixture was exposed to fenton-initiated reactions The amount of 8-hydroxy-2-deoxyguanosine (8-OHdG) was measured by HPLC.

Statistical analysis

All experiments were performed in triplicates (n=3) and the results expressed as the Mean±Standard

Deviation (SD). Data were analyzed using a statistical GraphPad Prism). One-way Analysis of Variance (ANOVA) followed by a post-hoc test to determine statistically significant differences among the means at a p<0.05 level of significance.

IV. RESULTS

In vitro antioxidant assessment

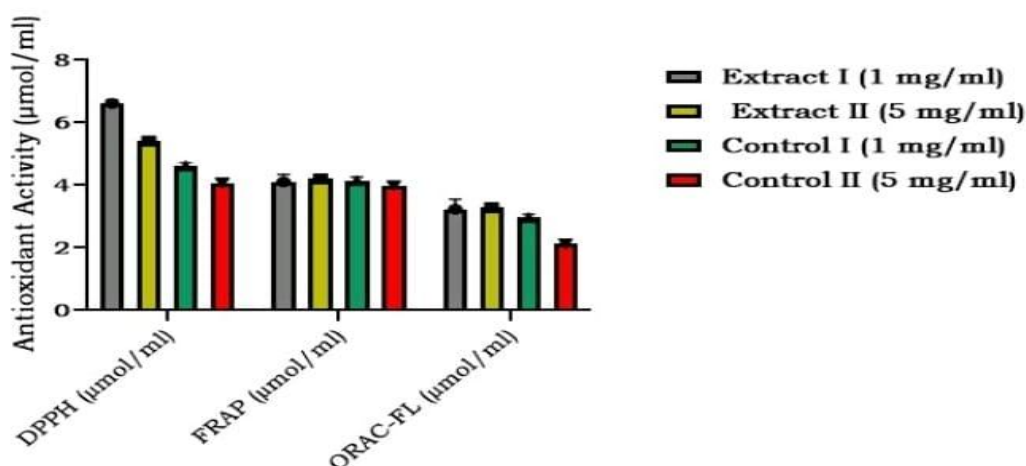


Figure1: In Vitro Antioxidant Activity of the Rauvolfia vomitoria Extract Measured by DPPH, FRAP, and ORAC-FL Assays.

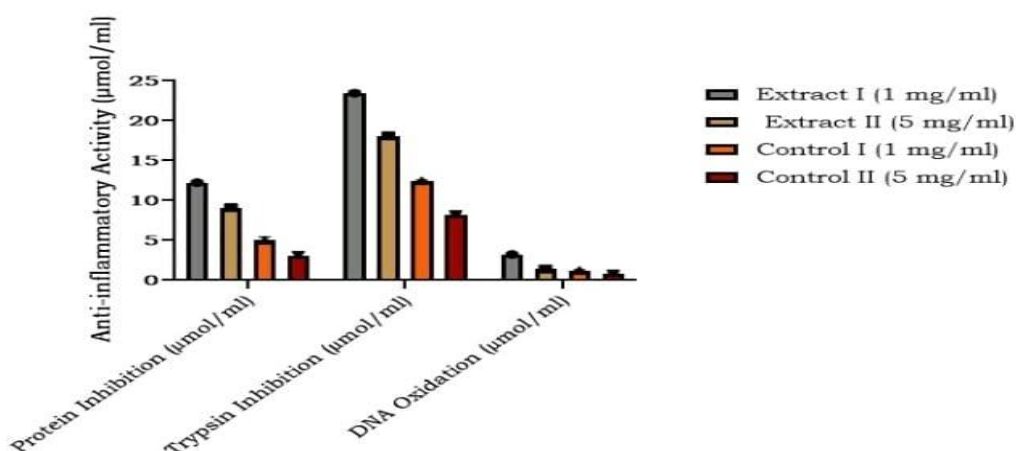


Figure 2: Evaluation of Anti-inflammatory Potential of the Rauvolfia vomitoria Extract Measured by Inhibition of Protein Denaturation, Trypsin Activity, and DNA Oxidation assays.

V. CONCLUSION

The present study evaluated the in vitro antioxidant and anti-inflammatory activities of extract of *Rauvolfia vomitoria* using multiple in vitro methods. The antioxidant potential was assessed through DPPH, FRAP, and ORAC-FL assays, while anti-inflammatory activity was evaluated via protein denaturation, trypsin inhibition, and DNA oxidation assays.

The DPPH assay revealed that both Extract I (1 mg/mL) and Extract II (5 mg/mL) exhibited significant radical scavenging activity, with mean absorbance values of 6.619 and 5.413 respectively, compared to the control values of 4.624 and 4.076 (fig. 4.1). This indicates a dose-dependent increase in antioxidant activity, as higher concentrations of the extract resulted in greater free radical neutralization. These findings were consistent with the previous reports that identified phenolic and alkaloid compounds in *R. vomitoria* as potent radical scavengers (Akinmoladun et al., 2010).

Similarly, the FRAP assay demonstrated that both extracts had ferric reducing capacities comparable to the standard, with Extract II (5 mg/mL) showing a slightly higher absorbance (4.2034) than Extract I (4.1123), and both exceeding the control II value (3.9865). This suggests that the extract possesses electron-donating properties, which are essential for reducing oxidized intermediates in biological systems (Benzie and Strain, 1996).

The ORAC-FL assay, which measures peroxyl radical scavenging capacity, further confirmed the antioxidant efficacy of the extract. Extract II recorded a higher fluorescence value (3.2856) than Extract I (3.2342), both of which surpassed the control values. These results underscore the extract's ability to inhibit oxidative degradation of biomolecules, likely due to the presence of flavonoids and other polyphenolic constituents (Lobo et al., 2010).

In the anti-inflammatory assays (figure 2), the extract demonstrated notable inhibition of protein denaturation, trypsin activity, and DNA oxidation. Extract I (1 mg/mL) showed the highest protein

inhibition (12.2456 $\mu\text{mol/mL}$), while Extract II (5 mg/mL) exhibited stronger trypsin inhibition (18.1117 $\mu\text{mol/mL}$) and DNA oxidation suppression (1.4123 $\mu\text{mol/mL}$). These results suggest that the extract may interfere with inflammatory pathways by stabilizing proteins and inhibiting proteolytic enzymes, mechanisms commonly associated with non-steroidal anti-inflammatory drugs (Chandra et al., 2012).

Collectively, the findings from this study align with earlier investigations that have highlighted the therapeutic potential of *R. vomitoria* in oxidative stress and inflammation-related conditions. The observed bioactivities can be attributed to the synergistic effects of its phytochemical constituents, particularly alkaloids, phenolics, and flavonoids, which are known to modulate redox homeostasis and inflammatory mediators (Ogbole et al., 2018).

The in vitro investigations conducted in this study provided compelling evidence of the antioxidant and anti-inflammatory potential of extracts from *Rauvolfia vomitoria*. The extract demonstrated significant free radical scavenging, ferric reducing, and peroxyl radical neutralizing activities, as well as inhibition of protein denaturation, trypsin activity, and DNA oxidation. These bioactivities suggest that *R. vomitoria* may serve as a valuable source of natural antioxidants and anti-inflammatory agents, with potential applications in the development of functional foods, nutraceuticals, and phytopharmaceuticals. Further studies, including in vivo evaluations and compound isolation, are recommended to elucidate the mechanisms of action and confirm the therapeutic efficacy of the extract.

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