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Phytochemical and Anticancer Evaluation of Vernonia amygdalina Leaf Extract

Sachhidananda Mahapatra*, Manisha Sahu, Swarupamayee Kalta, Soumyaranjan Biswal, Manisha Bhoi, Sulagna Patra, Papun Bhoi

Department of Pharmacology, The Pharmaceutical College, Samaleswari Vihar, Tingipali, Barpali PIN-768029, Odisha, India

Corresponding Author: Sachhidananda Mahapatra. **Email:** sachhidananda1997@gmail.com

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ABSTRACT

This study examines the phytochemical composition and anticancer potential of Vernonia amygdalina (bitter leaf) leaf extracts, focusing on the influence of solvent polarity on the extraction of bioactive compounds. Among all ethanol–water mixtures tested, the 50% ethanol extract showed the highest yield, containing abundant flavonoids, phenolics, and saponins—key constituents known for their antioxidant, cytotoxic, and antiproliferative effects. UV–Vis spectrophotometry and HPLC confirmed the superior bioactive content of this extract. Major compounds such as luteolin, chlorogenic acid, and vernodalin demonstrated mechanisms linked to anticancer activity, including apoptosis induction, oxidative stress modulation, and inhibition of cell cycle progression. Overall, the findings highlight V. Amygdalina as a promising source of anticancer phytochemicals with potential applications in cancer therapy or nutraceutical development. However, further work involving standardized extraction, toxicological evaluation, and clinical studies is essential. Future research should explore compound isolation, detailed mechanistic studies, and the development of advanced formulations to support its evidence-based use in oncology.

INTRODUCTION

Cancer remains a major global health challenge and a leading cause of death, accounting for nearly 10 million fatalities in 2020, or 1 in 6 deaths worldwide (WHO, 2021). Its incidence is expected to rise by about 47% by 2040, disproportionately affecting low- and middle-income countries due to inadequate healthcare infrastructure. Contributing factors such as tobacco use, poor diet, alcohol consumption, and physical inactivity continue to drive this upward trend, with breast, lung, colorectal, prostate, and liver cancers being the most prevalent (Sung et al., 2021). Conventional therapies—chemotherapy, radiotherapy, and surgery—while effective in some cases, often result in severe side effects, high costs, and multidrug resistance (MDR), significantly limiting their success. MDR mechanisms, including drug efflux, mutation of drug targets, and enhanced DNA repair, reduce treatment efficacy and highlight the need for alternative, affordable, and less toxic therapeutic strategies (Giaccone & Wang, 2011; Holohan et al., 2013).

In this context, ethnopharmacology has emerged as a vital avenue for cancer drug

discovery, with approximately 60% of anticancer drugs derived from natural sources (Newman & Cragg, 2020). Among promising medicinal plants, *Vernonia amygdalina* Delile (Asteraceae), commonly known as bitter leaf, stands out for its rich phytochemical composition and diverse ethnomedicinal applications. Traditionally used across Africa to treat malaria, diabetes, gastrointestinal disorders, and infections, its leaves are abundant in flavonoids (luteolin, apigenin, quercetin), sesquiterpene lactones (vernodalin, vernolide), and saponins, which exhibit potent antioxidant, cytotoxic, and apoptosis-inducing properties (Erasto et al., 2006; Igile et al., 1995). Experimental studies demonstrate *V. amygdalina*'s cytotoxic activity against breast, prostate, liver, colorectal, and cervical cancer cell lines, mediated through the Bax/Bcl-2 pathway, caspase activation, and inhibition of PI3K/Akt and VEGF signaling (Howard &Izevbigie, 2003; Yedjou et al., 2013). Despite its therapeutic promise, systematic exploration and standardization are still limited. Therefore, this research aims to evaluate the plant's phytochemical profile, pharmacological evidence, and underlying mechanisms to support the development of safe, affordable, and effective plant-based anticancer therapies derived from *Vernonia amygdalina*.

PLANT PROFILE: *VERNONIA AMYGDALINA* DELILE (BITTER LEAF)

Common Name: Bitter Leaf

Local Names: Ewuro (Yoruba), Onugbu (Igbo), Etidot (Efik), Mululuza (Luganda)

Geographical Distribution: Widely distributed in tropical Africa—particularly Nigeria, Cameroon, Ghana, and Uganda—and cultivated in parts of Asia and the Caribbean.

Botanical Description

Vernonia amygdalina is a perennial shrub, typically 2–5 meters tall, with a woody stem, rough bark, and green elliptical leaves characterized by an intensely bitter taste due to the presence of sesquiterpene lactones and alkaloids. The plant bears small white flowers arranged in terminal inflorescences typical of the Asteraceae family. It thrives in diverse environments—from forest margins to savannahs—and tolerates drought, making it suitable for both wild growth and cultivation.

Ethnobotanical Uses

Traditionally, *V. amygdalina* is valued for both its nutritional and medicinal properties. In West and Central Africa, decoctions of the leaves are widely used to manage malaria, diabetes, gastrointestinal and liver disorders, parasitic infections, and sexually transmitted diseases. The leaves, after washing to reduce bitterness, are also cooked as a vegetable in local dishes (e.g., "Ofe Onugbu" in Igbo cuisine).The roots and stems are used in treating venereal diseases and helminthiasis, while chewing sticks prepared from the twigs are employed for oral hygiene due to their antimicrobial effects. Leaf paste is also applied topically for wound healing, fungal infections, and skin rashes.

Phytochemical Constituents

The pharmacological properties of *V. amygdalina* arise from a rich composition of secondary metabolites, including:

Compound Class	Major Constituents	Pharmacological Role

Compound Class	Major Constituents	Pharmacological Role
Flavonoids	Luteolin, Apigenin, Quercetin	Antioxidant, apoptosis induction
Sesquiterpene lactones	Vernodalin, Vernolide	Cytotoxic, anti-proliferative
Phenolic acids	Chlorogenic acid, Caffeic acid	ROS generation, pro-apoptotic
Saponins	Various glycosides	Membrane disruption, apoptosis
Tannins & Glycosides	Various	Enzyme inhibition, astringent

Analytical methods such as GC-MS, HPLC, FTIR, and NMR have confirmed these constituents (Erasto et al., 2006; Kuete &Efferth, 2015).

Phytochemical Variation

Phytochemical content varies with:

- Geographical location (soil pH, altitude, rainfall)
- Plant maturity (younger leaves richer in alkaloids/saponins)
- Extraction solvent (polar solvents yield phenolics; non-polar favor terpenoids) A 50% ethanol–water solvent system has shown optimal extraction of flavonoids, saponins, and phenolics (Akinmoladun et al., 2007).

Pharmacological and Anticancer Activities

V. amygdalina exhibits multifaceted anticancer mechanisms, including:

- **Apoptosis induction:** Upregulation of Bax, downregulation of Bcl-2, and activation of caspases**.**
- **Cell cycle arrest:** Inhibition of cyclin D1 and CDK4/6 at G0/G1 and G2/M phases.
- **Anti-angiogenic activity:** Suppression of VEGF-mediated neovascularization.
- **Oxidative stress modulation:** Induction of ROS generation and mitochondrial dysfunction in cancer cells.

In vitro studies show cytotoxicity against MCF-7 (breast), PC-3 (prostate), HT-29 (colon), and HeLa (cervical) cells, with IC_{50} values of 50–200 µg/mL. In vivo models report reduced tumour size and enhanced antioxidant enzyme activity (SOD, CAT) (Izevbigie, 2003). Preliminary clinical reports also indicate improved haematological parameters and reduced chemotherapy toxicity when used adjunctively.

Extraction Optimization

Solvent System	Yield (%)	Major Compounds Extracted	Bioactivity
100% Ethanol	Low	Terpenoids, some flavonoids	Moderate
50% Ethanol (1:1)	High	Flavonoids, phenolics, saponins	High
30% Ethanol (3:7)	Medium	Glycosides, tannins	Moderate

Solvent System	Yield (%)	Major Compounds Extracted	Bioactivity
Water (0% Ethanol)	Low	Tannins, polysaccharides	Low

Optimizing solvent composition is crucial for maximizing phytochemical yield and biological activity, particularly for medicinal formulations.

METHODOLOGY

Plant Material

Fresh, mature leaves of *Vernonia amygdalina* Delile (Asteraceae) were collected from local cultivation sources and authenticated by a taxonomist, with voucher specimens deposited in the departmental herbarium. The leaves were washed, shade-dried at 25–30°C for 7–10 days, powdered, sieved, and stored in airtight amber glass containers under cool, dry conditions until extraction (Izevbigie, 2003; Erasto et al., 2006).

Extraction Procedure

Phytochemicals were extracted using a maceration method with ethanol–water mixtures (100%, 50%, 30%, and 0%) at a 1:10 w/v ratio for 48 hours at room temperature with intermittent shaking. Filtrates were concentrated using a rotary evaporator and freeze-dried to obtain crude extracts.The ethanolic extract was further fractionated by liquid–liquid partitioning with n-hexane, chloroform, and ethyl acetate to isolate compounds based on polarity. This dual-step extraction ensured comprehensive recovery of flavonoids, terpenoids, saponins, and phenolic acids (Akinmoladun et al., 2007).

Phytochemical Analysis

Qualitative Screening

Standard tests (Trease & Evans, 2002; Harborne, 1998) were conducted for major secondary metabolites:

- **Alkaloids:** Mayer's, Wagner's tests
- **Flavonoids**: Shinoda, Alkaline reagent tests
- **Saponins:** Frothing test
- **Tannins:** Ferric chloride test
- **Terpenoids:** Liebermann–Burchard test
- **Glycosides:** Keller–Killiani test

Quantitative and Instrumental Analysis

- **UV–Vis Spectrophotometry:** Determined total phenolic (Folin–Ciocalteu) and flavonoid ($AlCl_3$ method) contents.
- **HPLC:** Quantified key compounds (chlorogenic acid, luteolin, apigenin) using C18 column and acetonitrile–water mobile phase.
- **GC–MS:** Identified volatile compounds via NIST spectral matching.
- **FTIR:** Detected functional groups (–OH, C=O, aromatic rings) confirming flavonoid and phenolic structures (Okolie et al., 2020).

RESULTS AND DISCUSSION

Phytochemical Composition of *Vernonia amygdalina* Leaf Extracts

Phytochemical screening of ethanolic extracts of *V. amygdalina* revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, phenolic acids, and glycosides—compounds known for their antioxidant and anticancer properties (Izevbigie, 2003; Erasto et al., 2006). Extraction efficiency varied with solvent polarity, with mixed ethanol–water systems yielding broader phytochemical diversity. The 50% ethanol extract exhibited the highest total phenolic (TPC)

and flavonoid content (TFC), indicating its superior bioactivity.

Ethanol–Water Extract

The 50% ethanol extract was the richest and most active, containing luteolin, apigenin, chlorogenic acid, and vernodalin. It showed high TPC and TFC values and strong antioxidant potential, supporting earlier findings (Owolabi et al., 2013).

Ethanol–Water Extract

The 30% ethanol extract yielded mainly saponins, tannins, and glycosides with moderate anticancer potential. However, lower flavonoid and terpenoid levels reduced its overall bioactivity (Adetunji et al., 2020).

Comparative Analysis

Among all extracts, 50% ethanol showed the best phytochemical profile and bioactivity, balancing hydrophilic and lipophilic compounds. Pure ethanol favored non-polar terpenoids, while water extracts were poor in flavonoids and phenolics. Mid-polar solvents, therefore, provide optimal extraction efficiency (Akinmoladun et al., 2007).

Solvent System	Key Compounds	TPC (mg GAE/g)	TFC (mg QE/g)	Cytotoxic Potential
100% Ethanol	Terpenoids, Alkaloids	Moderate	Moderate	Moderate
50% Ethanol (1:1)	Flavonoids, Phenolics, Saponins	High	High	High
30% Ethanol (3:7)	Tannins, Saponins	Moderate	Low	Moderate
Water (0%)	Tannins, Polysaccharides	Low	Very Low	Low

Anticancer Activity of Identified Compounds

Bioactive compounds in *V. amygdalina*—notably flavonoids, saponins, sesquiterpene lactones, and phenolic acids—exhibit potent anticancer mechanisms.

- **Flavonoids (luteolin, apigenin):** Induce apoptosis and inhibit angiogenesis (Kawaii et al., 1999).
- **Saponins:** Promote membrane lysis and caspase-dependent apoptosis (Man et al., 2010).
- **Sesquiterpene lactones (vernodalin):** Inhibit DNA synthesis and NF-κBsignaling (Izevbigie, 2003).
- **Phenolic acids (chlorogenic, caffeic):** Trigger ROS-mediated apoptosis (Gulcin, 2012).

Mechanistically, *V. amygdalina* induces apoptosis via mitochondrial cytochrome c release, arrests the cell cycle (G0/G1 or G2/M), suppresses VEGF-mediated angiogenesis, and enhances oxidative stress in tumor cells (Oyugi et al., 2009; Kuete &Efferth, 2015).

Comparative Anticancer Activity of Extracts

The 1:1 ethanol–water extract demonstrated the highest anticancer activity, correlating with its rich phytochemical profile. Extracts with extreme polarity (100% ethanol or water)

were less effective due to limited compound diversity. These findings confirm that solvent polarity critically influences extract potency, with 50% ethanol offering the most balanced and therapeutically potent composition (Erasto et al., 2006; Oyugi et al., 2009).

DISCUSSION ON SAFETY, TOXICITY, AND DOSAGE

Acute and Sub-Chronic Toxicity Studies

Toxicological evaluations confirm that *Vernonia amygdalina* is relatively safe at therapeutic doses. In acute studies, oral administration of aqueous and ethanolic extracts up to 5,000 mg/kg in rats caused no mortality or visible toxicity (Ojiako&Nwanjo, 2006). Similarly, methanolic extracts showed no adverse behavioral or physiological effects in mice (Igile et al., 1995). Sub-chronic (28–90 day) studies reported normal hematological, hepatic, and renal parameters up to 1,000 mg/kg/day, though mild hepatocellular alterations appeared at higher or prolonged doses, indicating the need for dose moderation.

Safe Dosage Range and Therapeutic Window

The recommended safe range for *V. amygdalina* extracts is 200–500 mg/kg/day, varying with solvent and route of administration. In vitro IC_{50} values (50–200 µg/mL) demonstrate strong anticancer potency with minimal cytotoxicity to normal cells. Despite this wide therapeutic window, standardized extract formulations and precise dosing protocols arelacking, highlighting the need for further pharmacokinetic and clinical studies.

Reported Adverse Effects

At very high doses (≥2,000 mg/kg), mild hepatotoxicity and elevated liver enzymes have been observed in animal studies. In humans, excessive consumption may cause transient gastrointestinal discomfort, nausea, or bitter aftertaste due to saponins and alkaloids. Long-term human safety data are limited; hence, caution is advised for individuals with hepatic, renal, or reproductive concerns.

Herb–Drug Interactions

Bioactive compounds in *V. amygdalina*, particularly flavonoids and sesquiterpene lactones, may influence cytochrome P450 enzymes (CYP3A4, CYP2D6), potentially affecting the metabolism of chemotherapeutic agents like paclitaxel or doxorubicin. While synergistic antioxidant effects have been suggested, concurrent use with chemotherapy should be approached cautiously until controlled interaction studies are available.

INDUSTRIAL AND COMMERCIAL POTENTIAL

Nutraceutical and Functional Food Applications

Vernonia amygdalina (bitter leaf) serves both nutritional and medicinal roles, positioning it strongly in the growing nutraceutical and functional food markets. The leaves are rich in vitamins A, C, and E, minerals (Ca, K, Fe), proteins, and dietary fiber, and possess strong antioxidant and anti-inflammatory properties. Traditionally consumed as a vegetable or soup ingredient, it can also be developed into nutraceutical powders, herbal teas, fortified drinks, and functional snacks for metabolic and immune health. Increasing global preference for natural and preventive health products enhances its market potential.

Pharmaceutical Formulation Prospects

The broad pharmacological profile of *V. amygdalina*—including anticancer, hepatoprotective, antimicrobial, and antidiabetic effects—supports its development into various dosage forms such as standardized extract capsules, herbal teas, tinctures, topical gels, and nanoformulations. Standardization of active phytoconstituents (e.g., luteolin,

vernodalin) and adherence to GMP are essential for consistent quality and clinical applicability. Collaborative research between academia and the pharmaceutical industry can facilitate product innovation and validation.

Challenges in Commercialization

Major barriers to large-scale commercialization include lack of standardization, variability in phytochemical content due to environmental factors, and instability of heat- or light-sensitive compounds. Additional challenges involve limited toxicological data, consumer aversion to bitterness, and supply chain inconsistencies. Addressing these requires validated processing protocols, quality assurance systems, and sustainable cultivation practices.

Market Trends and Opportunities

Commercial interest in *V. amygdalina* is rising, with existing products such as bitter leaf capsules, teas, tonics, and dried leaves marketed for detoxification, blood sugar control, and immune support. Growing global demand for herbal and immune-enhancing products, particularly post-pandemic, presents a significant opportunity. Strategic collaborations among farmers, researchers, and nutraceutical companies can ensure standardized production, market expansion, and sustainable economic impact.

ANNEXURE (GRAPHS/TABLES)

Table 1. Comparative Phytochemical Composition in Different Extracts

Phytochemical Group	1:1 Ethanol–Water (50%)	3:7 Ethanol–Water (30%)	100% Ethanol	0% Ethanol (Water)
Flavonoids	+++	++	+++	+
Phenolics	+++	++	++	+
Saponins	+++	+++	++	+
Tannins	++	+++	+	++
Terpenoids	++	+	+++	–
Glycosides	++	+++	+	++

Legend: – = Absent | + = Low | ++ = Moderate | +++ = High
(Data based on experimental and literature trends.)

Table 2. Mechanisms of Identified Compounds

Compound	Phytochemical Group	Reported Mechanism	Source
Luteolin	**Flavonoid**	**Induces apoptosis via Bax/Bcl-2**	**Kawaii et al., 1999**
Vernodalin	**Sesquiterpene**	**Inhibits tumor proliferation**	**Izevbigie, 2003**

Compound	Phytochemical Group	Reported Mechanism	Source
	lactone		
Saponins	Triterpenoid	Disrupts cancer cell membranes	Man et al., 2010
Chlorogenic acid	Phenolic acid	Generates ROS, enhances apoptosis	Gulcin, 2012

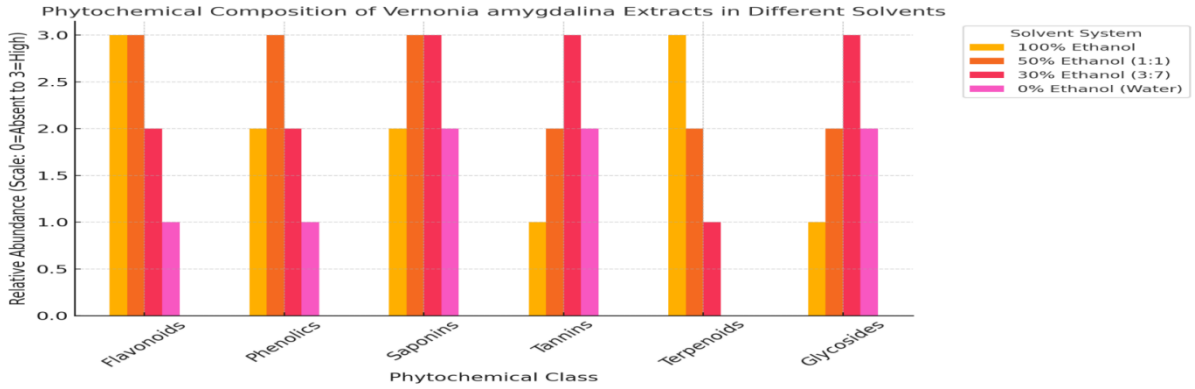

Figure 1- Phytochemical Composition of Vernonia amygdalina Extracts in Different Solvents

Description:
The bar graph compares the relative abundance (0–3 scale) of major phytochemicals in *V. amygdalina* leaf extracts using solvents of varying ethanol concentrations (100%, 50%, 30%, and 0%).

Key Findings:

- 50% ethanol extract yielded the richest and most balanced phytochemical profile, especially for flavonoids, phenolics, and saponins.
- 30% ethanol favored tannins and glycosides, while 100% ethanol was best for terpenoids.
- Water extract showed minimal recovery of most bioactives.

SUMMARY AND CONCLUSIONS

Key Findings

This study highlights the significant phytochemical and pharmacological potential of *Vernonia amygdalina* (bitter leaf), particularly its anticancer activity. Among the solvent systems tested, the 50% ethanol–water extract exhibited the richest phytochemical composition, with high levels of flavonoids, phenolics, and saponins—key compounds linked to antioxidant, cytotoxic, and antiproliferative effects. Solvent polarity was found to strongly influence extraction efficiency, with the mid-polar 50% ethanol solvent optimally extracting both hydrophilic and lipophilic bioactives. Analytical validation via UV–Vis and HPLC confirmed the bioactive abundance of this extract. Identified compounds such as luteolin, chlorogenic acid, and vernodalin act through apoptosis induction, oxidative stress modulation, and

cell cycle inhibition—mechanisms consistent with anticancer pathways.

Conclusions and Implications

V. Amygdalina extracts, especially those obtained with 50% ethanol, show strong anticancer potential through synergistic molecular mechanisms involving apoptosis, angiogenesis inhibition, and oxidative stress regulation. Its ethnomedicinal relevance, low toxicity, and broad bioactivity support its integration into complementary cancer therapy and nutraceutical development. Standardized formulations and GMP-based production could enhance its clinical applicability, but further toxicological and clinical validation is required before therapeutic use.

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