https://dx.doi.org/10.4314/dujopas.v10i4a.8
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 4a December 2024*

Evaluation of Pharmacognostic and Acute toxicity of *Vernonia amygdalina* Leaves

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Abstract
Vernonia amygdalina (Asteraceae) leaves have been traditionally used for managing numerous health conditions but lack standardized guidelines. This plant is known for its application in treating fever, cough, constipation, hypertension, vascular disorders, and diabetes. The current study seeks to assess the pharmacognostic features and acute toxicity of Vernonia amygdalina leaves, aiming to contribute to its standardization concerning quality, purity, and safety. Established methodologies for crude drug evaluation were employed to determine the pharmacognostic characteristics. Microscopic analysis showed anisocytic stomata and trichomes, while chemomicroscopy identified the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grains, and mucilage, but no calcium carbonate. Phytochemical analysis of the methanolic extract revealed the presence of flavonoids, alkaloids, tannins, saponins, steroids, triterpenes, carbohydrates, and phenols, with no glycosides or anthraquinones detected. The physico-chemical parameter averages were moisture content (5.10%), total ash (15.20%), acid-insoluble ash (15.20%), water-soluble ash (7.70%), alcohol extractive (2.60%), and water extractive (3.0%). The cytotoxicity assessment showed moderate cytotoxicity of the crude leaf extract (LC50: 29.65 µg/mL). Toxicity tests indicated LD50 values above 5000 mg/kg, with no fatalities in the test rats. These results indicate that Vernonia amygdalina extract contains phytochemical compounds that may be useful in managing various health conditions.

Keywords: Acute toxicity, Chemomicroscopical, Cytotoxicity Physicochemical, Phytochemical, Pharmacognostic

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INTRODUCTION

Vernonia amygdalina, also known as bitter leaf, has been traditionally recognized for its medicinal applications across various regions globally. With the rising interest in herbal medicine, it becomes increasingly important to scientifically investigate the pharmacognostic properties, antioxidant potential, and safety profile of this species. The antioxidant capabilities of *V. amygdalina* have drawn significant attention due to their potential health benefits (Oboh *et al.,* 2014). Antioxidants are vital in neutralizing free radicals and reducing oxidative stress, which are linked to the development of chronic conditions such as cardiovascular diseases, cancer, and neurodegenerative disorders. Research on the antioxidant capacity of *V. amygdalina* extracts and their active compounds contributes to our understanding of its possible role in mitigating oxidative damage and enhancing health. However, along with its medicinal properties, evaluating the safety of *V. amygdalina*, including its toxicity, is crucial. Although traditionally used for therapeutic purposes, thorough toxicity studies are essential to assess its safety, determine potential side effects, and establish appropriate dosage guidelines (Akinsola *et al.,* 2015). Understanding the toxicology of *V. amygdalina* is critical for its safe application as a medicinal herb and to minimize the risk of adverse reactions.

Vernonia amygdalina is a widely distributed plant, especially in West Africa, belonging to the Asteraceae family. Known for its bitter taste due to sesquiterpene lactones and other bitter compounds, this perennial herb typically thrives in open areas, often near streams or disturbed habitats. It is a bushy plant with broad, dark green, lance-shaped leaves, and can grow between 2-5 meters tall, producing clusters of small, purple tubular flowers (Ofori *et al.,* 2013). In traditional African medicine, bitter leaf has been utilized for its health benefits for generations, including treating malaria, diabetes, fever, and digestive disorders (Atinga *et al.,* 2021). Scientific research supports these uses, revealing that *V. amygdalina* possesses antioxidant, anti-inflammatory, antimicrobial, and antiplasmodial properties, likely due to its bioactive compounds such as alkaloids, flavonoids, terpenoids, and phenolic compounds (Ijeh *et al.,* 2011). In recent years, this plant has gained attention for its potential in the pharmaceutical and nutraceutical sectors. Extracts are being investigated for their pharmacological properties and possible uses in drug development. Despite its benefits, it's important to consume bitter leaf in moderation because excessive intake can cause gastrointestinal discomfort and other side effects (Okoli *et al.,* 2007).

A significant challenge in the use of herbal medicines is the lack of standardized pharmacognostic parameters, which are crucial for the identification and authentication of plant species, particularly in cases of substitution or adulteration (Atinga *et al.,* 2021). Standardization is key to ensuring the quality and purity of these herbal medicines. According to the World Health Organization, the initial step in establishing the identity and purity of medicinal plants involves macroscopic and microscopic examination, which should precede further testing (WHO, 2011). This study aim to evaluate the pharmacognostic characteristics and toxicity profile of *Vernonia amygdalina*, highlighting its potential as a natural medicinal resource and underscoring the importance of scientific research in confirming its therapeutic benefits and safety.

MATERIALS AND METHODS

Collection of Plant Materials

The *Vernonia amygdalina* leaves were carefully collected from the Botanical Garden at the Department of Plant Biology, Bayero University, Kano, located in Gwale Local Government Area, Kano State, Nigeria. The plant was identified and authenticated by botanical specialists

at the Herbarium of the same department, where it was assigned the voucher specimen number BUKHAN143

Macroscopical Evaluation
A comprehensive morphological assessment of the leaf samples was conducted, analyzing shape, apex, color, venation, and size. The examination adhered to the standard protocol outlined by the World Health Organization (WHO, 2011).

Microscopic evaluation
Sections of the plant sample were meticulously prepared following established procedures. The prepared material underwent clearing with 70% hypochlorite solution, followed by mounting in alcohol and dilute glycerol for observation of stomata and trichomes under a microscope, in accordance with Evans' guidelines (2002).

Micrometric Evaluation
Precise measurements of the dimensions (length and width) of various diagnostic microscopic characters of the leaves were conducted using a binocular microscope aided by graticles, as described by Kakote (1994).

Chemo-microscopic evaluation of *Vernonia amygdalina* leaves
The analysis of powdered *Vernonia amygdalina* leaves was conducted to identify cell wall components and cell inclusions. The finely powdered samples were treated with a 70% chloral hydrate solution and then boiled to eliminate any obscuring materials. The cleared samples were then mounted on microscope slides using diluted glycerol. The presence of cell wall materials and cell inclusions was determined using various detecting reagents, following WHO (2011) guidelines.

Cell Wall Materials

- **Test for Cellulose**: The powdered sample was treated with iodinated zinc chloride, which stained the cellulose in the cell walls blue to blue-violet.
- **Test for Lignin**: Phloroglucinol and hydrochloric acid were applied to the sample, resulting in a pink or cherry-red color, indicating the presence of lignin.
- **Test for Suberized or Cuticular Cell Walls**: Sudan red was used to detect suberin or cutin, which appeared as orange-red or red coloration.
- **Test for Gums and Mucilage**: The application of ruthenium red led to a pink coloration, confirming the presence of gums and mucilage.

Cell Inclusions/Cell Contents

- **Test for Starch Grains**: Starch was identified by adding N/50 iodine to the sample, which produced a blue-black or reddish-blue color.
- **Test for Calcium Oxalates and Calcium Carbonates**: Hydrochloric acid (HCl) was used to determine the presence of calcium oxalate and calcium carbonate. The absence of effervescence with crystal dissolution indicated calcium oxalate, while slow dissolution with effervescence suggested calcium carbonate (WHO, 2011).
- **Test for Inulin**: A mixture of 1-naphthol and sulfuric acid was used, with brownish-red spherical crystal aggregations confirming the presence of inulin.
- **Test for Tannins**: The addition of 5% ferric chloride solution resulted in a greenish-black coloration, indicating the presence of tannins.

Determination of Physicochemical Parameters of Powdered *Vernonia amygdalina* Leaves

The physicochemical properties of the powdered leaves were assessed according to the protocols described in the updated WHO guidelines for quality control of medicinal plant materials (WHO, 2011).

Moisture Content

Moisture content refers to the amount of water present in plant material. It was determined using the loss on drying method.

Approximately 3.0g of the powdered sample was precisely weighed and placed in clean, dry evaporating dishes of known weight. These dishes were heated in an oven at 105°C for one hour. After heating, the samples were cooled in a desiccator and re-weighed. This process of heating and weighing was repeated until a constant weight was achieved. The moisture content was then calculated using the following formula:

$$\% \text{ Moisture content} = \frac{\text{Initial Weight of Powder} - \text{Final Weight of Powder}}{\text{Initial Weight of Powder}} \text{ X } 100$$

Total Ash Value

Approximately 2g of the powdered plant material was carefully weighed and placed in a crucible with a known weight. The sample was heated gently at first, with the heat gradually increased until it turned white, indicating the complete removal of carbon. The crucible was then cooled in a desiccator and re-weighed. This process was repeated until a constant weight was achieved. The total ash content was calculated as a percentage using the following formula:

$$\% \text{ Ash Value} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Acid-Insoluble Ash

Acid-insoluble ash is the residue remaining after treating total ash with dilute hydrochloric acid.

To determine this for the powdered plant material, 25 mL of dilute hydrochloric acid was added to the crucible containing the ash. The mixture was covered with a watch glass and gently boiled for 5 minutes. After boiling, the watch glass was rinsed with 5 mL of hot water, and this rinse was added to the crucible. The insoluble residue was filtered using ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper with the residue was then placed back into the original crucible, dried in an oven, and ignited until a constant weight was achieved. The cooled residue was weighed immediately after removal from the desiccator (Evans, 2002). The percentage of acid-insoluble ash was then calculated using the appropriate formula.

$$\% \text{ Acid insoluble Ash} = \frac{\text{Weight of Residual Ash}}{\text{Original W} - \text{eight of Powder}} \text{ X } 100$$

Water Soluble Ash

To determine the water-soluble ash, 25 mL of water was added to the crucible containing the total ash and boiled for 5 minutes. The insoluble residue was collected in a sintered glass crucible, washed with hot water, and then ignited in an oven at 105°C for 15 minutes. The weight of this residue was subtracted from the weight of the total ash. The percentage of water-soluble ash relative to the air-dried powdered sample was then calculated and recorded (WHO, 2011).

$$\% \text{ Water Soluble Ash} = \frac{\text{Weight of Total Ash} - \text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Alcohol-Soluble Extractive Value

4g of plant material was weighed and placed in separate conical flasks. Each sample was treated with 100 of ethanol and allowed to macerate for 24 hours, with frequent shaking for the first 6 hours using a mechanical shaker. The mixture was then filtered, and 25 mL of the filtrate was transferred to an evaporating dish of known weight. This was evaporated to dryness using a water bath, and the residue was dried to a constant weight. The alcohol-soluble extractive value was then calculated as a percentage for each plant sample.

$$\text{Alcohol-Soluble Extractive Value (\%)} = \frac{\text{Weight of Extract in 25ml X 4}}{\text{Original Weight of Powder}} \text{ X } 100$$

Water-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with water. Same procedure as in alcohol-soluble extractive value was repeated here for the two plants, but solvent for extraction here was water.

$$\text{\% Water Extractive Value} = \frac{\text{Weight of Extract in 25ml X 4}}{\text{Original Weight of Powder}} \text{ X } 100$$

Extraction

Fresh leaves of *Vernonia amygdalina* were thoroughly washed, air-dried, and then ground into a coarse powder using a mechanical grinder. The powdered material was stored in airtight containers to preserve its quality for subsequent use. Two hundred grams of the powdered leaves were then soaked in 2 liters of methanol in a suitable container. The mixture was allowed to steep at room temperature (28 ± 2°C) for 72 hours, with regular stirring every hour to ensure effective extraction. After the extraction period, the liquid extract was separated from the plant residue. Initially, the mixture was strained through a muslin cloth to remove larger particles. The filtrate was then passed through Whatman No.1 filter paper to obtain a clear solution. This filtered extract was transferred into a clean evaporating dish and placed on a water bath at 50°C to evaporate the methanol. The process was continued until the solvent was completely removed, leaving a concentrated extract. This final extract was collected and stored in appropriate containers under controlled conditions for future analysis and experimentation (Namadina, 2021).

Qualitative Phytochemical Screening of Methanol Extract of *Vernonia amygdalina* Leaves

The methanol extract of *Vernonia amygdalina* leaves underwent qualitative phytochemical screening to identify its constituent phytochemicals using the following methods:

Carbohydrate

Molisch's Test: To 1 mL of the extract filtrate, 1 mL of Molisch's reagent was added in a test tube, followed by the careful addition of 1 ml of concentrated sulfuric acid to form a distinct lower layer. The presence of carbohydrates was indicated by a reddish color at the interface between the two layers (Evans, 2009).

Saponins

Frothing Test: Approximately 10 mL of distilled water was added to a portion of the extract, followed by vigorous shaking for 30 seconds. The tube was then allowed to stand upright for 30 minutes. The formation of a persistent honeycomb-like froth for 10-15 minutes signified the presence of saponins (Evans, 2009).

Flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2 mL of 50% methanol with heated metallic magnesium chips, and a few drops of concentrated hydrochloric acid were added. The appearance of a red color indicated the presence of flavonoids (Evans, 2009).

Alkaloids
Wagner's Test: A few drops of Wagner's reagent were added to a portion of the extract, leading to the formation of a whitish precipitate, which indicated the presence of alkaloids (Evans, 2009).

Steroids and Triterpenes
Liebermann-Burchard Test: Equal volumes of acetic anhydride were mixed with a portion of the extract. One milliliter of concentrated sulfuric acid was carefully added along the side of the test tube to create a lower layer. An immediate color change, followed by further changes, indicated the presence of steroids and triterpenes. A red, pink, or purple color suggested triterpenes, while a blue or green hue indicated steroids (Evans, 2009).

Cardiac-Glycosides
Keller-Killiani Test: A portion of the extract was dissolved in 1 mL of glacial acetic acid containing a trace amount of ferric chloride solution. This mixture was then transferred to a dry test tube, and 1 ml of concentrated sulfuric acid was added along the side of the tube to create a lower layer. The presence of deoxy sugars was indicated by a purple-brown ring at the interface, while a pale green color in the upper acetic acid layer indicated the presence of cardiac glycosides (Evans, 2009).

Tannins
Ferric Chloride Test: Three to five drops of ferric chloride solution were added to a portion of the extract. A greenish-black precipitate suggested the presence of condensed tannins, while hydrolyzable tannins produced a blue or brownish-blue precipitate (Evans, 2009).

Anthraquinones
Borntrager's Test: Five milliliters of chloroform were added to a portion of the extract in a dry test tube and shaken for at least five minutes. After filtration, the filtrate was mixed with an equal volume of 10% ammonium solution. The appearance of a bright pink color in the aqueous upper layer indicated the presence of free anthraquinones (Evans, 2009).

Cytotoxicity Assay

Hatching Brine Shrimp
Brine shrimp (*Artemia salina*) eggs were incubated in seawater to hatch. After 48 hours at room temperature, the larvae were attracted to one side of the container using a light source. They were then collected with a pipette and separated from the eggs by transferring them multiple times into small beakers containing seawater (Ibrahim and Abdullahi, 2015).

Brine Shrimp Lethality Assay
The cytotoxicity of the extract was assessed through a brine shrimp lethality assay, utilizing a modified version of the procedure outlined by Lilybeth *et al.* (2013). The extract was prepared in methanol at various concentrations, and lethality was assessed by counting the surviving shrimp after 24 hours. LC_{50} values were calculated using SPSS version 20

RESULTS
The leaves exhibited dark green coloration on the upper surface and light green on the lower surface, had a slightly rough texture with granules, a distinctive odor, a strong bitter taste, reticulate venation, and measured between 6-10 cm in size

Table 1: Physical Characteristics of Vernonia amygdalina Leaves

Features	Description
Shape	Simple , lanceolate
Size	6-10 cm
Apex	Acuminate
Venation	Reticulate/palmate
Petiole	Long petiole
Margin	Serrate
Base	Attenuate
Upper epidermis	Dark green
Lower epidermis	Light green
Odour	Strong bitter smell
Taste	Bitter taste
Cell shape	Circular
Texture	Smooth
Arrangement	Alternate

Table 2. Microscopic examination of *Vernonia amygdalina* leaves

Features	Description
Upper and lower epidermis	trichomes is present
Cells	uniseriate and homogenous
Stomata	Stomata are abundant 10-15 stomata per mm2, Anisocytic type

Chemo-microscopical examination of powdered *Vernonia amygdalina* leaves revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage but calcium carbonate was absent (Table 3).

Table 3: Chemomicroscopical studies of *Vernonia amygdalina* powdered leaves

Constituents	Inference
Starch	+
Gum and Mucilage	+
Cellulose cell walls	+
Lignin	+
Aleurone grain	+
Calcium oxalate crystals	+
Calcium carbonate	-
Suberized/Cuticular cell wall	+
Inulin	+

Keys: + = present, - = absent

Table 4 showed the result of physicochemical constants of *Vernonia amygdalina* leaves, average moisture contents was calculated to be 5.10% and the percentage yield of total ash, acid insoluble and water soluble matter were recorded in percentage values as 15.90%, 15.20%, 7.70% and 5.22% respectively. The extractives obtained were 2.60% and 3.0% for alcohol and water solvents respectively.

Table 4. Physicochemical Constituents of Powdered Leaves of *Vernonia amygdalina*

Parameters	Values (%w/w) ± SEM*	B.H.P Standard
Moisture content	5.1	10-12%
Ash content	15.9	6-19%
Acid insoluble ash	15.2	>1
Water soluble ash	7.7	-
Water extractive value	3.0	-
Ethanol extractive value	2.6	-

*Average values of three determinations. B.H.P (British Herbal Pharmacopeia)

The methanolic extract was found to contain flavonoids, alkaloids, tannins, saponins, steroids, triterpenes, carbohydrates, and phenols, while glycosides and anthraquinones were not detected (Table 5).

Table 5. Qualitative Phytochemical Analysis of Methanolic Extract of *Vernonia amygdalina* Leaves

Metabolite	Inferences ; Presence (+) / Absence (-)
Alkaloid	+
Flavonoid	+
Saponins	+
Cardiac glycoside	-
Tannins	+
Steroid	+
Triterpenes	+
Phenol	+
Anthraquinones	-
Carbohydrate	+

Table 6 displays the findings from the Brine Shrimp Lethality Assay (BSLA) performed on the methanolic extract of *Vernonia amygdalina* leaves. The data suggest that the extract exhibits a toxic effect on brine shrimp larvae in a dose-dependent manner. Higher concentrations, such as 100 µg/mL and 1000 µg/mL, resulted in increased mortality rates, with 96.6% mortality observed at the 1000 µg/mL concentration. The LC_{50} value was determined to be 29.645 µg/mL, indicating the concentration at which 50% of the larvae are expected to succumb, which reflects the extract's toxicity. Table 6: Brine Shrimp Lethality Assay of methanol extract of *Vernonia amygdalina* leaves

	Conc. (µg/mL)	Number of Surviving Nuplii After 24 hrs			Total Number of Death	% Mortality	LC_{50} (µg/mL)
		T1	**T2**	**T3**			
Vernonia amygdalina extract	10	07	06	04	13	43.3	29.645
	100	07	05	03	15	50	
	1000	01	0	0	29	96.6	

Table 7 presents the outcomes of the acute toxicity study. During the initial phase, no fatalities were observed. Similarly, in the second phase, where doses of 1500, 2250, 3250, and 5000 mg/kg were administered, no deaths occurred. Based on these results, the oral median lethal dose (LD_{50}) for the methanolic extract of *Vernonia amygdalina* leaves is estimated to be above 5000 mg/kg. Additionally, no behavioral changes were noted during the study. Table 7. Acute Toxicity Assessment of Methanolic Extract from *Vernonia amygdalina* Leaves

Treatment phase	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24hrs
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

DISCUSSION

Research on the organoleptic properties of *Vernonia amygdalina* leaves has uncovered unique features that differentiate it from other plants in the Asteraceae family. The leaves display

shades of green and light green on the outer and inner surfaces, accompanied by a slightly coarse texture and reticulate venation patterns. Typically measuring between 6.0 to 10 cm in length, they emit a distinctive odor and possess a notably bitter taste, a characteristic shared by some other Asteraceae species. Microscopic analysis revealed important diagnostic features such as anisocytic stomata and trichomes, which are essential for identifying and verifying both whole and powdered crude drug materials, as well as detecting possible adulteration (Atinga *et al.,* 2021). Chemo-microscopic evaluation of powdered *Vernonia amygdalina* leaves identified the presence of compounds like cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grains, and mucilage, while calcium carbonate was absent. These findings are crucial pharmacognostic markers for distinguishing various cell types and inclusions, thereby facilitating the authentication of powdered drugs (Atinga *et al.,* 2021).

Moisture content analysis, conducted using the loss on drying method, revealed an average value of 5.10%, while the percentage yields of total ash, acid-insoluble ash, and water-soluble matter were 15.90%, 15.20%, and 7.70%, respectively. Additionally, the extractive values obtained using alcohol and water solvents were 2.60% and 3.0%, respectively. These metrics serve as benchmarks for assessing the identity and purity of crude drugs, highlighting the presence of various inorganic materials such as carbonate, oxalate, and silicate (Evans, 2009; WHO, 1996; Atinga *et al.,* 2021). The observed moisture content of 5.11% falls within the acceptable limits, as per recommendations by B. H. P (1990) and WHO (2011). Lower moisture content is advantageous as it hinders microbial growth and enhances the stability of the material during storage, while also indicating the presence of impurities like carbonate, oxalate, sand, and silicate (Kaneria and Chanda, 2011; Atinga *et al.,* 2021). The total ash value, which includes both physiological and non-physiological ash, is a critical parameter for evaluating the identity and purity of crude drugs (WHO, 2011; Atinga *et al.,* 2021).

The higher extractive value observed with water (3.0%) compared to alcohol (2.60%) is attributed to the high polarity and universal solvent properties of water. Despite alcohol's lower extraction efficiency, it is preferred in certain research contexts due to its preservative properties and ease of handling (Ajazuddin and Shailendra, 2010; Atinga *et al.,* 2021). Phytochemical analysis of the methanolic extract revealed the presence of flavonoids, alkaloids, tannins, saponins, steroids, triterpenes, carbohydrates, and phenols, while glycosides were absent. These compounds contribute to various biological activities, such as antibacterial, anti-inflammatory, and antioxidant properties, and can serve as chemotaxonomic markers in phylogenetic studies (Atinga *et al.,* 2021).

Bioassays demonstrated significant cytotoxic activity in *Vernonia amygdalina* leaf extracts, with lethality increasing in proportion to extract concentration. The extract exhibited an LC_{50} of 27.65 µg/mL, while the negative control (DMSO) showed no mortality. These results are consistent with previous studies that correlated LC_{50} values with oral toxicity in rats, suggesting the bioactive potential of the plant's secondary metabolites (Nazifi *et al.,* 2019).
Acute toxicity testing in rats indicated a median lethal dose (LD_{50}) value above 5000 mg/kg when administered orally, implying that the extract is non-toxic as no fatalities occurred. According to OECD guidelines, substances with LD_{50} values exceeding 5000 mg/kg are considered practically non-toxic, further confirming the safety of *Vernonia amygdalina* leaf extracts (Namadina *et al.,* 2020). These results highlight the plant's potential as a safe therapeutic agent, warranting further investigation for medicinal use.

CONCLUSION

This study offers valuable insights into the pharmacognostic traits, phytochemical profile, and potential medicinal applications of *Vernonia amygdalina* leaves. The organoleptic analysis

identified distinct characteristics that set *V. amygdalina* apart from other members of the Asteraceae family, aiding in its identification and verification. Chemo-microscopic evaluations revealed specific cellular components, which are crucial for distinguishing powdered drugs and detecting any adulteration.

Chemical analyses confirmed the presence of various bioactive phytochemicals, including flavonoids, alkaloids, tannins, and saponins, which contribute to the plant's medicinal properties. These compounds have shown antibacterial, anti-inflammatory, and antioxidant activities, indicating that *V. amygdalina* could be a promising source of natural therapeutic agents.

Bioassays have demonstrated notable cytotoxic effects in *V. amygdalina* leaf extracts, suggesting potential applications in cancer treatment. Additionally, acute toxicity studies showed that the plant extracts have a high safety margin, supporting their potential use in medicine.

In summary, the findings underscore the pharmacological importance of *Vernonia amygdalina* leaves and highlight the need for further research to fully understand their therapeutic properties and explore their use in contemporary medicine. Continued investigation is essential to unlock the complete therapeutic potential of this valuable plant.

REFERENCES

Abubakar, K., Danjuma, N.M., Maiha, B.B., Anuka, J.A., Yam, M.F., Bello, S.O., Yusoff, A., Hor, S.Y., Mariam, A. and Zaini, M.A. (2015). A 28- Day Oral Toxicity Study of *Pseudocedrela kotschyi* Methanol Extract in Sprague-Dawley Rats, *European Journal of Medicinal Plants,*10 (3), 1-11.

Ajazuddin, N. and Shailendra, S. (2010). Evaluation of Physicochemical and Phytochemical Properties of *Safoof-E-sana*, a Unani polyherbal Formulation. *Journal of Pharmacognosy Research,* 2(5): 318-322.

Alhassan, M. A., Ibrahim, M., and Musa, I. A. (2014). Phytochemical Screening and Antimicrobial Evaluation of Stem Bark Extract of *Pseudocedrela kotschyi* (Herms). *British Journal of Pharmaceutical Research,* 4(16): 1937-1944.

Atinga, V, Musa, T.L., Ibrahim, H.M., Ayeni, E.A., Namadina, M.M., Jajere, U.A. and Ali, U.Y. (2021). Standardization of stem bark of *Pseudocedrela kotschy*i (Meliaceae). *Journal of Pharmacognosy and Phytochemistry*, 10(6): 19-23.

British Herbal Pharmacopoeia (1990). *British Herbal Medicine Association*. Bournemouth: Dorset. 1st edition. Vol.1. Pp. 1-2.

Evans, W. C. (2009). *Trease and Evans pharmacognosy,* 16th edition, W. B. Saunders Ltd., London, 10 – 11.

Hamid, A.A., Aiyelaagbe, O.O. and Usman, L.A. (2011). Essential oils: Its medicinal and pharmacological uses. *International Journal of Current Research*, **3**(2):086-098.

Kaneria, M., and Chanda, S. (2011). Phytochemical and Pharmacognostic Evaluation of the Leaves of *Psidium guajava L* (*Mrytaceae*). *Journal of Pharmacognosy;* 3 (23): 41-45.

Kibiti, M. and Afolayan, J. (2015).Preliminary Phytochemical Screening and Biological Activities of Bulbine abyssinica Used in the Folk Medicine in the Eastern Cape Province, South Africa. *Journal of Evidence-Based Complementary and Alternative Medicine*, 2015.

Namadina, M. M., Shawai, R. S., Musa, F. M., Sunusi, U., Aminu, M. A., Nuhu, Y. and Umar, A. M. (2020). Phytochemical and Antimicrobial Activity of *Securidaca longipedunculata* Root against Urinary Tract Infection Pathogens. *BUK ChemSearch Journal*, 11(2): 90 – 98.

Nazifi S. I., Namadina, M.M., Abubakar, K.R., Maryam, Y.B**.** (2019). Cytotoxicity, Antioxidant and Antimicrobial Activity of the Leaves and Stem bark of *Vitellaria paradoxa. FUDMA Journal of Sciences (FJS),* 4(1): 562-567

Njoku, P.C. and Akumufula, M.I. (2007).Phytochemical and nutrient evaluation of *Spondias mombi* Leave. *Pakistani Journal of Nutrition,* 6(6):613-615.

Ofori, D. A., Anjarwalla, P., Jamnadass, R., Stevenson, P. C. and Smith, P. (2013). Pesticidal Plant Leaflet *Vernonia amygdalina*. Del. World Agroforestry Center. University of Greenwich, *Royal Botanical Garden.* ISBN 978-92-9059-348-5.

Sharall, V., Verma M., Sharma, S. and Malik, A. (2013).Determination of Phytocomponents of *Jatropha curcas* root by GC-MS Analysis and their Termcidal activity.

WHO (1996): *Quality Assurance of Pharmaceuticals:* A Compendum of Guidelines and Related Materials, Good Manufacturing Practices and Inspection. World Health Organization, Geneva, Switzerland. Pp 2-18.

World Health Organization (2011). *Quality Control Methods for Medicinal Plants*. WHO, Geneva, Switzerland, Pp. 31.