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PHYTOCHEMICAL SCREENING AND COMPARATIVE BRINE SHRIMP CYTOTOXICITY EVALUATION OF ETHANOLIC CRUDE EXTRACT AND FRACTIONS OF *Parquetina nigrescens* LEAVE

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ABSTRACT

Parquetina nigrescens is extensively used in African traditional medicine, yet a systematic, solvent-polarity-guided evaluation of its cytotoxic potential is lacking. This study aimed to validate its ethnomedicinal use by investigating the phytochemical constituents and cytotoxic activity of its extracts, with a focus on how solvent polarity influences bioactivity. The P. *nigrescens* leaves was exhaustively extracted with absolute ethanol then sequentially fractionated using solvents of increasing polarity (n-hexane, chloroform, ethyl acetate, n-butanol, and water). Phytochemical profiling was conducted via standard qualitative methods, and cytotoxicity was evaluated using the Brine Shrimp Lethality Assay (BSLA). Phytochemical screening confirmed a rich profile of alkaloids, flavonoids, and triterpenoids in the plant. The BSLA revealed that all extracts demonstrated significant cytotoxicity (LC_{50} < 1000 µg/mL). Most importantly, a statistically significant difference in potency was observed across the fractions ($p < 0.0001$), revealing a critical solvent-polarity dependency. The butanol and aqueous fractions exhibited superior and remarkably similar potency (LC_{50} = 70 and 72 µg/mL, respectively), significantly more cytotoxic than the mid-polar ethyl acetate (142 µg/mL) and non-polar fractions (n-hexane: 366 µg/mL; chloroform: 555 µg/mL). The crude ethanol extract was the least potent (LC_{50} = 660 µg/mL) obviously due to the masking effect of other compounds in the matrix. This study provides robust evidence that the cytotoxic potential of *P. nigrescens* is significantly influenced by solvent polarity ($p < 0.0001$). The high potency of the butanol and aqueous fractions validates the plant's traditional use in water-based preparations.

Keywords: Brine Shrimp Lethality, Medicinal Plants, Cytotoxicity, *parquetina nigrescens*, LC_{50}, Solvent Polarity

INTRODUCTION

The use of medicinal plants for treating human and animal ailments is a practice deeply rooted in human history, as evidenced by archaeological findings such as the Lascaux cave paintings in France, dated to 13,000–25,000 BC (Kamanja *et al.*,2018). Long before the advent of modern medicine, traditional healers relied extensively on botanical sources for patient care. This legacy continues today, with plant-derived medicines serving as vital alternatives and complements to conventional drugs globally. It is estimated that about 25% of pharmaceutical drugs in current clinical use and approximately 50% of drugs approved in the last three decades are derived, either directly or indirectly, from natural products (Newman & Cragg, 2020). In biodiversity-rich regions like Nigeria, medicinal plants constitute a significant component of the flora, making tropical forests a particularly promising reservoir for novel bioactive lead compounds. Consequently, the World Health Organization advocates for the validation and use of medicinal plants not only for prophylactic and therapeutic purposes but also as a crucial source for new drug development (WHO, 2013). This has led to a surge in the use of herbal medicines worldwide, particularly in developing countries, driven by their perceived safety, accessibility, and economic benefits (García-Cortés *et al.*,2008).

The Brine Shrimp Lethality Assay (BSLA)

The investigation of plant secondary metabolites for cytotoxicity is essential for two primary reasons: to assess potential adverse effects associated with their traditional use and to identify promising candidates for anticancer drug development (Fatope *et al.*,1995). Cytotoxicity, defined as the capacity of a substance to disrupt vital metabolic processes or cause cellular damage leading to abnormal function or death, is a critical parameter for establishing a drug's toxicity profile and is a prerequisite for anticancer agents (Fatope *et al.*,1995). Among the various models available for preliminary cytotoxicity screening—such as crown gall tumor inhibition on potato discs and frond proliferation inhibition in duckweed (McLaughlin *et al.*, 1998)—the Brine Shrimp Lethality Assay (BSLT) remains one of the most widely utilized and cited in the literature. The assay, introduced by Michael *et al*. (1956) and further refined by Vanhaecke *et al*. (1981), evaluates the lethality of plant extracts against the larvae (nauplii) of *Artemia salina* (Ameen *et al.*,2011). Its relevance in anticancer screening stems from the proposed analogy between the rapid, undifferentiated cell division of brine shrimp nauplii and the proliferation of cancer cells, suggesting a valuable correlation with antitumor activity in mammalian systems (Solis *et al*., 1993). Therefore, the ability of an extract to induce mortality in brine shrimp is a useful preliminary indicator of cytotoxic and potential anticancer properties.

The enduring popularity of the BSLA is attributed to its simplicity, reliability, cost-effectiveness, and minimal sample requirement (2–20 mg), making it an accessible tool for initial bioactivity screening (Meyer *et al*., 1982; Alluri *et al.*,2005). An extract is generally considered bioactive or cytotoxic if it demonstrates an LC_{50} (lethal concentration for 50% of the nauplii) value of less than 1000 µg/mL (Meyer *et al.*, 1982). The model's utility is well-established across diverse applications, from screening Indian medicinal plants (Alluri *et al.*, 2005) and evaluating betel leaves for anticancer potential (Nerdy *et al.*,2021) to assessing the toxicity of

environmental contaminants like heavy metals and nanoparticles (Carballo *et al.*,2002; Ghosh *et al*.,2015). This broad applicability underscores that the selection of the brine shrimp model is a well-justified and non-arbitrary decision in pharmacological research.

Parquetina Nigrescens

Parquetina nigrescens (Afzel.) Bullock is a perennial evergreen woody climber belonging to the *Apocynaceae family,* subfamily *Periplocoideae.* It is native to equatorial West Africa, thriving in diverse habitats such as secondary forests, savannas, and gallery forests, and can grow on various soil types, including marshy areas (Alvarez, 2012). The plant is characterized by its twining stem, cordate leaves (6.5–18.3 cm long and 5.4–16.8 cm wide), and a corolla that is typically maroon, pink, or deep crimson internally (Ayoola *et al.*,2018). This plant holds a prominent place in African ethnomedicine, with its leaves, roots, and latex being used for centuries to treat a wide spectrum of ailments (Owoyele *et al.,* 2011). Its traditional uses across countries like Ghana, Nigeria, and Senegal are extensive, including the management of fatigue, diabetes, anaemia, ulcers, menstrual disorders, asthma, and diarrhoea, among others (Ayoola *et al.*,2011; Imaga *et al.*,2010). For instance, a leaf decoction mixed with honey is traditionally used in baths to alleviate fatigue (Adase *et al.*,2015). The plant is known by various local names, such as *Ewe Ogbo* (Yoruba, Nigeria) and *Abakamo* (Twi in Ghana), reflecting its widespread ethnobotanical and cultural significance (Kayode *et al*.,2017).

Given its extensive traditional use and the preliminary scientific evidence of its bioactivity, a systematic investigation into the phytochemical and cytotoxic properties of *P. nigrescens* is warranted. This study therefore aims to assess the phytochemical constituents of its ethanol extract and evaluate the comparative cytotoxicity of its fractions, obtained using solvents of varying polarity, through the brine shrimp lethality assay.

Figure 1: *P. nigrescens* plant

Figure 2: Adaxial view of *P. nigrescens*

A Review of Phytochemicals and Pharmacological Assessment of *P. nigrescens*

A growing body of evidence confirms that the diverse pharmacological profile of *Parquetina nigrescens* is a direct manifestation of its rich and complex phytochemistry. Comprehensive phytochemical analyses across different plant parts have consistently identified a potent arsenal of bioactive compounds, including flavonoids, alkaloids, tannins, Saponins, cardiac glycosides, and terpenoids (Ayoola *et al*., 2011; Moses *et al*., 2014; Onyegeme-Okerenta *et al*., 2018). This chemical diversity is evident in extracts from leaves, roots, stem bark, and latex, which contain phenols, alkaloids, and cardiac glycosides, among others (Sopeyin *et al*., 2016), with hydroethanolic and methanolic extracts being particularly rich in these constituents (Airaodion *et al.*, 2019; Ajayi *et al.*, 2021; George *et al*., 2014).

The therapeutic significance of this chemical arsenal is profound. These identified phytochemicals are the active principles responsible for the plant's wide-ranging and scientifically validated pharmacological activities. This includes well-documented antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects, as well as more specific actions such as antisickling, hematopoietic (blood-boosting), and uterotonic properties (Odetola *et al*., 2006; Onyegeme-Okerenta *et al*., 2018). Crucially, this established bioactivity profile extends to the cellular level, with studies confirming the antiproliferative, cytostatic, and cytotoxic actions of its phytochemicals against cancer cells (Cordaliza *et al*., 2007). Therefore, the traditional use of *P. nigrescens* for a multitude of ailments is firmly grounded in the verifiable presence of specific, bioactive compounds that underpin its medicinal efficacy. Thus, the therapeutic potential of *Parquetina nigrescens*, deeply rooted in ethnomedicine, is supported by a substantial body of scientific evidence which reveals a wide spectrum of pharmacological activities. These activities are validated through various *in vitro* and *in vivo* models, confirming its traditional uses and revealing new therapeutic avenues:

Metabolic and Hematological Activities

P. nigrescens exhibits significant antidiabetic and antihyperlipidemic properties, as demonstrated in several studies (Saba *et al.*, 2010; Faloye *et al.*, 2018; Adeyomoye *et al.*, 2018; Ojuade *et al.*, 2021). Its efficacy is further highlighted in a polyherbal formulation with *Erythrina senegalensis*, which showed potential in managing streptozotocin-induced diabetes in Wistar rats (Romaric *et al.*, 2021). Furthermore, the plant is renowned for its hematinic effects. Multiple investigations confirm its ability to stimulate red blood cell production and accelerate recovery from anaemic conditions, solidifying its reputation as a potent "blood booster" in traditional practice (Agbor *et al.*, 2001, 2005; Saba *et al.*, 2010; Oyewole *et al.*, 2011; Angele *et al.*, 2019; Ighadoro *et al.*, 2025). This antianaemic activity is complemented by antisickling properties, with research indicating its effectiveness, both in polyherbal preparations and alone, against sickle cell anemia (Kade *et al.*, 2003; Kple´ *et al.*, 2020).

Anti-infective and Gastroprotective Properties

The plant's traditional use against infections is well-supported. Its antimicrobial efficacy spans a range of Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Salmonella typhi*, and *E. coli* (Azeez *et al.,* 2010; Oloyede *et al.,* 2017; Adeyemi *et al.,* 2019; Adaze *et al.,* 2022), with notable antityphoid activity in vivo (Akinyemi *et al.,* 2014). Antimalarial activity has also been documented for a polyherbal preparation containing *P. nigrescens* (Nafiu *et al.,* 2014). For gastrointestinal health, the plant demonstrates strong antidiarrheal (Kola *et al.,* 2019; Banwo *et al.,* 2019; Mahmud *et al.,* 2021) and antiulcerogenic activities. Extracts have been shown to protect the gastric mucosa by reducing acid secretion and enhancing mucus production (Otedola *et al.,* 2006).

Anti-inflammatory, Analgesic, and Organ-System Effects

The anti-inflammatory and analgesic (pain-relieving) properties of *P. nigrescens* provide a scientific basis for its use in treating conditions like arthritis, headaches, and fever (Oyewole *et al.,* 2009; Okunrobo *et al.,* 2014). Its aqueous leaf extract has been validated for antiasthmatic activity, showing comparative efficacy to standard treatments (Terlarbi *et al.,* 2000). Beyond these, the plant exhibits diuretic effects that can ameliorate hypertension (Konan *et al.,* 2022) and possesses insecticidal properties (Adesola *et al.,* 2016; Owolabi *et al.,* 2014).

Reproductive, Neurological, and Endocrine Activities

P. nigrescens significantly influences the reproductive and endocrine systems. It demonstrates uterotonic activity, justifying its traditional use to induce labour (Datte *et al.,* 1996), and has shown potential in treating polycystic ovarian syndrome (PCOS) (Femi-Olabisi *et al*., 2020). Conversely, it also possesses male fertility-boosting activities (Oyelowo *et al.,* 2014; Kayode *et al*., 2017; Adedokun *et al.,* 2021). Neurologically, a methanol stem extract has confirmed memory-enhancing potential (Bukari *et al.,* 2019).

Antioxidant and Cytotoxic Activities

A cornerstone of its bioactivity is its potent antioxidant capacity. Several studies confirm the presence of active antioxidant principles, with one report highlighting that its essential oil has a superior free radical scavenging ability compared to the standard antioxidant butylated hydroxyl anisole (Ayoola *et al.,* 2011; Akinrinmade *et al.,* 2016; Oghenejoboh *et al.,* 2018). This is intrinsically linked to its cytotoxic and anticancer potential. The plant's cytotoxicity has been consistently observed in various models, including in Wistar rats and via essential oils (Adu-Amoah *et al.,* 2014; Oghenejoboh *et al.,* 2018). Most notably, an ethanol extract demonstrated significant antitumoral activity against a panel of human cancer cell lines, including MCF-7 (breast), C4-2WT (prostate), and HCT 116 (colourectal) carcinomas, marking it as a promising anticancer agent (Onyegeme-Okerenta *et al.,* 2018).

Toxicological Considerations

Despite the broad bioactivity, several toxicity studies suggest a relatively safe profile for the aqueous leaf extract, with no significant toxicity reported in subacute models (Nsiah *et al.,* 2006; Adeyomoye *et al.,* 2018; Olatunbosun *et al.,* 2018).

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh leaves of *Parquetina nigrescens* were harvested from Odo Eri, in the Yagba West Local Government Area of Kogi State, Nigeria. The plant specimen was identified, authenticated and vouchered by Mr. Namadi Sanusi at the Department of Biological Sciences, Ahmadu Bello University with herbarium Number: ABU09881. A specimen was deposited at the University Herbarium for future reference.

Sample Preparation and Extraction

The collected leaves were air-dried at room temperature (approximately 25°C) for two weeks to a constant weight. The dried leaves were then pulverized into a fine powder using a laboratory mortar and pestle. The powdered plant material (830.0 g) was subjected to exhaustive extraction via cold maceration with 3000 mL of absolute ethanol. The mixture was allowed to stand for 72 hours at room temperature with occasional shaking. This maceration process was repeated thrice with fresh solvent to ensure complete extraction. The combined extracts were filtered using Whatman No. 1 filter paper. The filtrate was concentrated and allowed to dry at room temperature in an evapourating dish, yielding the crude ethanol extract.

Fractionation of the Crude Extract

The crude ethanol extract was fractionated using solvents of increasing polarity via liquid-liquid partitioning. Briefly, 100 g of the crude extract was dissolved in 200 mL of distilled water and introduced into a separating funnel. An equal volume (200 mL) of n-hexane was added, and the mixture was vigorously shaken with intermittent venting to release pressure. The mixture was allowed to stand until complete separation into distinct layers occurred. The n-hexane fraction (upper layer) was carefully collected. The partitioning process was repeated three times with fresh n-hexane to ensure exhaustive defatting. The same procedure was subsequently employed to partition the aqueous layer successively with chloroform, ethyl acetate, and n-butanol (Adeyemi *et al*., 2021). Each fraction was concentrated using a rotary evapourator, and the resulting residues were stored in airtight containers at 4°C until further use. The percentage yield of the crude extract and each fraction was calculated using the following formulae:

$$\% \text{ Yield} = \frac{\text{mass of crude extract} \times 100}{\text{mass of leave}}$$

While the percentage yields of each fraction is estimated from;

$$\% \text{ Yield} = \frac{\text{mass of fraction} \times 100}{\text{Mass of crude extract}}$$

Phytochemical Screening

Qualitative phytochemical analysis of the crude ethanol extract was performed to identify the presence of major secondary metabolite classes using standard procedures (Harborne, 1998: AOAC, 2015). The specific tests conducted are summarized below:

Test for Alkaloids (Wagner's Test)

Approximately 1.00 g of the powdered plant material was extracted with 20 mL of ethanol using warm maceration. The mixture was filtered, and the filtrate was concentrated to a small volume. The concentrate was then treated with 10 mL of 10% sulfuric acid (H_2SO_4) to acidify the medium. The acidic solution was alkalinized with 10% ammonium hydroxide (NH_4OH) and subsequently extracted with chloroform. The chloroform layer was separated and

evapourated to dryness. The residue was reconstituted in a small volume of ethanol (Adebayo *et al.* 2024). A few drops of Wagner's reagent (iodine in potassium iodide) were added to 1.00 mL of this solution. The formation of a reddish-brown precipitate was recorded as a positive indication for the presence of alkaloids.

Tests for Flavonoids

The extract was defatted by shaking with petroleum ether. The defatted residue was then dissolved in 20.00 mL of 80% ethanol and filtered. The filtrate was used for the following confirmatory tests:

Ammonia Test

A strip of filter paper was dipped into the ethanolic extract and subsequently exposed to ammonia vapour. The observation of a yellow colouration on the filter paper was considered a positive result for flavonoids.

Shinoda Test

To 2.00 mL of the ethanolic extract, a few fragments of magnesium turnings were added, followed by the dropwise addition of concentrated hydrochloric acid. The appearance of a pink, red, or magenta colour indicated the presence of flavonoid aglycones.

Vanillin-HCl Test

2.00 mL of vanillin-HCl reagent (a mixture of vanillin and concentrated HCl) were added to 5.00 mL of the ethanolic extract. The development of a pink or red colour confirmed the presence of flavonoids (Adebayo *et al*., 2024; Vinoth *et al*., 2012).

Tests for Tannins

About 0.50 g of the plant extract was boiled in 20.00 mL of distilled water in a test tube and then filtered. The filtrate was used for the following tests

Ferric Chloride ($FeCl_3$) Test

To the aqueous filtrate, a few drops of 0.10% ferric chloride solution were added. The formation of a blue-black or greenish colouration was taken as evidence of tannins.

Bromine Water Test

A few drops of bromine water were added to a small portion of the extract. The decolourization of bromine water or the formation of an orange or red precipitate was considered a positive test for tannins.

Tests for Saponins

Froth Test (Frothing Test)

Exactly 0.50 g of the plant extract was dissolved in 10.00 mL of boiling distilled water in a test tube. After cooling, the mixture was shaken vigorously for 30 seconds and then allowed to stand for 15 minutes. The formation of a stable, persistent foam layer of at least 1 cm in height was interpreted as a positive result for Saponins.

Haemolysis Test

A drop of fresh animal blood was placed on a glass slide and mixed with a few drops of an aqueous solution of the plant extract. The slide was observed under a microscope for the lysis of red blood cells, which indicates the presence of saponins (Josephine *et al*., 2009).

Test for Steroids and Triterpenoids (Liebermann-Burchard Test)

10.00 mL of the ethanol extract were evapourated to dryness. The residue was extracted with 5.00 mL of chloroform ($CHCl_3$). To this chloroform extract, a few drops of acetic anhydride were added, followed by the careful addition of 1 mL of concentrated sulfuric acid (H_2SO_4) down the side of the test tube to form a layer. The development of a blue-green ring at the interface indicated the presence of steroids, while the formation of a red or purple ring suggested the presence of triterpenoids (Vinoth *et al*., 2012).

Test for Cardiac Glycosides (Keller-Killiani Test)

To 10.00 mL of an alcohol extract of the crude plant material, 10.00 mL of distilled water was added, followed by 0.50 mL of a strong lead acetate solution. The mixture was shaken and filtered to remove precipitates. The filtrate was then extracted with 10.00 mL of chloroform. The chloroform extract was evapourated to dryness, and the residue was dissolved in 3.00 mL of glacial acetic acid. A few drops of 15% ferric chloride ($FeCl_3$) solution were added to this. Finally, 1.00 mL of concentrated sulphuric acid was carefully added down the side of the test tube. The formation of a reddish-brown ring at the interface between the acetic acid and sulphuric acid layers confirmed the presence of cardiac glycosides (Adebayo *et al*. 2024).

Test for Anthraquinones (Modified Bontrager's Test)

1.00 g of the powdered sample was boiled with 5.00 mL of dilute sulphuric acid (H_2SO_4) for 10 minutes in a water bath. After cooling, the mixture was filtered. The filtrate was then extracted with 10.00 mL of chloroform. The chloroform layer was separated, and 5.00 mL of 10% ammonia solution was added. The appearance of a rose-pink colour in the ammoniacal (upper) layer was recorded as a positive test for free anthraquinones (Adebayo *et al*. 2024).

Test for Volatile Oils

2.00 mL of the sample extract were treated with a few drops of a 10% sodium hydroxide (NaOH) solution, followed by the addition of a few drops of diluted hydrochloric acid (HCl). The formation of a white precipitate was considered indicative of the presence of volatile oils (Vinoth *et al*., 2012).

Brine Shrimp Lethality Assay (BSLA)

The cytotoxicity of the extracts was evaluated using the Brine Shrimp Lethality Assay (BSLT) as described by Meyer *et al.* (1982).

Hatching of Brine Shrimp

Artificial seawater was prepared by dissolving 35 g of commercial sea salt in 1.00 L of distilled water (NT Labs, 2015). Brine shrimp (*Artemia salina*) eggs were sprinkled into a hatching chamber filled with the seawater and aerated continuously. The setup was illuminated for 24-48 hours at 24-28°C to hatch the eggs and release the larvae (nauplii).

Preparation of Test Solutions

A stock solution of each extract (2000 µg/mL) was prepared by dissolving 20 mg of the extract in 10 mL of a suitable solvent (e.g., dimethyl sulfoxide, DMSO, not exceeding 1% v/v in the final test solution). From this stock, a series of graded concentrations (10, 100, 200, 500, and 1000 µg/mL) were prepared via serial dilution with artificial seawater.

Bioassay Procedure

For each test concentration, 4.00 mL of the solution was transferred into a vial. 10 actively swimming nauplii were added to each vial using a Pasteur pipette, and the volume was adjusted to 5.00 mL with artificial seawater to achieve the final test concentrations. A control group containing nauplii in artificial seawater with an equivalent amount of solvent was included. All tests were performed in triplicate. The vials were maintained under illumination, and the number of dead nauplii (immobile at the bottom) in each vial was counted after 24 hours.

Determination of LC_{50}

The percentage mortality at each concentration was calculated. The LC_{50} value (the lethal concentration that kills 50% of the nauplii) was determined by plotting the logarithm of concentration against the percentage mortality, which was transformed to probits. Regression analysis was performed using Finney's probit analysis method with Biostat 2009 software. Percentage mortality (M %) was calculated from the expression below:

$$\%M = \frac{\text{(Total No of nauplii used-No of nauplii alive)} \times 100}{\text{Total No of nauplii}}$$

Statistical Analysis

The data obtained from bioassay assays were subjected to statistical analysis using IBM SPSS Statistics Software (Version 20). One-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test was used for multiple comparisons at a 5 % probability level ($P \leq 0.0001$). Values are presented as mean ± SEM. Other software used includes Microsoft Excel 2019, Graphpad prism, Origin and maetronova

RESULTS AND DISCUSSION

Yield of the Extract

12.06 % Ethanol extract was obtained from *Parquetina nigrescens* leave sample which was then extracted successively to obtain different extract fractions with n-hexane, chloroform, ethyl acetate, butanol, aqueous.

Table 1: % Fraction Yields from *P. nigrescens* Extract

Extract fraction	% Yield
N-hexane	35.30
Chloroform	36.94
Ethyl acetate	6.57
Butanol	6.16
Aqueous	15.11

Table 2: Phytochemical Screening of *P. nigrescens* Extract and Fractions

Phytochemicals	Crude	n-hex	Chl	But	EA	AQ
Alkaloids	+++		+++			
Flavonoids	+++			+++	+++	+
Flavanol	+++		+	+++	+++	++
Steroidal	++	++	++			++
Triterpenoid	+++	+++				
Saponin	+			+		
Tannin	+++		++	+++		
Coumarin Glycoside	++			++		+
Phlobatannins	++		+	++		+
Cardiac glycosides	++			++		
Free anthraquinone	++		++			++
Anthraquinone-o-glycoside	+++			+++		+
Anthraquinone-c-glycoside	++			++		
Free Reducing sugar	++					++
Volatile oil	++	+++				
Cardenolides	++			++		

Table 3: Brine Shrimp Lethality Assay (BSLA)

Extract/Fraction	Conc. (ppm)	Nauplii Used (n)	Dead nauplii (Mean ± SD in 24hr)	% Mortality ± SD	LC_{50} (µg/mL)
Chl	10	20	4.0 ± 0.2	20.0 ± 0.03	555
	100	20	7.0 ± 0.10	35.0 ± 0.02	
	200	20	11.0 ± 0.51	55.0 ± 0.03	
	500	19	10.0 ± 0.11	52.6 ± 0.01	
	1000	19	12.0 ± 0.01	63.2 ± 0.02	
But	10	19	5.0 ± 0.13	26.3 ± 0.02	70
	100	16	13.0 ± 0.02	81.3 ± 0.04	
	200	19	14.0 ± 0.20	73.7 ± 0.03	
	500	19	13.0 ± 0.10	68.4 ± 0.01	
	1000	20	15.0 ± 0.21	75.0 ± 0.0	
EA	10	19	5.0 ± 0.04	26.3 ± 0.03	142

Extract/Fraction	Conc. (ppm)	Nauplii Used (n)	Dead nauplii (Mean ± SD in 24hr)	% Mortality ± SD	LC_{50} (µg/mL)
	100	19	16.0 ± 0.30	84.2 ± 0.06	
	200	19	13.0 ± 0.10	68.4 ± 0.01	
	500	19	14.0 ± 0.40	73.7 ± 0.2	
	1000	20	14.0 ± 0.13	70.0 ± 0.02	
n-Hex	10	18	6.0 ± 0.01	33.3 ± 0.03	366
	100	16	7.0 ± 0.02	43.8 ± 0.01	
	200	16	10.0 ± 0.0	62.5 ± 0.01	
	500	19	12.0 ± 0.0	63.2 ± 0.06	
	1000	19	10.0 ± 0.0	52.6 ± 0.05	
Crude	10	20	7.0 ± 0.02	35.0 ± 0.01	660
	100	19	8.0 ± 0.02	42.1 ± 0.03	
	200	18	8.0 ± 0.01	44.4 ± 0.12	
	500	20	11.0 ± 0.10	55.0 ± 0.03	
	1000	18	13.0 ± 0.20	72.2 ± 0.02	
Aqueous	10	20	5.0 ± 0.03	25.0 ± 0.40	72
	100	19	15.0 ± 0.40	78.9 ± 0.01	
	200	20	14.0 ± 0.10	70.0 ± 0.03	
	500	19	16.0 ± 0.12	84.2 ± 0.01	
	1000	16	15.0 ± 0.12	93.8 ± 0.02	

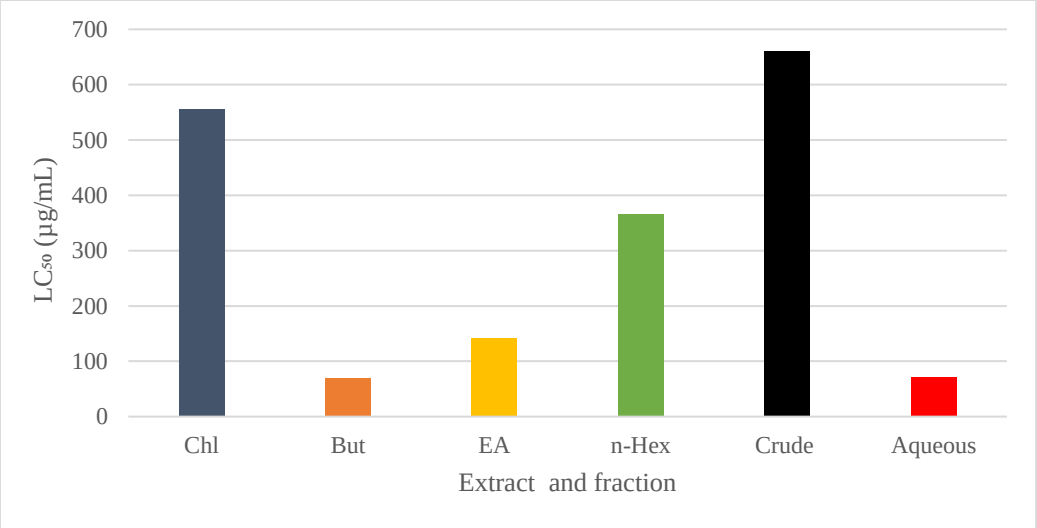

Figure 3: Cytotoxicity Potency (BSLA) of Crude and Fractions of *P. nigrescens* (Expressed in LC_{50})

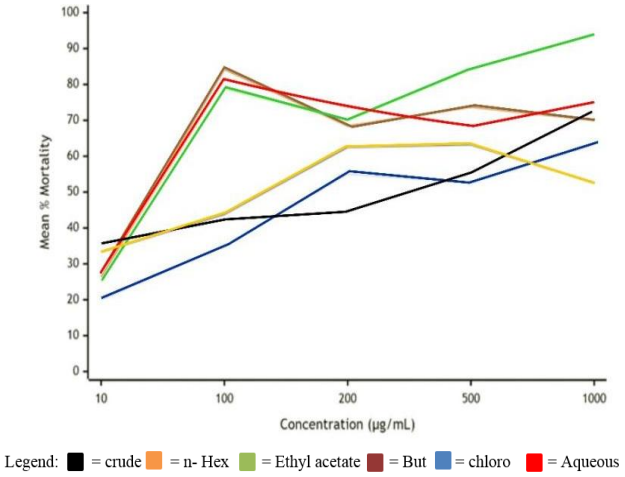

Figure 4: Concentration-Dependent Mortality of Crude and Solvent Extracts

Discussion

The investigation into the phytochemical composition and cytotoxic potential of *Parquetina nigrescens* leaves elucidates a compelling narrative on the strategic importance of solvent polarity in bioactivity-guided fractionation.

Crude ethanol extract was found to give 12.06% yield, then subsequent fractionation of the crude extract yielded 35.30% of n-hexane, 36.94% chloroform 15.11% of aqueous, 6.57 % of ethyl acetate and 6.16 % butanol fractions (Table 1). Phytochemical screening of the crude revealed a rich matrix of secondary metabolites, with abundant alkaloids, flavonoids, flavanols, and triterpenoids (Table 2). This profile is consistent with previous studies on *by* Ayoola *et al*. (2011) and Onyegeme-Okerenta *et al*. (2018) who reported high concentrations of phenolics and flavonoids, which are well-established for their antioxidant and cytotoxic properties. The significant presence of alkaloids and triterpenoids is particularly noteworthy, as these classes include some of the most potent anticancer agents discovered from plants, such as vinblastine and betulinic acid (Cordaliza, 2007; Kintzios, 2006). Therefore, our phytochemical data does not merely list constituents, it gives a credible chemical basis for the plant's purported medicinal efficacy, aligning with and reinforcing the existing body of literature. setting the stage for a comparative bioactivity assessment. *P. nigrescens* is endowed with a potent arsenal of cytotoxic compounds, and their efficient extraction is governed by a precise interplay with solvent polarity. This study not only corroborates the plant's ethnomedicinal value but also provides a clear roadmap for the isolation of its bioactive principles.

The Brine Shrimp Lethality Assay (BSLA) results universally confirmed the cytotoxic nature of all extracts, with LC_{50} values significantly below the 1000 µg/mL threshold established for potential bioactivity (Meyer, 1982). The lower the value of LC_{50}, the higher the cytotoxicity. However, the data revealed a critical, non-random efficacy gradient directly correlated with solvent polarity: Butanol (LC_{50} = 70 µg/mL) ≈ Aqueous (72 µg/mL) > Ethyl Acetate (142 µg/mL) > n-Hexane (366 µg/mL) > Chloroform (555 µg/mL) > Crude Extract (660 µg/mL) (Table 3). This gradient is a direct manifestation of the solubility of the active cytotoxic compounds. Our results firmly place *P. nigrescens* in the category of plants with significant cytotoxic potential. Although cytotoxicity is not the same as antitumoral activities, yet it's a preliminary consideration for antitumoral active principle. Thus, this finding directly corroborates the work of Onyegeme-Okerenta *et al*. (2018) who demonstrated the antitumoral activity of an ethanol extract against human cancer cell lines.

The superior potency of the butanol and aqueous fractions unequivocally identifies the primary bioactive agents as highly polar to mid-polar molecules. This is strongly supported by the phytochemical data (Table 2), which shows the concentration of polar glycosides, saponins, and tannins in these fractions. The remarkable activity of the aqueous fraction, in particular, provides a robust scientific validation for the traditional use of water-based *P. nigrescens* decoctions (Owoyele *et al*. 2011). The concentration-response curve (Figure 2) for the butanol fraction, characterized by a sharp ascent to over 80% mortality at 100 µg/mL, is indicative of a potent, fast-acting cytotoxic agent, probably a saponin or a flavonoid glycoside acting through rapid membrane disruption (Cordell, et *al.* 2005).

Conversely, the moderate activity of the ethyl acetate fraction aligns with its enrichment of mid-polarity flavonoids and flavanol aglycones. Also the lower potency of the n-hexane and chloroform fractions, despite their high yields and content of non-polar compounds like triterpenoids and steroidal compounds (Table 2), suggests these constituents play a secondary role in the observed cytotoxicity within this model. The concentration- response curve of n-hexane fraction's is more gradual. Such a linear dose-response may indicate a different mechanism, such as the disruption of cellular membranes or slower metabolic inhibition, which is consistent with the mode of action of many triterpenoids (Anderson *et al*., 2017). The crude extract's position as the least potent is a classic demonstration of the "dilution effect," where active principles are diluted by inert plant material, thereby underscoring the indispensability of fractionation for bioactivity concentration (Cragg, *et al.,* 2013).

The correlation among the phytochemical profile, fractionation polarity, and cytotoxic potency is thus clearly defined: the most potent fractions (Butanol, Aqueous) are those enriched with polar, glycosylated compounds. While the BSLA is an excellent preliminary screening tool (Solis, *et al.,* 1993) for anticancer study its results do not directly imply antitumoral activities. There are a number of other considerations required. However, a positive result for cytotoxicity study warrant further investigation using specific human cancer cell lines to validate potential antitumor activities.

In summary, this study moves beyond simply confirming the bioactivity of *P. nigrescens*. It provides a sophisticated, solvent-based roadmap for unlocking its therapeutic potential. By identifying the high-polarity fractions as the most potent, we have not only validated a key aspect of its traditional use but have also provided a focused and efficient strategy for the discovery of novel anticancer lead compounds from this promising medicinal plant.

CONCLUSION

In conclusion, this study successfully demonstrates that *P. nigrescens* is a promising source of cytotoxic compounds. The significant finding is that solvent polarity is a critical factor in isolating these bioactive principles, with butanol and water being the most effective for extracting the potent cytotoxic components. At $p < 0.001$, there is statistical significant difference in the BSLA cytotoxicity across the fractions of different polarity. This not only provides a scientific basis for the plant's ethno medicinal use but also guides future research towards the bioassay-guided isolation of the specific anticancer compounds present in the most active fractions. A more focused scientific approach towards the maximization of the pharmacological properties of *P. nigrescens* is potentially a roadmap towards the discovery of novel lead compounds with potential anticancer applications. The following recommendations are worthy of note

i. All fractions exhibit cytotoxicity therefore there is need to carry out isolation using techniques like VLC, MPLC, and HPLC to separate the complex mixtures in each fractions.
ii. There is need for characterisation of compounds present in each solvent fraction
iii. In-depth cytotoxicity profiling of the crude, fractions and purified compounds against a panel of human cancer cell lines (e.g., MCF-7, C4-2WT, HT 29 and HTC 116 cell lines) to confirm if the cytotoxicity indicated by BSLA correlates with anticancer potential.

4. Investigating the mode of action of the most potent isolates, exploring pathways such as apoptosis induction, cell cycle arrest, and anti-angiogenesis.

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