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# Assessment of Effects of Ethanolic Extracts of Psidium Guajava Andvernonia Amygdalina on the Gut Microbiota of Albino Rats

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## Abstract

Medicinal plants are rich in phytochemicals with various therapeutic potentials, including antibacterial, antioxidant, and haematinic properties. In this study, the effects of ethanolic extracts of Vernonia amygdalina (bitter leaf) and Psidium guajava (guava) on the intestinal microbiota of albino Wistar rats were investigated. Fresh leaves were collected, authenticated, processed, and extracted using ethanol. One hundred and twenty rats were divided into control groups and treatment groups receiving 200 mg/kg and 400 mg/kg of extract for 28 and 56 days, respectively. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, glycosides, and steroids in both plants, while anthraquinones were present only in Vernonia amygdalina. Microbial evaluation of stool samples showed that beneficial bacteria (species of lactic acid bacteria) did not change appreciably, while harmful bacteria and fungi (Salmonella spp., Proteus spp., Candida species) were significantly reduced. Statistical analysis revealed a significant reduction ($p < 0.05$) in total bacterial and fungal load without affecting beneficial flora. These findings support the ethnomedical applications of Vernonia amygdalina and Psidium guajava leaf extracts and highlight their potential as natural antimicrobial agents that modify gut microbiota by depleting harmful bacteria while retaining beneficial ones.

**Keywords**: Antibacterial Activity; Gut Microbiota; Phytochemicals; Psidium Guajava; Vernonia Amygdalina; Wistar Rats.

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## INTRODUCTION

Despite the great nutritional values of plants, they have also been found to possess certain therapeutic potentials, hence are often referred to as 'medicinal plants'. Medicinal plants are laden with numerous biologically and pharmacologically active substances or chemicals known as phytochemicals, which are effective in the prevention, treatment or management of various pathological conditions. Some have been shown to possess antimicrobial (Umedum & Oyeka, 2016), cardio-protective (Obi-Ezeani et al., 2020), and haematinic (Eze et al., 2019) properties among others. Extracts from medicinal plants have been used since ancient times in folk medicine to promote general health, prevent and manage certain ailments

Bacteria and their host must interact for normal host physiological activity. However, factors like diabetes, obesity, hypertension, chronic kidney disease, inflammatory bowel disease, and colon cancer can occur when the flora in the intestine is out of balance (Alexander et al., 2017; Eom et al., 2018; Lau & Wong, 2018; Rukavina et al., 2020). Sharing this are a number of disease processes that show alterations in haematological, biochemical, and histological characteristics.

The high medicinal value of plants combined with their presumed low toxicity, low cost, and easy accessibility has led to the present surge in plant research. However, some of these plants can be poisonous, especially if used in amounts that are higher than the suggested amounts.

Leaves of *Psidium guajava* (guava) and *Vernonia amygdalina* (bitter leaf) have been used for various medicinal and defense purposes in the traditional system of medicine. The effect of these plant extracts on visceral organs, gut flora, reproductive process and haematological parameters, however, remains unknown on the other hand.

Thus, this study aims to assess the antibacterial activity of ethanolic extracts of P. guajava and V. amygdalina leaves using an animal model

## LITERATURE/THEORETICAL UNDERPINNING

Previous studies have demonstrated that P. guajava leaves possess potent antioxidant activity due to their rich phenolic and flavonoid composition (Ekerette, 2015). V. amygdalina leaf extract is known to exhibit strong antibacterial activity, including activity against multidrug-resistant Salmonella typhi (Usman et al., 2025). It is also recognized to contain anthraquinones, which have long been associated with gastrointestinal benefits. These findings provide the theoretical basis for investigating the role of P. guajava and V. amygdalina leaves in regulating gut flora.

## METHODOLOGY

Fresh samples of leaves of *P. guajava* and *V. amygdalina* were collected from Awka.

It was verified by Professor C.G. Ukpaka, cleaned, air- dried for 14 days at room temperature under shade, and crushed into a fine powder using an electric blender.

40g of the powdered leaves were extracted in 400mL of ethanol on a Soxhlet for 6 hours.Before being used, the extracts were concentrated under reduced pressure using a rotary evaporator,

dried to constant weight in a vacuum oven, and kept at 4°C (Azwanida, 2015; Umedum & Oyeka, 2016; Pandey et al., 2016; Pratama, Hutapea, & Ambarwati, 2024) .

**Phytochemical Examination of the Extracts was Carried Out as an Initial Stage**.

This was conducted along the methodology adapted from Poornima (Umedum & Iheukwumere 2013).

**Adopting and Taking Care of Animals**

One hundred and twenty (120) mature albino Wistar rats (60 males and 60 females) were obtained from the CONIG-SIMMONNE BIOMEDICALS animal house, Awka. The rats were given two weeks to acclimatize prior to the commencement of the experiment, in order to reduce any stress phenomena that might otherwise affect their physiological stability (Festing & Altman, 2002)."

The animals were housed in metal cages under a light-dark period of 12 hours, a controlled temperature range of 22-25$^{o}$C and controlled relative humidity of 50-60 %. Male and female were separated to keep them from mating and meeting to cause stress. They were fed with pelletized broiler finisher feed (Vital Feeds, Jos) and were provided with free choice of water. Each technique performed was done according to international requirements for the use and care of laboratory animals as well as institutional requirements (National Research Council, 2011; World Health Organization, 2009).

**Experimental Design and Administration of Extracts**

Both the designs and administration of extracts are experimental.

Randomly, 120 Wistar rats were allocated into two main groups (I and II) with 60 rats in each. In Group I, animals received ethanolic extracts obtained from *Psidium guajava* leaves and in Group II, animals received ethanolic extracts obtained from the leaves of *Vernonia amygdalina*. One and two months of oral gavage are considered the most reliable route of exposure to simulate humans; therefore, extracts were administered orally to the rats daily via gavage.

Six groups (A–F) of ten rats each were created by further dividing each major group based on sex:

Group A: Male Control Group – Distilled water 5 ml/kg b.w.

Female Control Group (5 ml/kg b.w. distilled water) (Group B).

Group C – Male Test group (200mg/kg b.w. extract).

Group D: Test group for females (200 mg/kg b.w extract)

Group E: Test group for males (400 mg/kg b.w extract)

Group F: Test group fed with 400 mg/kg b.w extract (Female)

The doses of 200 mg/kg and 400 mg/kg body weight were selected based on previous toxicity and pharmacological studies. For Psidium guajava, 200 mg/kg has been reported as safe for long-term administration, while 400 mg/kg produces mild but tolerable hepatic changes (Akinmoladun et al., 2015; Ojewole, 2006). For Vernonia amygdalina, doses up to 500 mg/kg

are considered safe, with pharmacological efficacy demonstrated at 100–400 mg/kg (Adedapo et al., 2009; Farombi & Owoeye, 2011).

**Periodic Stool Sample Collection and Processing**

Fecal collection was carried out on days 28 and 56 during the treatment. Fresh fecal pellets were obtained directly from each rat (from the cage floor immediately after defecation). Each fecal sample was weighed to ensure accurate dilution.

The sample was then crushed and homogenized in 10 mL of phosphate-buffered saline (PBS) per 1 g of feces**,** following Cheesbrough (2006).

The homogenized suspension was subjected to ten-fold serial dilutions to reduce microbial density and allow accurate colony counting. Aliquots (0.1 mL) from the $10^3$ dilution were then plated, in triplicate, on various media namely Nutrient agar (HiMedia, India), de Man, Rogosa, Sharpe (MRS) agar (HiMedia, India), Sabouraud dextrose agar (HiMedia, India) and CHROMagar Candida (HiMedia, India). Spread onto selective and non-selective agar plates (Nutrient agar, MRS agar, Sabouraud Dextrose Agar, and CHROMagar Candida). Plates were incubated at 37°C for 5 days to allow colony growth. Colonies were counted and expressed as colony -forming units per millilitre (CFU/mL), adjusted for dilution factor. Replicates were averaged and log-transformed for statistical analysis

Standard microbiological techniques were used to count and identify colonies (Atlas, 2010). The rationale for selecting 28 and 56 days was to capture both intermediate and longer-term effects of extract administration on gut microbiota. Day 28 represents a sub-chronic exposure period, while day 56 reflects a more extended exposure, allowing for assessment of cumulative or adaptive changes. Stool samples were collected repeatedly from the same animals at both time points. This repeated-measures design minimized inter-animal variability and reduced the number of animals used, in line with ethical principles of animal research.

**Enumeration and Identification of Microorganisms**

The number of colonies was counted after an incubation period to determine the microbial load present in the rat faeces samples. Colony- forming units per millilitre (CFU/mL) was used to represent colony numbers. This was calculated by dividing the number of colonies by the volume plated (times the dilution factor). For this investigation, stool samples were ten-fold serially diluted and 0.1 mL from the $10^3$ dilution tube was spread onto Chrom agar, Nutrient agar, Sabouraud Dextrose Agar (SDA) and MRS agar plates in triplicate. The average colony was taken between replicates and represented as mean ± standard deviation (SD).

The number of CFU/mL was then transformed into $\log_2{}^1$ CFU/mL and used for statistical analysis and easy comparison. Each replicate was log- transformed and the mean ± SD of the log data was calculated. This modification eliminates variation and allows the distribution of the data to take on a normal distribution, making it suitable for using a parametric test such as a t -test (Cappuccino & Sherman, 2014).

Representative colonies were identified by using standard microbiological techniques.

**Identification of Bacteria**

Gram staining allowed the differentiation of the Gram-negative and Gram-positive organisms.

The first distinction was made on an oxidase and catalase test.

The general bacteria were isolated by growing on nutrient agar (HiMedia).

Lactic acid bacteria were isolated on MRS agar (HiMedia) and characterised.

Biochemical tests were then performed (IMViC and sugar fermentation) for further characterisation.

**Identification of Fungi**

Macroscopic examination of Sabouraud dextrose agar (HiMedia) colonies revealed the morphology (colour, texture, growth pattern) of the colonies.

Fungi were isolated from Hyphae, spores, and conidia by taking subsamples from the original samples and then examining them under the microscope using lactophenol cotton blue staining.

To identify yeasts the germ tube test was employed.

*Candida* species were determined by their growth on CHROMagar Candida (HiMedia) with regard to the morphology and colour of the colonies (Odds & Bernaerts, 1994).

**Analysis of Data**

In each group, the colony counts were expressed as the mean ± standard deviation (CFU/g stool). The data were analyzed using PAIRED T TEST and the criteria for statistical significance were set at $< 0.05$ (Umedum & Oyeka, 2016).

**Ethical Considerations**

Ethical permission was given by the University Research and Ethics Committee, and all procedures were in accordance with the institution's rules for care and use of animals. (See Appendix I).

## RESULTS

Preliminary phytochemical screening of the extracts of *Psidium guajava* and *Vernonia amygdalina* revealed the presence of several major secondary metabolites (Table 1). Both the plants contained various bioactive compounds including alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, glycosides, and steroids in varying concentrations. The concentration of alkaloids and phenols was higher in *Psidium guajava* than in *Vernonia amygdalina*, while tannins, saponins, phenols, and anthraquinones were more in *Vernonia amygdalina*, than in *Psidium guajava*. It is interesting to note that anthraquinones were present in *Vernonia amygdalina*, but not in *Psidium guajava*. From these results, it can be seen that these plants have both a high content of bioactive compounds and the *Vernonia amygdalina*, generally had a more extensive profile of these compounds than *Psidium guajava*.

Analysis of the microbial counts [Tables 2, 3 and 4] showed different patterns in the different culture media. The colony counts in Chrom Agar, which is selective for Candida species, showed that counts decreased slightly more from Days 28-56, but were also not statistically significant ($p>0.05$). However, there was a significant reduction in the overall bacterial growth measured by nutrient agar in treated groups ($P < 0.05$), indicating significant antibacterial

activity of the extracts. The constant and statistically significant inhibition of wider fungal growth on Sabouraud Dextrose Agar (SDA) in almost all treatment groups confirmed the antifungal efficacy of extracts ($p < 0.05$). Finally, counts on MRS, favouring lactic acid bacteria, were also steady or slightly higher, but no significant changes were observed ($p > 0.05$). This stability implies that, in spite of the antibiotic effects on pathogenic bacteria and fungi, beneficial gut flora were maintained.

There were significant differences between the faecal microbiota in the Wistar rats of the different experimental groups at 28 days (Table 7). The lactic acid bacteria and *E. coli* were present in most groups at all times, indicating that these were stable components of the gut. Pathogenic organisms like *Salmonella* and *Proteus* were not found in several groups (IC, ID, IID, IF, and IIF), which signifies that there was less colonization or clearance over a period of time. The ability of capsule -forming Enterobacteriaceae to persist in the environment is demonstrated by the presence of *Klebsiella* spp. in multiple populations. The presence of opportunistic fungi (*C. albicans, C. tropicalis, C. parapsilosis* and *C. glabrata)* was variable, with more opportunity in group A, B and IF and less opportunity in other groups such as IIC and IIF. Colonisation is likely to have been a transient phenomenon in the early groups as *Aspergillus niger* and *Aspergillus flavus,* two moulds occurring in the environment, were only detected in these. The results indicated that the composition of the microbiota communities varied, being more diverse in groups A and B during the early phase, but becoming more restricted in the later phase. However, lactic acid bacteria remained dominant across all groups.

After prolonged therapy (Day 56), the presence (+) or absence (–) of certain bacteria are given in Table 8. As observed on Day 28, lactic acid bacteria are still dominant, but *E. coli* remain predominant, with *Salmonella* and *Proteus* species being consistently absent. Examples of group-specific organisms with selective persistence in individual treatment groups are *Aspergillus niger* and *Candida glabrata*.

**Table 1. Results of preliminary phytochemical screening showing the presence of major secondary metabolites in guava (*Psidium guajava*) and bitter leaf (*Vernonia amygdalina*) extracts.**

| Phytochemicals | *Psidium guajava* | *V. amygdalina* |
|---|---|---|
| Alkaloids | | |
| Flavonoids | ++ | ++ |
| Tannins | + | +++ |
| Saponins | ++ | +++ |
| Phenols | +++ | +++ |
| Terpenoids | + | + |
| Anthraquinones | - | + |
| Glycosides | + | + |
| Steroids | + | + |

***Keys:***

–: Not detected

+: Present in trace/little amount

++: Moderately present

+++: Highly present

**TABLE 2: Microbial Colony Counts $Log_{10}$ CFU/mL (Mean ± SD) from Rat Stool Samples Cultured on Different Media at Day 28(Mean ± SD, CFU/mL)**

| Group | Chrom agar | Nutrient agar | SDA | MRS |
|---|---|---|---|---|
| A | 6.09 ± 0.09 | 6.07 ± 0.05 | 6.24 ± 0.04 | 5.77 ± 0.06 |
| B | 6.26 ± 0.05 | 6.09 ± 0.03 | 6.27 ± 0.02 | 5.89 ± 0.02 |
| IC | 6.01 ± 0.05 | 6.26 ± 0.03 | 6.23 ± 0.03 | 6.00 ± 0.01 |
| IIC | 6.07 ± 0.06 | 6.27 ± 0.02 | 6.22 ± 0.03 | 6.04 ± 0.02 |
| ID | 6.12 ± 0.04 | 6.30 ± 0.05 | 6.21 ± 0.02 | 6.04 ± 0.02 |
| IID | 6.16 ± 0.02 | 6.30 ± 0.02 | 6.22 ± 0.03 | 6.09 ± 0.01 |
| IE | 6.12 ± 0.03 | 6.08 ± 0.02 | 6.17 ± 0.02 | 5.96 ± 0.01 |
| IIE | 6.14 ± 0.02 | 6.09 ± 0.02 | 6.18 ± 0.02 | 5.98 ± 0.01 |
| IF | 6.12 ± 0.01 | 6.09 ± 0.02 | 6.17 ± 0.01 | 5.97 ± 0.01 |
| IIF | 6.08 ± 0.02 | 6.09 ± 0.01 | 6.16 ± 0.04 | 5.99 ± 0.01 |

**Table 3: Microbial Colony Counts $Log_{10}$ CFU/mL (Mean ± SD) from Rat Stool Samples Cultured on Different Media at Day 56(Mean ± SD, CFU/mL)**

| Group | Chrom agar | Nutrient agar | SDA | MRS |
|---|---|---|---|---|
| A | 6.08 ± 0.04 | 6.07 ± 0.05 | 6.08 ± 0.05 | 5.76 ± 0.04 |
| B | 6.14 ± 0.04 | 6.08 ± 0.04 | 6.22 ± 0.05 | 5.89 ± 0.03 |
| IC | 6.00 ± 0.04 | 6.21 ± 0.03 | 6.23 ± 0.02 | 6.07 ± 0.05 |
| IIC | 6.04 ± 0.06 | 6.21 ± 0.04 | 6.14 ± 0.06 | 6.09 ± 0.03 |
| ID | 6.09 ± 0.04 | 6.23 ± 0.05 | 6.11 ± 0.03 | 6.10 ± 0.03 |
| IID | 6.09 ± 0.03 | 6.20 ± 0.05 | 6.09 ± 0.04 | 6.12 ± 0.02 |
| IE | 6.09 ± 0.03 | 6.05 ± 0.04 | 6.09 ± 0.03 | 6.05 ± 0.04 |
| IIE | 6.10 ± 0.02 | 6.05 ± 0.03 | 6.09 ± 0.02 | 6.02 ± 0.03 |
| IF | 6.09 ± 0.02 | 6.05 ± 0.04 | 6.11 ± 0.02 | 6.05 ± 0.02 |
| IIF | 6.04 ± 0.05 | 6.01 ± 0.02 | 6.09 ± 0.03 | 6.10 ± 0.03 |

***Keys***:

A: Male rat control group

B: Female rat control group

IC: Male rats treated with guava leaves extract (200 mg/kg body weight)

ID: Female rats treated with guava leaves extract (200 mg/kg body weight)

IIC: Male rats treated with bitter leaves extract (200 mg/kg body weight)

IID: Female rats treated with bitter leaves extract (200 mg/kg body weight)

IE: Male rats treated with guava leaves extract (400 mg/kg body weight)

IF: Female rats treated with guava leaves extract (400 mg/kg body weight)

IIE: Male rats treated with bitter leaves extract (400 mg/kg body weight)

IIF: Female rats treated with bitter leaves extract (400 mg/kg body weight)

Values represent colony -forming units per milliliter (CFU/mL) calculated from rat stool samples. Samples were diluted using ten-fold serial dilution, and 0.1 mL from the $10^{-3}$ dilution tube was plated in triplicate on Chrom agar, Nutrient agar, SDA, and MRS media. Counts

shown are mean ± standard deviation for each experimental group (see Keys for group definitions).

**Table 4: Comparison of Microbial Counts ($log_{10}$ CFU/mL, Mean ± SD)**

| Group | Chrom agar (Day 28 → Day 56, p) | Nutrient agar (Day 28 → Day 56, p) | SDA (Day 28 → Day 56, p) | MRS (Day 28 → Day 56, p) |
|---|---|---|---|---|
| A | 6.09 ± 0.09 → 6.08 ± 0.04 (p = 0.72, ns) | 6.07 ± 0.05 → 6.07 ± 0.05 (p = 0.65, ns) | 6.24 ± 0.04 → 6.08 ± 0.05 (p = 0.04, sig) | 5.77 ± 0.06 → 5.76 ± 0.04 (p = 0.81, ns) |
| B | 6.26 ± 0.05 → 6.14 ± 0.04 (p = 0.08, ns) | 6.09 ± 0.03 → 6.08 ± 0.04 (p = 0.59, ns) | 6.27 ± 0.02 → 6.22 ± 0.05 (p = 0.03, sig) | 5.89 ± 0.02 → 5.89 ± 0.03 (p = 0.77, ns) |
| IC | 6.01 ± 0.05 → 6.00 ± 0.04 (p = 0.11, ns) | 6.26 ± 0.03 → 6.21 ± 0.03 (p = 0.02, sig) | 6.23 ± 0.03 → 6.23 ± 0.02 (p = 0.99, ns) | 6.00 ± 0.01 → 6.07 ± 0.05 (p = 0.06, ns) |
| IIC | 6.07 ± 0.06 → 6.04 ± 0.06 (p = 0.09, ns) | 6.27 ± 0.02 → 6.21 ± 0.04 (p = 0.01, sig) | 6.22 ± 0.03 → 6.14 ± 0.06 (p = 0.04, sig) | 6.04 ± 0.02 → 6.09 ± 0.03 (p = 0.07, ns) |
| ID | 6.12 ± 0.04 → 6.09 ± 0.04 (p = 0.07, ns) | 6.30 ± 0.05 → 6.23 ± 0.05 (p = 0.03, sig) | 6.21 ± 0.02 → 6.11 ± 0.03 (p = 0.02, sig) | 6.04 ± 0.02 → 6.10 ± 0.03 (p = 0.05, borderline) |
| IID | 6.16 ± 0.02 → 6.09 ± 0.03 (p = 0.06, ns) | 6.30 ± 0.02 → 6.20 ± 0.05 (p = 0.02, sig) | 6.22 ± 0.03 → 6.09 ± 0.04 (p = 0.01, sig) | 6.09 ± 0.01 → 6.12 ± 0.02 (p = 0.09, ns) |
| IE | 6.12 ± 0.03 → 6.09 ± 0.03 (p = 0.08, ns) | 6.08 ± 0.02 → 6.05 ± 0.04 (p = 0.04, sig) | 6.17 ± 0.02 → 6.09 ± 0.03 (p = 0.02, sig) | 5.96 ± 0.01 → 6.05 ± 0.04 (p = 0.12, ns) |
| IIE | 6.14 ± 0.02 → 6.10 ± 0.02 (p = 0.07, ns) | 6.09 ± 0.02 → 6.05 ± 0.03 (p = 0.03, sig) | 6.18 ± 0.02 → 6.09 ± 0.02 (p = 0.01, sig) | 5.98 ± 0.01 → 6.02 ± 0.03 (p = 0.11, ns) |
| IF | 6.12 ± 0.01 → 6.09 ± 0.02 (p = 0.09, ns) | 6.09 ± 0.02 → 6.05 ± 0.04 (p = 0.04, sig) | 6.17 ± 0.01 → 6.11 ± 0.02 (p = 0.02, sig) | 5.97 ± 0.01 → 6.05 ± 0.02 (p = 0.10, ns) |
| IIF | 6.08 ± 0.02 → 6.04 ± 0.05 (p = 0.06, ns) | 6.09 ± 0.01 → 6.01 ± 0.02 (p = 0.02, sig) | 6.16 ± 0.04 → 6.09 ± 0.03 (p = 0.01, sig) | 5.99 ± 0.01 → 6.10 ± 0.03 (p = 0.08, ns) |

***Note:***

All values are expressed as $log_{10}$ CFU/mL (Mean ± SD)

P-values are from paired t-tests comparing Day 28 vs. Day 56 for each group and medium.

sig= statistically significant ($p < 0.05$).

ns = not significant ($p \geq 0.05$).

Chrom agar (Candida): Declines observed, but $p > 0.05$ → not significant.

Nutrient agar (general bacteria): $p < 0.05$ in treated group's → significant reduction.

SDA (fungi): $p < 0.05$ in nearly all treated groups → significant suppression.

MRS (lactic acid bacteria): $p > 0.05$ → no significant change, confirming stability of beneficial flora.

**Table 5: Morphological and Biochemical Identification of Bacteria Isolated from Wistar Rat Feces**

| Key Tests / Media | Morphological & Biochemical Features | Identification Outcome |
|---|---|---|
| Growth on MRS agar Gram staining; Catalase test; ; Sugar fermentation | creamy convex colonies; Gram-positive rods; catalase-negative; acid production in sugars | Identified as Lactobacillus species |
| Gram staining; IMViC tests: Indole (+), Methyl Red (+), Voges–Proskauer (–), Citrate (–); Sugar fermentation | Gram-negative rods; lactose fermenter; gas in lactose broth | Identified as Escherichia coli |
| Gram staining; IMViC tests: Indole (–), Methyl Red (–), Voges–Proskauer (+), Citrate (+); $H_2S$ production | Gram-negative rods; non-lactose fermenter; $H_2S$ positive | Identified as Salmonella spp. |
| Gram staining; IMViC tests: Indole (–), Methyl Red (–), Voges–Proskauer (+), Citrate (+); Growth on MacConkey agar | Gram-negative rods; non-motile; lactose fermenter; large mucoid colonies due to capsule | Identified as Klebsiella spp. |
| Gram staining; IMViC tests: Indole (+/– depending on species), Methyl Red (+), Voges–Proskauer (–), Citrate (+); Urease test; Motility test | Gram-negative rods; highly motile with characteristic swarming growth on agar; non-lactose fermenter; urease positive | Identified as Proteus spp. |

**TABLE 6: Morphological and Biochemical Identification of Fungi Isolated from Wistar Rat Feces**

| Key Tests / Media | Morphological & Biochemical Features | Identification Outcome |
|---|---|---|
| Growth on CHROMagar Candida | Green colonies; yeast cells; germ tube positive | Identified as Candida albicans |
| Growth on CHROMagar Candida | Blue colonies; yeast cells; pseudohyphae formation possible | Identified as Candida tropicalis |
| Growth on CHROMagar Candida | Purple colonies; yeast cells; smooth colonies | Identified as Candida parapsilosis |
| Growth on CHROMagar Candida | Milky-white colonies; small yeast cells; no pseudohyphae | Identified as Candida glabrata |
| Growth on Sabouraud Dextrose Agar (SDA) | Black colonies with white edges; septate hyphae; conidial heads radiate | Identified as Aspergillus niger |
| Growth on CHROMagar | Milky-white colonies; small | Identified as Candida glabrata |

| | | |
|---|---|---|
| Candida | yeast cells; no pseudohyphae | |
| Growth on Sabouraud Dextrose Agar (SDA) | Black colonies with white edges; septate hyphae; conidial heads radiate | Identified as Aspergillus niger |
| Growth on Sabouraud Dextrose Agar (SDA) | Yellow-green colonies; septate hyphae; rough conidia; produces aflatoxins | Identified as Aspergillus flavus |

**TABLE 7: Variation in Microbial Populations After 28 Days of Treatment**

| GROUP | *E.coli* | *Salmonella* spp | Lactic acid bacteria | *Klebsiella* spp | *Proteus* spp | C.A | C.T | C.P | C.G | A.N | AF |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | + | + | + | + | + | + | + | + | + | + | + |
| B | + | + | + | + | + | + | + | + | + | + | + |
| IC | + | - | + | + | - | - | - | - | - | - | - |
| IIC | + | - | + | + | -- | + | + | - | + | - | - |
| ID | + | - | + | + | - | - | - | - | - | - | - |
| IID | + | - | + | + | - | - | - | + | - | - | - |
| IE | + | - | - | - | - | - | - | + | + | - | - |
| IIE | + | - | + | - | - | - | - | - | + | - | - |
| IF | - | - | + | - | - | - | - | - | + | - | - |
| IIF | - | - | + | - | - | - | - | - | + | - | - |

**TABLE 8: Variation in Microbial Populations after 56 Days of Treatment**

| GROUP | *E.coli* | *Salmonella* spp | Lactic acid bacteria | *Klebsiella* spp | *Proteus* spp | C.A | C.T | C.P | C.G | A.N | AF |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | + | + | + | + | + | + | + | + | + | + | + |
| B | + | + | + | + | + | + | + | + | + | + | + |
| IC | + | - | + | + | - | - | - | - | - | - | - |
| IIC | + | - | + | + | - | + | + | - | + | - | - |
| ID | + | - | + | + | - | - | - | - | - | - | - |
| IID | + | - | + | + | - | - | - | + | - | - | - |
| IE | + | - | - | - | - | - | - | + | + | - | - |
| IIE | + | - | + | - | - | - | - | - | + | - | - |
| IF | - | - | + | - | - | - | - | - | + | - | - |
| IIF | - | - | + | - | - | - | - | - | + | - | - |

***Keys:***

CA *Candida albicans*

CT *Candida tropicalis*

CP *Candida parasilopsis*

CG *Candida glabrata*

AN *Aspergillus niger*

AF *Aspergillus flavus*

## DISCUSSION

In an initial phytochemical screening, extracts of *P. guajava* and *V. amygdalina* were determined to contain several secondary metabolites including alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, glycosides and steroids. *V. amygdalina* is found to be richer in alkaloids and tannin, saponins, phenols and anthraquinones than *P. guajava*. The results confirm the ethnomedical use of these herbs for the remedy of metabolic disorders, oxidative stress and infections.

The amount of phenols and flavonoids found in guava is in line with the findings of Ekerette (2015), who reported that guava leaves present a high amount of phenols and flavonoids which make them antioxidant. Findings by Usman et al. (2025), which revealed that extracts of *V. amygdalina* exhibited significant antibacterial activity against multidrug- resistant strains of *S.typhi* would further buttress the implication of bitter leaves given its significant content of tannins and saponins. Anthraquinones have been found in *V. amygdalina* but not in *P. guajava,* which is in line with previous findings that *V. amygdalina* contains laxative chemicals and is used traditionally to treat gastrointestinal issues (Springer Nature, 2024).

In general, the comparative phytochemical profile shows that bitter leaf appears to possess high antibacterial and antiparasitic properties while guava has great potential for antioxidant uses. These metabolites' joint existence in both plants emphasises their complementary medicinal functions and encourages their ongoing usage in ethnomedicine.

The present study revealed that *Vernonia amygdalina* and *Psidium guajava* extracts caused changes in the intestinal microbial community in the Wistar rats. From day 28 to 56, the level of Candida decreased on Chrom agar but was not significant at $p > 0.05$. This result is in line with data from Nigeria that indicate *P. guajava* extracts have a dose-dependent antifungal activity by inhibiting *Candida albicans* only at higher doses (Ogunjobi et al., 2024). However, the overall bacterial load in the nutrient agar showed significant reduction ($p < 0.05$) ,which is in line with the research conducted in West Africa where *Vernonia amygdalina* extracts inhibited *Salmonella typhi* and *E. coli* (Akinmoladun et al., 2021). In terms of SDA, as reported previously in Namibia, *P. guajava* leaves phytochemicals reduced both the bacterial and fungal infections and here too, caused a dramatic reduction in SDA ($p < 0.05$), which demonstrated the wide antifungal activity of *P. guajava* and *Vernonia amygdalina* leaves extracts (Shikongo et al., 2025). Finally, there was no change ($p > 0.05$) in the number of MRS agar, indicating the preservation of lactic acid bacteria. The selective impact aligns with earlier studies indicating that plant extracts are effective against pathogenic microorganisms while leaving the health-enhancing gut microorganisms untouched (Eze et al., 2020).

Although multiple studies conducted in Africa have demonstrated that *Psidium guajava*, and *Vernonia amygdalina* leaves extracts possess antimicrobial properties against bacterial and fungal pathogens, most of those studies used laboratory pathogens or clinical isolates. (Ogunjobi et al., 2024; Akinmoladun et al., 2021; Shikongo et al., 2025). Few studies have been conducted on the long-term control of microbial communities in the gut of in vivo animals, particularly in rodents, receiving treatments of different plant extracts. Moreover, the potential use of beneficial lactic acid bacteria identified in this study has not been widely reported for African conditions, although the role of lactic acid bacteria in the control of fungi and bacteria has already been demonstrated. This gap poses a connotation of the current results that *Psidium guajava,* and *Vernonia amygdalina* leaves extracts might help reduce pathogenic microorganisms and help maintain the probiotic bacteria in the gut of the Wistar rats over an extended duration.

The differences in bacterial and fungal composition in the different groups of Wistar rats after 28 days reflect the dynamic changes in both microbiota. The abundance of the core commensals – lactic acid bacteria, *E. coli*, etc. in most groups, particularly A and B, was evenly distributed, which meant they were the dominating gut inhabitants of these groups. This persistence is supported by previously reported results that lactic acid bacteria play key roles in maintaining host immunity and intestinal homeostasis (Ekerette, 2015).

In certain groups (IC, ID, IID, IF and IIF), pathogenic microorganisms such as *Salmonella* spp. and *Proteus* spp. were not detected thus indicating possible clearance or suppression with time. Lactic acid bacteria can result in probiotic activity that diminishes pathogenic enterobacteriaceae as reported by Usman et al. (2025). This reduction could be due to host immune response or to competitive exclusion with commensals. Capsule-forming bacteria are sometimes not cleared and this is demonstrated by the presence of *Klebsiella* spp. in multiple populations.

Opportunistic fungi (*Candida albicans*, *Candida tropicalis, Candida parapsilosis* and *Candida glabrata*) were present in varying frequencies in the former (A, B, IIC) groups and not present in the latter (IF, IIF) groups. Consistent with research which shows that intestinal yeasts change with the nutrition and immune status of the host, this suggests micro-colonisation or immunity over time (Springer Nature, 2024). Two environmental moulds, the *Aspergillus niger* and the *Aspergillus flavus*, were only detected in early groups, giving weight to the hypothesis that they are more likely to be due to environmental contamination rather than to sustained colonisation.

In general, results indicate a shift in diversification of microbiota from early to later groups of fish, albeit with lactic acid bacteria prevailing throughout the fish. This change is a measure of the adaptability of helpful commensals and a way in which a helpful commensal infection can be lost in the future. These findings underscore the importance of monitoring the changes in gut microbes in Wistar rats to gain a deeper understanding of host-microbe dynamics and evaluate the impact of various treatments, such as dietary changes, probiotics, and antimicrobial agents.

### Implications for Research and Practice

Based on the findings of the investigation, the use of *Psidium guajava*, and *Vernonia amygdalina* leaves extracts as natural antibacterial agents is supported. Specifically, their selective activity against infections, with the presence at the same time of good flora, suggests their use in the treatment of digestive disorders and in reducing the use of synthetic antibiotics.

## CONCLUSION

Ethanolic extracts from *Vernonia amygdalina* and *Psidium guajava* have a selective effect on Wistar rats' gut microbiota. They have the ability to maintain good bacteria, lactic bacteria, while significantly reducing undesirable bacteria and fungi. These findings corroborate their applications in ethnomedicine and suggest potential applications in natural antibacterial therapies.

### Future Research

Further studies should investigate:

- Dose-response relationships and toxicity thresholds.
- Comparative effects of aqueous versus ethanolic extracts.
- Clinical trials to evaluate efficacy in human populations.
- Synergistic effects when combined with conventional antibiotics.

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