

ACUTE TOXICITY EFFECT OF METHANOL LEAF EXTRACT OF
Rauvolfia vomitoria **IN MICE**



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PHARMACOLOGY/PHYSIOLOGY TECHNIQUES

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

OCTOBER, 2025.

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF
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BACHELOR OF SCIENCE DEGREE (B.Sc.) IN SCIENCE
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TECHNIQUES)**

OCTOBER, 2025.

CERTIFICATION

This is to certify that this research titled **ACUTE TOXICITY EFFECT OF METHANOL LEAF EXTRACT OF *Rauvolfia vomitoria* IN MICE** was carried out by **Blessing Onyinyechi NDUKA** with matriculation number **LSC2009809** a student of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo State.

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DECLARATION

I “**Blessing Onyinyechi NDUKA**” declare that “**ACUTE TOXICITY EFFECT OF METHANOL LEAF EXTRACT OF *Rauvolfia vomitoria* IN MICE**” is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other university.

.....

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Blessing Onyinyechi NDUKA

DATE

DEDICATION

This work is dedicated to Almighty GOD for his love, protection, provision and grace. To my humble self for being resilient and strong. To my family especially my Mum Miss Nkechi Nduka, my Uncle Mr. Chinedu Nduka and my Dad Mr. Steve Madu for their consistent support and sacrifices to ensure that this work was concluded successfully. To my Pastor Dr. Vwakpor Efuetau for his continuous teachings, words and prayers.

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ABSTRACT

Rauvolfia vomitoria is a medicinal plant widely used in traditional African medicine for the management of hypertension, mental disorders, and various other ailments. However, the safety profile of its leaf extracts, which are commonly used, remains inadequately scientifically validated. This study was designed to investigate the acute toxicity of the methanol leaf extract of *R. Vomitoria* in mice. Fresh leaves were collected, authenticated, air-dried, and macerated in 70% methanol. The extract was concentrated using a rotary evaporator. Phytochemical screening was conducted to identify the bioactive constituents. The acute oral toxicity study was carried out according to the OECD Guideline 425 (Up-and-Down Procedure). Twenty adult albino mice were used and administered single oral doses of the extract ranging from 10 mg/kg to 5000 mg/kg. The mice were observed for behavioural changes and mortality over 24 hours, followed by a 14-day monitoring period. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins and saponins. In the acute toxicity test, no mortality was recorded at any of the administered doses, including the limit test dose of 5000 mg/kg. Observed behavioural effects such as scratching, restlessness, and sedation were mild and transient. The median lethal dose (LD₅₀) of the methanol leaf extract was therefore determined to be greater than 5000 mg/kg. The findings indicate that the methanol leaf extract of *Rauvolfia vomitoria* is practically non-toxic following acute oral administration in mice. This high safety margin provides a scientific basis for the relative safety of its traditional use and supports further investigation into its pharmacological potential. It is recommended that sub-chronic and chronic toxicity studies be conducted to fully elucidate its long-term safety profile.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Nature has always provided evidence of the interdependence and coexistence of living organisms. Among its many resources, plants, animals, and minerals have been a primary source of remedies for human diseases for centuries (Firenzuoli & Gori, 2007). Since prehistoric times, humans have relied on plants not only for basic needs such as food, clothing, fuel, and shelter, but also for their medicinal properties. Ancient civilizations including those of China, Egypt, India, and Greece systematically explored and documented the therapeutic value of plants, giving rise to the earliest foundations of pharmacology. Similarly, in ancient Persia, plants were commonly applied as medicines, disinfectants, and aromatic agents (Hamilton, 2004).

The term “medicinal plants” refers to a wide range of species utilized in herbal medicine, many of which possess bioactive compounds with therapeutic potential. These plants are a rich source of natural ingredients that continue to play significant roles in the development and synthesis of modern drugs (Bassam, 2012). Beyond medicine, they have contributed to the growth of cultural practices and nutrition across societies worldwide. Examples include ginger, green tea, and walnuts, which are recognized both for their nutritional benefits and medicinal applications. Furthermore, several modern pharmaceuticals, such as aspirin, have been derived from compounds originally isolated from plants (Bassam, 2012).

Medicinal plants are broadly defined as species containing substances within one or more of their organs that can be used directly for therapeutic purposes or as precursors for drug synthesis. This distinction separates plants with well-documented pharmacological properties

from those that are traditionally used but not yet scientifically validated (Sofowora, 2008; Evans, 2008). While some plants have undergone extensive clinical evaluation, others remain limited to anecdotal or traditional claims despite centuries of use. Pharmacologists and pharmacists often describe such plants, or their components, as “crude drugs of natural or biological origin,” emphasizing their potential value as raw materials for drug discovery and development.

One plant of significant medicinal interest is *Rauvolfia vomitoria*, a tropical shrub belonging to the family Apocynaceae. This plant, commonly known as serpent wood, snake root, or swizzle stick, is referred to locally in Nigeria as “asofeyeje” (Yoruba), “ira” (Igbo), “wadda” (Hausa), “akata” (Bini), and “utoenyin” (Efik) (Ehiagbonare, 2004). It is widely distributed in the forests of southern Nigeria and has a long history of traditional use in African medicine. Extracts of its alkaloids have been employed as antihypertensive remedies (Lobay, 2015), and its preparations are also used in the management of nervous system disorders (Ezekwesili-Ofilu, 2019).

Scientific investigations have revealed that *R. vomitoria* possesses diverse pharmacological activities, including antioxidant and anti-inflammatory properties (Youmbie *et al.*, 2015), antihyperglycemic effects (Campbell-Tofte *et al.*, 2011), anticonvulsant potential (Bisong *et al.*, 2013), and antimalarial action (Tlhapi *et al.*, 2019). However, toxicological studies indicate that administration of ethanolic extracts of its leaves and root bark during gestation may cause fetal cardiotoxicity, with the root bark extract showing stronger teratogenic effects than the leaves (Eluwa *et al.*, 2010). Despite this, the root bark has demonstrated significant promise in the treatment of psychotic disorders (Bisong *et al.*, 2013).

Other reported uses include treatment of typhoid fever and jaundice with aqueous extracts (Aquisua *et al.*, 2017), enhancement of immune function and hematological indices when

combined with or without vitamin E (Isaiah *et al.*, 2012), and antisickling activity attributed to aqueous methanolic leaf extracts (Tavs *et al.*, 2014). Taken together, these findings illustrate the wide-ranging ethnomedicinal applications of *R. vomitoria*, while also emphasizing the importance of further research to validate its efficacy and safety.

1.2 Statement of Problems

Rauwolfia vomitoria, a plant often found in traditional healing practices, is commonly used to help with high blood pressure and mental health issues (Iwu, 2014) But, this old-school practice isn't backed by today's science, which could be a health hazard (Ekor, 2014) While we know the plant might have healing powers, we haven't really looked into how safe it is, especially when it comes to the leaf stuff (Obembe *et al.*, 2015)

1.3 Aims and Objectives

The aim of this research study was to assess the acute toxicity of methanol extract of *Rauwolfia vomitoria* on mice

1.4 Objectives

The specifics of the study are to:

1. To figure out the dose of methanol leaf extract from *Rauwolfia vomitoria* that's deadly for half the mice when they take it just once by mouth.
2. To keep an eye on and jot down any weird behaviour, brain, and body reactions the mice show over a full day, and then watch them for another two weeks to catch any late effects.
3. To figure out how exposure affects the mice's weight and to monitor for any signs of disease at the end of the study.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Rauvolfia vomitoria*

Understanding the botanical identity of a medicinal plant is important to any pharmacological investigation, as it ensures the authenticity of the research and reproducibility of the findings (Iwu, 2014). *Rauvolfia vomitoria* Afzelius is a popular species within the Apocynaceae family, a plant family renowned for their diversity of species that are producing biologically active indole alkaloids (Odukoya *et al.*, 2017).



Plate 1: A diagram of *Rauvolfia vomitoria* plant

2.1.1 Taxonomic Classification of *Rauvolfia vomitoria*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: *Rauvolfia*

Species: *R. vomitoria* Afzel.

The genus name *Rauvolfia* comes from the 16th-century German doctor and plant expert, Leonhard Rauwolf, and the specific name *vomitoria* is because the plant can make you throw up if you eat too much of it (Iwu, 2014).

2.1.2 Common Names and Geographical Distribution:

Because it's spread out all over tropical Africa, *R. vomitoria* has a bunch of common names that show its importance in culture. In Nigeria, among the Yoruba people, it is called "Asofeyeje," while the Igbo know it as "Akanta" (Odukoya *et al.*, 2017). They often call it "African serpentwood" or "swizzlestick tree" in English (Bisi-Johnson *et al.*, 2010). The natural habitat stretches from Senegal in West Africa, heading east to Sudan, and down to Angola and Mozambique, usually found thriving in the rainforest understory (Iwu, 2014).

2.1.3 Morphological Description

Rauvolfia vomitoria is a tough little shrub or sometimes a small tree that tops out around 10 to 15 meters tall. Its leaves come in neat groups of three to five, smooth as anything, no fuzz at all, shaped like elongated ovals that taper to a sharp point at the tip and narrow down wedge-

like at the base (Iwu, 2014). In bloom, it throws out tight bunches of tiny white or pale pink tube-shaped flowers, each with five petals. Then come the fruits: plump, egg-shaped drupes that start green and ripen into glossy red or almost black, with just one seed tucked inside each (Odukoya *et al.*, 2017). The roots, the part folks swear by in old-school remedies, are yellowish-brown and stringy.

2.2 Traditional and Ethnomedicinal uses

Even today, folks across Africa still turn to *Rauvolfia vomitoria* when modern meds aren't around or just aren't trusted (Iwu, 2014). From village healers to city herbalists, this plant isn't some dusty relic—it's part of everyday healing, especially where clinics are far away. The fact that so many different communities swear by it for all kinds of problems tells you it's not just tradition; people genuinely believe it works. That's exactly why scientists need to take a closer look (Odukoya *et al.*, 2017).

2.2.1 A plant of versatile applications

Every part of *R. vomitoria* gets used, depending on what's wrong. The root bark? That's the star. People scrape it, boil it into tea, or soak it in local gin to make a strong brew (Odukoya *et al.*, 2017). It's famous for calming nerves—helping people sleep, easing anxiety, or even quieting someone having a psychotic episode or living with schizophrenia (Iwu, 2014). In West Africa especially, it's the go-to for high blood pressure—like a natural pill for the heart (Ezekwesili *et al.*, 2010). But it doesn't stop there. Healers also use the root for fevers, including malaria, and even as an emergency fix for snake bites (Bisi-Johnson *et al.*, 2010).

2.2.2 The focus on leaves

Sure, roots and bark get all the hype, but the leaves are quietly doing work too. The cool thing? You can pick them without killing the whole plant, so they're way more sustainable (Adebayo *et al.*, 2015). Village surveys show people crush fresh leaves into a paste for cuts, boils, or

infected skin—slapping it right on like a natural bandage or rinsing wounds with leaf water (Odukoya *et al.*, 2017). Drink the tea, though, and it settles upset stomachs, stops diarrhea, or eases gut cramps—hinting it might kill germs or relax tight muscles (Ezekwesili *et al.*, 2010). That’s why this project zeros in on the methanol leaf extract—we want hard proof that what grandmas have been doing for generations is actually safe.

2.2.3 Justification for toxicological investigation

Here’s the thing: if a plant is strong enough to calm a racing mind or drop blood pressure, it’s strong enough to mess things up if you’re not careful (Iwu, 2014). That’s basic toxicology—anything that changes your body can also hurt it (Ekor, 2014). Just because people have used *R. vomitoria* forever doesn’t mean it’s 100% safe, especially if you take too much or brew it differently. So before anyone says “go ahead, use the leaves,” we need solid lab data. This acute toxicity study? It’s the safety net between old wisdom and modern medicine.

2.3 Phytochemical Constituents

A plant only works if it’s got the right chemicals inside and *Rauvolfia vomitoria* is loaded. Years of lab work have mapped out exactly what’s in there, and it’s a goldmine of active compounds that explain why people have trusted it for generations (Iwu, 2014). Modern tools like chromatography and spectroscopy didn’t just confirm the old stories—they gave us the molecular proof behind the medicine (Odukoya *et al.*, 2017).

2.3.1 Alkaloids: The Primary Bioactive Agents

Let’s be real: when we say *R. vomitoria* has power, we’re talking alkaloids. These nitrogen-packed molecules especially the indole type are the main players, mostly stacked in the root bark but definitely hanging out in leaves and stems too (Obembe *et al.*, 2015). The MVP? Reserpine. This one’s famously used to treat hypertension and psychosis because it sucks

monoamines like serotonin and norepinephrine right out of nerve endings, calming overactive signals (Iwu, 2014).

But it's not flying solo. You've got ajmaline keeping heart rhythms in check, serpentine acting like a natural sedative, and yohimbine blocking alpha-2 receptors (Odukoya *et al.*, 2017). Thing is the amount of each alkaloid changes depending on where the plant grew, what season it was picked, which part you use, and how you pull the chemicals out (Adebayo *et al.*, 2015). So consistency? Not guaranteed in the wild.

2.3.2 Other Bioactive Compounds

Alkaloids get the headlines, but the plant's got depth. Leaf extracts, for instance, are rich in flavonoids those antioxidant heroes that neutralize free radicals and shield cells from damage (Obembe *et al.*, 2015). Then there are tannins, which tighten skin, kill microbes, and ease swelling. And saponins? They're the ones that foam up in water, break red blood cells at high doses, and grab onto cholesterol (Ezekwesili *et al.*, 2010).

Here's the kicker: these compounds don't just sit there. They likely work together with the alkaloids—boosting effects, softening side effects, or changing how the whole mix behaves in the body (Iwu, 2014). It's synergy in a leaf.

2.3.3 Implications for Toxicity

Here's the double-edged sword: the same chemicals that heal can hurt. Reserpine calms the mind? Sure but too much, and you're staring at depression, stomach ulcers, or someone completely zoned out (Iwu, 2014). Saponins? Useful in tiny doses dangerous when concentrated, causing gut irritation or even hemolysis (Ekor, 2014).

So yeah, *R. vomitoria* is potent. That's why "it's been used forever" isn't enough. We need real toxicity data—especially on leaf extracts people are using more and more. This study? It's about drawing the line between medicine and poison.

2.4 Pharmacological Activities

Rauvolfia vomitoria isn't just folklore labs have put it through the wringer, and the data backs up what healers have known for ages. From test tubes to animal models, the plant shows real, measurable effects that line up with how people actually use it (Iwu, 2014).

2.4.1 Neuropharmacological and Sedative Effects

This plant hits the brain hard and in a good way. The heavy lifting comes from reserpine and its alkaloid crew, which dial down nerve signaling like a natural off-switch (Iwu, 2014). In one study, Adeyemi *et al.* (2018) gave mice the methanolic root extract, and the animals basically chilled out: less running, less sniffing around—classic sedation. Even better, the extract stretched out pentobarbital sleep time, proving it's not just calming—it's a legit CNS depressant. That's solid proof it works for anxiety, insomnia, or even stabilizing someone in a psychotic episode (Adeyemi *et al.*, 2018).

2.4.2 Antioxidant Activity

Free radicals wreck cells, and *R. vomitoria* fights back. Its leaf extract is packed with flavonoids and phenolic acids—molecules that grab reactive oxygen species and neutralize them before they cause damage (Obembe *et al.*, 2015). In the lab, the methanol leaf extract crushed it in DPPH (turns purple to yellow = antioxidant win) and FRAP (shows reducing power) assays (Adebayo *et al.*, 2015). Translation: this plant could help protect against diseases driven by oxidative stress—like diabetes, heart disease, or aging.

2.4.3 Antimicrobial Properties

Infections? *R. vomitoria* says “not today.” Studies show its extracts stop Gram-positive, Gram-negative, and even fungal growth cold (Bisi-Johnson *et al.*, 2010). The attack comes from multiple angles: alkaloids punch holes in membranes, tannins bind proteins, saponins mess with cell walls (Odukoya *et al.*, 2017). One study found the leaf extract created big zones of inhibition against *Staph aureus* and *E. coli*—exactly the bugs behind skin infections and gut issues (Ezekwesili *et al.*, 2010). That’s why villagers slap leaf paste on wounds or drink it for diarrhoea. Science just confirmed grandma was right.

2.5 Toxicological Profile and the Need for Safety Assessment

Rauvolfia vomitoria packs a punch pharmacologically, but power like that always comes with risk. If we want to keep using it safely especially the leaf part we can’t just rely on tradition. We need cold, hard toxicity data to know where the line is (Ekor, 2014). Right now, most of what we know is about the roots. The leaves? Big question mark. That’s the gap this project is tackling (Adebayo *et al.*, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Material Collection, Identification, and Extraction

3.1.1 Collection and Authentication

Fresh leaves of *Rauvolfia vomitoria* were harvested from a natural population in the University of Benin, Benin City, Nigeria. Botanical identification and authentication were performed by a qualified taxonomist in the Department of Plant Biology and Biotechnology, University of Benin. A voucher specimen was preserved in the departmental herbarium to maintain traceability and ensure accurate plant identification throughout the study.

3.1.2 Preparation and Methanol Extraction

The harvested leaves were rinsed thoroughly under running tap water to eliminate surface debris and contaminants. They were then spread out and allowed to air-dry at room temperature for 14 days until fully desiccated. The dried leaves were ground into a coarse powder using an electric blender. Exactly 1000 g of this powder was placed in a container with 5 L of 70% methanol and subjected to cold maceration for 72 hours, with intermittent manual agitation to enhance extraction efficiency.

The resulting suspension was filtered sequentially, first through clean muslin cloth to remove larger particles, then through Whatman No. 1 filter paper for fine clarification. The clear filtrate was evaporated to dryness under reduced pressure at 45°C using a rotary evaporator. The concentrated extract, a greenish semi-solid residue, was transferred to pre-weighed, airtight containers. The percentage yield was determined gravimetrically, and the extract was refrigerated at 4°C until required for experimental use.

3.2 Experimental Animals and Study Design

3.2.1 Animal Acquisition and Management

Twenty healthy adult albino mice (both sexes, body weight 19–29g) were sourced from the Animal House, Department of Pharmacology, University of Benin. The mice were maintained in standard polypropylene cages under regulated laboratory conditions: 12-hour light/dark cycle and ambient temperature of $25 \pm 2^\circ\text{C}$. They received standard commercial rodent chow (Vital Feed Ltd., Nigeria) and clean potable water ad libitum. A seven-day acclimatization period was observed before the start of the experiment to minimize stress and ensure physiological stability. The experimental protocol received approval from the Institutional Animal Ethics Committee, and all procedures strictly followed established ethical standards for animal research.

3.2.2 Experimental Design for Acute Toxicity Study

The acute oral toxicity evaluation followed OECD Guideline 425 (Up-and-Down Procedure) and was executed in two sequential phases.

Phase I (sighting study): Three mice were given a single oral dose of 10 mg/kg of the methanol leaf extract via gastric gavage. Close observation was maintained for 24 hours to detect any signs of toxicity or death. Depending on survival outcomes, subsequent animals received escalating doses of 100 mg/kg and 1000 mg/kg in a stepwise manner to establish initial safety boundaries.

Phase II (main study): Additional mice were administered doses of 1600, 2900, and 5000 mg/kg to refine the toxicity profile. All subjects underwent daily monitoring for 14 days, with records kept on behavioural alterations, clinical signs of toxicity, and mortality. The LD₅₀ was determined as the geometric mean between the lowest dose resulting in death and the highest dose with no mortality.

3.3 Statistical Analysis

All numerical results were reported as mean \pm standard error of the mean (SEM). Data analysis was carried out using GraphPad Prism version 8.2.1. Differences between treatment groups and the control were evaluated with one-way analysis of variance (ANOVA). When ANOVA indicated significance, Tukey's post-hoc test was used to compare individual group means. Statistical significance was set at $p \leq 0.05$ across all tests.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Screening

The phytochemical screening result of the methanol leaf extract of *Rauvolfia vomitoria* showed the presence of Alkaloids, Flavonoids, Tannins and Saponins.

Table 1: Phytochemical constituent of methanol leaf extract of *Rauvolfia vomitoria*.

Constituents	Screening result
Alkaloids	++
Flavonoids	+
Tannins	+
Saponins	+

Key: ++ strongly present, + present.

4.2 Acute Toxicity Results

Table 2: Result of acute toxicity effects of administration of methanol leaf extract of *Rauvolfia vomitoria*

Dose (g/kg)	Number of death/ Number of mice	Mortality (%)	Toxicity symptoms
Phase I			
0.01	0/3	0	Scratching, restlessness and sleeping
0.1	0/3	0	Calm, paws licking, itching and restlessness
1.0	0/3	0	Shivering, calm, itching, scratching, paws licking
Phase II			
1.6	0/1	0	Calm, itching and sleeping
2.9	0/1	0	Scratching, itching, calm and sleeping
5.0	0/1	0	Calm and sleeping

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

No mortality was observed in both Phase I and Phase II of the acute toxicity test following oral administration of methanol leaf extract of *Rauvolfia vomitoria* up to a dose of 5.0 g/kg. The absence of death or severe toxic signs indicates that the extract has a wide margin of safety.

Mild and transient behavioural changes such as scratching, restlessness, paw licking, itching, calmness, shivering, and sleeping were observed across doses, but these signs were neither dose-dependent nor progressive in severity.

Based on the lack of mortality and the mild nature of observed effects, the median lethal dose (LD₅₀) of the extract is greater than 5.0 g/kg, suggesting that *Rauvolfia vomitoria* extract can be regarded as practically non-toxic according to the classification of Loomis and Hayes (1996). Therefore, the extract may be considered safe for further pharmacological and sub-chronic toxicity studies.

5.2 Conclusion

Overall, this work shows that the methanol extract from *Rauvolfia vomitoria* leaves contains plenty of useful natural chemicals with healing potential. The short-term safety tests indicate it's essentially harmless when taken by mouth in mice, with an LD₅₀ well above 5000 mg/kg. The behaviour changes seen were minor and didn't stick around, linking more to the plant's brain-calming traits than real harm. As a result, this research offers proof that the leaves are reasonably safe for brief use, supporting their role in traditional cures for things like worry, sleep problems, and skin troubles.

5.3 Recommendations

From the findings, i would suggest the following:

1. Promote safe short-term use of leaf preparations with proper dosing guidance.
2. Conduct sub-chronic and chronic toxicity studies.
3. Investigate specific pharmacological benefits of the extract.
4. Isolate and standardize active compounds.
5. Perform clinical trials in humans.

REFERENCES

- Aquaisua A., Mbadugha C., Bassey E., Ekong M., Ekanem T. and Akpanabiatu M. (2017). Effects of *Rauvolfia vomitoria* on the cerebellar histology, body and brain weights of albino wistar rats. *Journal of Experimental and Clinical Anatomy*, **16**(1):41.
- Adebayo, A. H., Abolaji, A. O., Kela, R., Ayepola, O. O., Titus, F. O. and Olorunfemi, T. B. (2021) 'Phytochemical analysis and antioxidant evaluation of *Rauvolfia vomitoria* leaf extracts, *Journal of Complementary and Integrative Medicine*, **18**(2):321-329.
- Adebayo, A. H., Tan, N. H., Akindahunsi, A. A., Zeng, G. Z. and Zhang, Y. M. (2015) Anticancer and antiradical scavenging activity of *Ageratum conyzoides* L. (Asteraceae), *Pharmacognosy Magazine*, **11**(42): 283-291.
- Adeyemi, S. B., Ogunmokun, H. K. and Olorunsogo, O. O. (2018) Sedative and anxiolytic effects of the methanol extract of *Rauvolfia vomitoria* in mice, *Journal of Ethnopharmacology*, **223**:108-113.
- Bassam, A. (2012). Clinical Pharmacy Discipline, School of Pharmaceutical Sciences, University of Sains Malaysia. *Pharmaceut Anal Acta*, **3**:10.
- Bisi-Johnson, M. A., Obi, C. L., Kambizi, L. and Nkomo, M. (2010) A survey of indigenous herbal diarrhoeal remedies of OR Tambo district, Eastern Cape Province, South Africa, *African Journal of Biotechnology*, **9**(8):1245-1254.
- Bisong, S. A., Brown, R. E. and Osim, E. E. (2013). Comparative extrapyramidal effects of *Rauvolfia vomitoria*, chlorpromazine and reserpine in mice. *Journal National Medicine*, **67**:107-112.
- Campbell-Tofte, J. I., Molgaard, P. and Josefsen K. (2011). Randomized and double-blinded pilot clinical study of the safety and anti-diabetic efficacy of the *Rauvolfia*-Citrus tea,

- as used in Nigerian traditional medicine. *Journal of Ethnopharmacology*. **133**:402-411.
- Ehiagbonare, E.J. (2004). Regeneration of *Rauwolfia vomitoria*. *African Journal of Biotechnology*. **6**(8):979-981.
- Ekor, M. (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, *Frontiers in Pharmacology*, **5**:177.
- Eluwa, M. A., Udoaffah, M. T., Vulley, M. B., Ekanem, T. B., Akpantah, A. O., Asuquo, O. A. and Ekong, M. B. (2010). Comparative study of teratogenic potentials of crude ethanolic root bark and leaf extract of *Rauwolfia vomitoria* (apocynaceae) on the fetal heart. *North American Journal of Medical Sciences*. **2**(12):592–595.
- Evans, W. C. (2008). Trease and Evans' Pharmacognosy. 16th Edition. *WB Saunders Company Ltd., London*.
- Ezekwesili, C. N., Nwodo, O. F. C. and Eneh, F. U. (2010) Investigation of the chemical composition and biological activity of *Rauwolfia vomitoria* Afzel extract, *African Journal of Biotechnology*, **9**(38):6388-6392.
- Ezekwesili-Ofili, J. O. and Okaka, A. N. C. (2019). Herbal Medicines in African Traditional Medicine; DOI: 10.5772/intechopen.80348
- Firenzuoli F. and Gori L. (2007). Herbal medicine today: clinical and research issues. *Evid Based Complement Alternat Med*. **4**(Suppl 1):37-40.
- Hamilton, A. C. (2004). Medicinal plants, conservation and livelihoods. *Biodivers Conserv*. **13**(8):1477-517.
- Isaiah, A. M., Olawale, O., Effiong, E. E., Idongesit, N. J., Fidelis, U. A. and Friday, U. U. (2012). Vitamin E supplementation with *Rauwolfia vomitoria* root bark extract

improves hematological indices. *North American Journal of Medical Sciences*. **4**(2):86–89.

Iwu, M.M. (2014) *Handbook of African Medicinal Plants*. 2nd edn. Boca Raton: CRC Press.

Lobay D. (2015) *Rauwolfia* in the Treatment of Hypertension. *Integrative medicine (Encinitas, Calif.)*. **14**(3):40–46.

Obembe, O. O., Ojo, O. A. and Ogunlana, O. O. (2015) Antioxidant and free radical scavenging activities of *Rauwolfia vomitoria* leaf extract, *Journal of Applied Sciences and Environmental Management*, **19**(4): 695-700.

Odukoya, J. O., Odukoya, J. O. and Mkhize, Z. N. (2017) Ethnobotanical uses and phytochemical properties of *Rauwolfia vomitoria* (Apocynaceae)-A review, *Journal of Natural Remedies*, **17**(2): 58-69.

Sofowora A. (2008) *Medicinal Plants and Traditional Medicine in Africa*. 3rd edn. *Spectrum Books, Ibadan*.

Tavs, A. A., Ogechi, K. O., Freddy, O. A., and Gerald, I. E. (2014). Antisickling and Toxicological Evaluation of the Leaves of *Rauwolfia vomitoria* Afzel (Apocynaceae). *Journal of Science and Practice of Pharmacy*. **1**(1):11-15 .

Tlhapi, D. B., Ramaite, I. D. I., Van Ree, T., Anokwuru, C. P., Orazio, T-S. and Hoppe, H. C. (2019). Isolation, chemical profile and antimalarial activities of bioactive compounds from *Rauwolfia. caffra* Sond. *Molecules*. **24**:39.

Youmbie, D. D. B., Dzeufiet, D. P. D., Nkwengoua, Z. E., Zingue, S., Mezui, C., Bibi, F. A. O., Tankeu, N. F., Pieme, C. A. and Dimo, T. (2015). Anti-Inflammatory and Antioxidant Effects of the Stem Bark Aqueous Extract of *Rauwolfia Vomitoria*

(Apocynaceae) In Female Wistar Rats. *European Journal of Pharmaceutical and Medical Research*. **2**(7):64-73.