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Phytochemical Profiling and Anti-Diarrhoea Activity of a Bi-herbal Aqueous Extract of *Psidium guajava* and *Ocimum gratissimum*

Joseph Omorogiuwa Erhabor* and Oghenekevwe Otoberise

Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Edo State, Nigeria

*Corresponding author Email: joseph.erhabor@uniben.edu; Tel: +234(0) 807 797 9390

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ABSTRACT: Diarrhoeal diseases remain a global health challenge, with rising antimicrobial resistance driving the need for plant-based therapies. This study evaluated the phytochemical composition and anti-diarrhoeal activity of a bi-herbal aqueous extract derived from *Psidium guajava* and *Ocimum gratissimum*. The phytochemical analyses were performed using standard methods. The in vitro anti-diarrhoeal activity of the extract was assessed against selected diarrhoeagenic microorganisms (*Escherichia coli*, *Salmonella* sp., *Shigella dysenteriae*, and *Aspergillus niger*) using the agar well diffusion technique. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, phenolics, terpenoids, glycosides, reducing sugars, and proteins. Quantitative analysis showed that proteins (2013.00 mg/100 g) and tannins (698.20 mg/100 g) were the most abundant constituents, while flavonoids, saponins, and phenolics were present in moderate amounts. The bi-herbal extract exhibited concentration-dependent antimicrobial activity, with zones of inhibition ranging from 1.00 to 1.70 cm across the tested organisms. The MIC was 16.67 mg/mL for all isolates, while the MBC was greater than 50 mg/mL, indicating predominantly bacteriostatic activity. The bi-herbal extract showed rich phytochemistry and inhibitory activity against diarrhoeagenic pathogens, indicating potential as a natural antidiarrhoeal agent, though further in vivo and mechanistic studies are needed.

Keywords: Diarrhoea, Bi-herbal extract, Medicinal plants, Phytochemistry, Antimicrobial activity

Introduction

Diarrhoea is defined as an alteration in normal bowel movement characterised by an increase in the frequency, volume, and fluidity of stool output (Guerrant *et al.*, 2001). It remains a major global health challenge, particularly in developing countries, where it contributes significantly to morbidity and mortality, especially among children under five years of age (Shoba and Thomas, 2001). Despite advances in modern medicine, diarrhoeal diseases continue to impose a substantial public health burden, largely due to limited access to healthcare, poor sanitation, and the increasing emergence of antimicrobial resistance among enteric pathogens. However, there has been continued reliance on medicinal plants for the management of diarrhoeal diseases, underscoring their therapeutic relevance. Medicinal plants have long served as a valuable source of bioactive compounds, many of which have formed the basis of modern pharmacotherapy (Chaachouay and Zidane, 2024). In many developing regions, including sub-Saharan Africa, plant-based remedies remain the primary form of healthcare due to their accessibility, affordability, and perceived safety. Furthermore, international health bodies such as the World Health Organisation (WHO) have encouraged the scientific validation of traditional medicinal

practices for the treatment and prevention of diarrhoeal diseases (Maikere-Faniyo *et al.*, 1989). Recent studies have further reinforced the importance of medicinal plants as alternative therapeutic agents, particularly in combating multidrug-resistant pathogens and addressing the limitations of conventional antibiotics (Gonelimali *et al.*, 2018; Kanarek *et al.*, 2025).

The pharmacological potential of medicinal plants is largely attributed to their rich secondary metabolite content, including flavonoids, tannins, alkaloids, and saponins, which have been reported to exhibit antimicrobial and anti-diarrhoeal activities. These phytochemicals exert their effects through multiple mechanisms, such as disruption of microbial cell membranes, inhibition of nucleic acid synthesis, interference with enzymatic systems, and modulation of intestinal motility and secretion. Recent evidence indicates that plant-derived secondary metabolites demonstrate selective toxicity against microbial cells by targeting essential cellular processes, thereby enhancing therapeutic efficacy while minimising host toxicity (Latif and Nawaz, 2025). In addition, contemporary reviews have shown that the antidiarrhoeal activity of medicinal plants is strongly associated with the presence of bioactive constituents such as flavonoids, tannins, alkaloids, and saponins, which may act individually or synergistically to inhibit diarrhoeagenic pathogens and regulate gastrointestinal function (Damtie *et al.*, 2023). Notably, polyherbal formulations have attracted increasing attention due to their potential for synergistic interactions among phytoconstituents, which may enhance therapeutic efficacy compared to single-plant extracts. Such synergistic effects have been shown to improve antimicrobial potency and broaden the spectrum of activity against pathogenic microorganisms, making polyherbal approaches particularly relevant in ethnopharmacological research.

Psidium guajava L. (Myrtaceae) and *Ocimum gratissimum* L. (Lamiaceae) are widely used in traditional medicine for the treatment of gastrointestinal disorders, including diarrhoea. *P. guajava* leaves are rich in tannins and flavonoids, which are known for their astringent and antimicrobial properties. At the same time, *O. gratissimum* contains essential oils and phenolic compounds with established antimicrobial and anti-inflammatory activities. Although both plants have been individually reported to possess anti-diarrhoeal properties, there is limited scientific evidence on the combined (bi-herbal) effects of their aqueous extracts, particularly regarding their phytochemical composition and integrated antimicrobial and anti-diarrhoeal activities. Given the increasing global interest in plant-based therapeutics, the growing challenge of antimicrobial resistance, and the need for safe, effective, and affordable treatments for diarrhoeal diseases, it is imperative to validate the efficacy of bi-herbal formulations scientifically. Therefore, this study was designed to evaluate the phytochemical composition and anti-diarrhoeal activity of a bi-herbal aqueous extract of *Psidium guajava* and *Ocimum gratissimum*. Specifically, the study aimed to determine the qualitative and quantitative phytochemical constituents of the bi-herbal extract and assess their *in vitro* antimicrobial activity against selected diarrhoeagenic microorganisms.

Materials and methods

Collection of plant materials: The plant materials used in this experiment were the leaves of *Psidium guajava* L. and *Ocimum gratissimum*, collected from the Faculty of Life Sciences, University of Benin, Ugbowo, Edo State. The identification and authentication of these plants was done by Prof. H. A. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria with the voucher number UBH-P378 for *Psidium guajava* L. and voucher number UBH-O333 for *Ocimum gratissimum* L.

Preparation of crude extract: The plant samples were washed with sterile distilled water first to avoid contamination. The plant materials were chopped into pieces, air-dried for two (2) weeks and ground using a locally fabricated grinding machine. The extraction was performed by mixing 60 g each of the two powdered plant materials with 900 ml of distilled water, stirring and shaking continuously, and then straining through a cloth filter. The mixture was then concentrated into a solid form in an oven at 45 °C for 3 hours, after which a dry weight of 100g was recorded. It was then stored in a refrigerator for further use.

Phytochemical screening of the bi-herbal sample:

Qualitative phytochemistry: A mixture of 6 grams of the powdered samples of *Ocimum gratissimum* and *Psidium guajava* was used for the phytochemical analysis. The phytochemical examination of the plant extract was carried out using standard methods described by Tiwari *et al.* (2011), Sagayaraj *et al.* (2015), and Edeoga *et al.* (2005), with minor modifications.

Detection of alkaloids: This was done by first evaporating 2.0ml of the plant extract to dryness. Then the resultant residues were dissolved in 5 mL of HCl (2mol/dm³) and filtered. The filtrate was divided into two test tubes. To the first test tube, a few drops of Mayer's reagent were added, and the formation of a yellow-coloured

precipitate indicates the presence of alkaloids. The second test tube was treated with a few drops of Wagner's reagent, and the brownish-red precipitate formation indicates alkaloids.

Detection of glycoside: This was done by dissolving 0.5 mg of the extract in about 1 mL of water, then adding an aqueous NaOH solution. The formation of a yellow colour indicates the presence of glycosides.

Detection of tannins: To 1.0 ml of the extract, 1.0 ml of 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicates the presence of tannins.

Detection of phenols: This was done by treating 1.0 ml of the plant extract with 4 drops of ferric chloride solution. The formation of a bluish-black colour indicates the presence of phenols.

Detection of saponins: The foam test and froth test methods were used to detect saponins. In the foam test method, 0.5 g of the plant extract was shaken with 2.0 ml of distilled water. The formation of foam that persists for 10 minutes indicates the presence of saponins. In the froth test method, 5.0 mL of the extract was diluted to 20.0 mL with distilled water, and the mixture was shaken in a 50 mL graduated cylinder for 15 minutes. The formation of a 1cm layer of foam indicates the presence of saponins.

Detection of flavonoids: This was done using the alkaline reagent test and the lead acetate test. In the alkaline reagent test, the extract was treated with a few drops of a 2 mol/dm³ solution of sodium hydroxide. The formation of an intense yellow colour, which becomes colourless with the addition of dilute hydrochloric acid (2 mol/dm³), indicates the presence of flavonoids. In the lead test, the plant part extract was treated with a few drops of lead acetate solution. The formation of a yellow colour precipitate indicates the presence of flavonoids.

Detection of eugenols: About 2 mL of the extract was mixed with 5 mL of 5% KOH solution. The aqueous layer was separated and filtered. A few drops of HCl were added to the filtrate. A pale-yellow precipitate indicated a positive test.

Detection of steroids: 2 mL of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H₂SO₄. The colour changed from violet to blue or green in some samples, indicating the presence of steroids.

Detection of terpenoids: 0.2 g of the plant sample extract was mixed with 2 ml of chloroform (CHCl₃), and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish-brown colouration at the interface indicates the presence of terpenoids.

Quantitative phytochemistry

Determination of total phenolic content: The amount of total phenols in the extract was determined with Folin–Ciocalteu reagent according to the method of Lamuela-Raventós (2018), with slight modifications, using tannic acid as a standard. Briefly, 1.0 ml of extract solution (250 Ug/ml) was added to a test tube. Then, 1.0 ml of Folin–Ciocalteu reagent was added, and the contents of the flask were mixed thoroughly. After 5 min, 15.0 ml of Na₂CO₃ (20 %) was added, and the mixture was allowed to stand for 2 hours. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, UK). The total phenolic content was determined as Ug of tannic acid equivalent (TAE) using an equation obtained from the standard tannic acid calibration graph.

Determination of alkaloid content: The total alkaloid content was measured using the method described by Harborne (1973). 5 g of the extract was weighed into a 250 mL beaker, and 100 mL of 20 % acetic acid in ethanol was added; the mixture was then covered and allowed to stand for 2 h. This was filtered, and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected by filtration, washed with 1% ammonia solution, dried and weighed. All samples were analysed in triplicate.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of residue} \times 100}{\text{Weight of sample}}$$

Flavonoid content determination: The flavonoid content was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) (Ilahy *et al.*, 2011). Thirty-microliter aliquots of the extract were used for flavonoid determination. Samples were diluted with 90 µL methanol, 6 µL of 10 % Aluminium chloride (AlCl₃), 6 µL of 1 mol/L Sodium acetate (CH₃CO₂Na), and, finally, 170 µL of methanol was added. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard to calculate flavonoid content (Ug Qe/g).

Estimation of total saponin content: The total saponin content was estimated using the method described by Makkar *et al.* (2007), based on the vanillin-sulphuric acid colourimetric reaction, with some modifications. About 50 µL of plant extract was added to 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800 mg of vanillin in 10 ml of 99.5% ethanol) was added. Then 2.5 ml of 72% sulphuric acid was added, and the mixture was mixed well. This solution was kept in a water bath at 60 °C for 10 min. After 10 min, it was cooled in ice-cold water, and the absorbance was read at 570 nm. 0-25 ppm standard saponin solutions were prepared from the saponin stock solution. The standard solutions were treated in the same manner as the test samples. The values were expressed as ppm.

Estimation of tannin content: Exactly 0.20 mL of sample was added to 20 mL of 50% methanol, placed in a water bath at 77 - 80 °C for 1 h, and shaken. The extract was quantitatively filtered using a double-layered

Whatman No. 1 filter paper. 20 mL of distilled water, 2.5 mL of Folin-Denis reagent, and 10 mL of 17% Na₂CO₃ were added and mixed. The mixture was allowed to stand for 20 min. A series of standard tannic acid solutions was prepared in methanol, and their absorbance, as well as the samples, was measured after colour development on a UV/Visible spectrophotometer at 760 nm. Total tannin content was calculated from the calibration curve.

In vitro anti-diarrheal activity: The antimicrobial activity of the crude extract was determined using the method described by NCCLS (2002) with slight modifications. The pre-identified test microorganisms (local clinical isolates, including *Escherichia coli*, *Shigella dysenteriae*, *Salmonella* sp., and *Aspergillus niger*) were obtained from the Microbiology Laboratory at the University of Benin. The antimicrobial activity of the bi-herbal extract was tested against the selected microorganisms using the Agar well diffusion technique as outlined by NCCLS (2002). The bi-herbal extract concentration ranges from 12.50 mg/mL to 50.00 mg/mL. The controls included 30 µg/ml ampicillin for the bacterial culture and 30 µg/ml ketoconazole for the fungal culture, with sterile distilled water as the negative control. The minimum inhibitory concentration (MIC) of the extracts was determined using the two-fold serial microdilution method with saline solution. The MIC values were taken as the lowest extract concentration in the wells that showed no turbidity after the appropriate incubation time against the respective microorganisms. The turbidity of the wells in the plate was interpreted as visible microbial growth.

Data analysis: All data were presented as mean±SEM. The groups' means were compared using one-way analysis of variance (ANOVA), followed by Dunnett's test; P < 0.05 was considered significant.

Results

Phytochemical analysis of the biherbal sample: The qualitative phytochemical analysis in Table 1 revealed that in the aqueous extract of the biherbal (*Ocimum gratissimum* and *Psidium guajava*) sample, the following phytochemicals were present: glycosides, saponins, phenolics, terpenoids, steroids, alkaloids, flavonoids, reducing sugars, tannins, and proteins. Eugenols and steroids were absent.

Table 1: Results of the qualitative phytochemical screening for the bi-herbal sample.

S/N	Parameters	Test Methods	Inference
1	Glycosides	General Test	+
2	Saponins	Frothing Test	++
3	Phenols	Ethanol/Ferric Chloride	+
4	Eugenols	Ethanol/Ferric Chloride	-
5	Terpenoids	Salkowski Test	+
6	Steroids	KOH test	-
7	Alkaloids	Picric Acid	+
8	Flavonoids	Lead Acetate	+
9	Tannins	Ferric Chloride	+++
10	Reducing Sugar	Fehling's A and B	+
11	Protein	NaOH/CuSO ₄	+

For the quantitative phytochemical analysis, metabolites, proteins (2013 mg/100g), and tannins (698 mg/100g) had the highest quantities, respectively (Table 2). Phenol, saponin and flavonoids had considerable quantities ranging between 38 and 74 mg/100 g

Table 2: Results of the quantitative phytochemical analysis of the bi-herbal (*Ocimum gratissimum* and *Psidium guajava*) sample

S/N	Parameters	Unit	Quantitative Phytochemistry
1	Alkaloids	%	7.01±0.76
2	Tannins	mg/100g	698.20±20.67
3	Phenolic	mg/100g	37.74±1.93
4	Saponins	mg/100g	46.06±1.42
5	Flavonoids	mg/100g	74.42±6.23
6	Protein	mg/100g	2013.00±25.54

Result expressed in mean ± SEM, n=3

In vitro- antidiarrheal activity of the Bi-herbal extract: The minimum inhibitory concentration (MIC) of the bi-herbal extract against the tested microbial isolates is presented in Table 3. The extract exhibited concentration-dependent antimicrobial activity against both bacterial and fungal organisms. Among the bacterial isolates, *Escherichia coli* showed inhibition zones ranging from 1.50 ± 0.00 cm at 50 mg/mL to 1.10 ± 0.01 cm at 16.67 mg/mL, with no inhibition at 12.5 mg/mL. Similarly, *Salmonella* sp. and *Shigella dysenteriae* exhibited measurable inhibition at 50 mg/mL, 25 mg/mL, and 16.67 mg/mL, but activity was absent at 12.5 mg/mL. The fungal isolate *Aspergillus niger* demonstrated the highest susceptibility to the extract, producing inhibition zones of 1.70 ± 0.10 cm, 1.40 ± 0.40 cm, and 1.10 ± 0.01 cm at 50 mg/mL, 25 mg/mL, and 16.67 mg/mL, respectively. Overall, antimicrobial activity declined with decreasing extract concentration, and the absence of inhibition at 12.5 mg/mL across all isolates indicates that the minimum inhibitory concentration (MIC) of the bi-herbal extract was approximately 16.67 mg/mL for the tested microorganisms.

Table 3: Minimum inhibitory concentration (MIC) of the bi-herbal extract expressed as zones of inhibition (cm) against tested microbial isolates

Microorganisms	Positive Control (cm)	Negative Control (cm)	50.00 mg/mL	25.00 mg/mL	16.67 mg/mL	12.50 mg/mL	MIC (mg/mL)
<i>Escherichia coli</i>	1.90 ± 0.10^a	0.00 ± 0.00	1.50 ± 0.00^b	1.20 ± 0.01^b	1.10 ± 0.01^b	0.00 ± 0.00	16.67
<i>Salmonella</i> sp.	0.00 ± 0.00^a	0.00 ± 0.00	1.30 ± 0.00^b	1.10 ± 0.11^b	1.00 ± 0.00^b	0.00 ± 0.00	16.67
<i>Shigella dysenteriae</i>	1.80 ± 0.08^a	0.00 ± 0.00	1.30 ± 0.30^b	1.10 ± 0.02^b	1.00 ± 0.00^b	0.00 ± 0.00	16.67
<i>Aspergillus niger</i>	2.00 ± 0.20^a	0.00 ± 0.00	1.70 ± 0.10^b	1.40 ± 0.40^b	1.10 ± 0.01^b	0.00 ± 0.00	16.67

Values are expressed as Mean \pm SEM (n = 3). Different superscripts indicate statistically significant differences between treatments ($p \leq 0.05$). Positive control for bacterial isolates- ampicillin, positive control for fungal isolates- ketoconazole, negative control-distilled water.

The results of the minimum bactericidal concentration (MBC) of the aqueous bi-herbal extract against the tested bacterial isolates are presented in Table 4. The MBC assay revealed that none of the tested concentrations of the extract (12.5–50 mg/mL) completely inhibited bacterial growth in the tested organisms. In *Escherichia coli*, microbial growth was observed at all extract concentrations (50 mg/mL, 25 mg/mL, 16.67 mg/mL, and 12.5 mg/mL), indicating that the extract lacked bactericidal activity within the tested concentration range. Similarly, *Salmonella* sp. showed visible growth across all tested concentrations, suggesting that the extract did not achieve bactericidal effects under the experimental conditions. A comparable trend was observed for *Shigella dysenteriae*, where bacterial growth persisted at all tested extract concentrations. Consequently, the minimum bactericidal concentration for all tested bacterial isolates was observed to be greater than 50 mg/mL, the highest concentration evaluated in this study.

Table 4: Minimum Bactericidal Concentration (MBC) of the aqueous Bi-herbal extract against the tested bacterial isolates

Microorganisms	Positive Control	Negative Control	50.00 mg/mL	25.00 mg/mL	16.67 mg/mL	12.50 mg/mL	MBC (mg/mL)
<i>Escherichia coli</i>	NG	G	G	G	G	G	>50
<i>Salmonella</i> sp.	G	G	G	G	G	G	>50
<i>Shigella dysenteriae</i>	NG	G	G	G	G	G	>50

Key: G = Growth observed; NG = No growth observed; Positive control – 30 µg/ml ampicillin, negative control-distilled water

Discussion

Infectious diarrheal diseases are the second leading cause of morbidity and mortality worldwide. (LeDuc and Hughes, 1999). In the search for newer remedies for infectious diarrhoea and dysentery, this study aimed to investigate the bioactive constituents and antidiarrheal activities of *Ocimum gratissimum* and *Psidium guajava* against a range of microbial species associated with diarrhoea and dysentery. The antimicrobial activity

observed in the present study may be attributed to the presence of several bioactive phytochemicals identified in the bi-herbal extract. Plant secondary metabolites, such as flavonoids, tannins, alkaloids, and saponins, have been widely reported to exhibit antimicrobial activity through diverse mechanisms, including disruption of microbial cell membranes, inhibition of nucleic acid synthesis, and interference with microbial metabolic pathways (Cowan, 1999; Gonelimali *et al.*, 2018).

Qualitative phytochemical screening carried out in this study reveals that the aqueous extracts contain saponins, phenols, alkaloids, flavonoids, glycosides, eugenols, terpenoids, steroids, and reducing sugars. The presence of these phytochemicals could be due to their polarity, which makes them readily soluble in water. According to previous research by Omidiwura (2017), the presence or absence of metabolites may be due to differences in solvent polarity during extraction. The combined presence of these phytochemical classes in the bi-herbal formulation likely results in synergistic antimicrobial effects, thereby enhancing the extract's inhibitory activity against pathogenic microorganisms (Gonelimali *et al.*, 2018). This synergistic interaction among phytochemical constituents may therefore explain the broad-spectrum antimicrobial activity observed against both bacterial isolates (*Escherichia coli*, *Salmonella* sp., and *Shigella dysenteriae*) and the fungal isolate (*Aspergillus niger*) in the present study.

The antimicrobial activity study revealed that the aqueous extract of the combined leaves of *Ocimum gratissimum* and *Psidium guajava* possessed inhibitory activity against the tested pathogenic strains that cause diarrhoea and dysentery. The *in vitro* antimicrobial activity of the bi-herbal leaf extract was lower than that of ampicillin and ketoconazole, the standards, as indicated by the zone of inhibition range (1.80–2.00 cm) against the tested isolates. Additionally, the results of the study indicated that the aqueous extract of *Ocimum gratissimum* and *Psidium guajava* leaves exhibited antimicrobial activity against all the isolates (*Escherichia coli*, *Shigella dysenteriae*, *Salmonella* sp and *Aspergillus niger*). The positive controls (ampicillin and ketoconazole) showed the highest zones of inhibition against *Escherichia coli*, *Shigella dysenteriae*, and *Aspergillus niger*, respectively, whereas the bi-herbal aqueous extract exhibited the best zone of inhibition against *Escherichia coli* and *Aspergillus niger*, measuring 1.5 cm and 1.7 cm, which aligns with previous reports by Suresh *et al.* (2008) using the same agar well diffusion method. The minimal inhibitory concentration (MIC), the lowest concentration at which a substance inhibits the growth of a microorganism, was determined for the tested microorganisms. The minimum inhibitory concentration of the bi-herbal extract against *E. coli*, *Salmonella* sp., *Shigella dysenteriae*, and *Aspergillus niger* was approximately 16.67 mg/mL, as no measurable inhibition occurred at 12.5 mg/mL. This MIC result is similar to that reported by Ouedraogo *et al.* (2024). The minimal bactericidal concentration (MBC) is the lowest concentration of a substance that can completely kill bacteria. The MBC of the bi-herbal extract against *E. coli*, *Salmonella* sp., and *Shigella dysenteriae* was greater than 50 mg/mL, indicating that the extract exerted bacteriostatic rather than bactericidal effects at the tested concentrations. The positive control had a bacteriocidal effect on the three bacterial isolates, while the test treatment had no bacteriocidal effect at the following concentration on any of the bacteria. These findings on the antimicrobial activity of the bi-herbal extract suggest that although it demonstrated inhibitory activity in the MIC assay, its antimicrobial effect may be primarily bacteriostatic rather than bactericidal at the tested concentrations. The persistence of microbial growth following subculture indicates that the extract likely inhibits microbial proliferation without eliminating the organisms at the tested concentrations. The antibacterial activity of plant leaves, especially when aqueous extracts are used as solvents, has been shown to be effective in eradicating microbes. This study showed that aqueous extracts exhibited antimicrobial activity. The observed antimicrobial activity may be attributed to the synergistic effects of bioactive phytochemicals, particularly flavonoids, tannins, and alkaloids, which are known to disrupt microbial cell membranes and inhibit essential metabolic processes. The results of this study are consistent with those of Ogbueke *et al.* (2000), who found that aqueous and ethanolic extracts of plant seeds also contain active ingredients that are responsible for their antimicrobial effects.

Conclusion

In summary, given that the aqueous extract exhibited antidiarrheal activity following the antimicrobial activity, the extract could be useful as a possible treatment for diarrhoea. Based on these findings, *Psidium guajava* L. and *Ocimum gratissimum* leaves could be a potential source for a novel 'lead' discovery for antidiarrheal drug development.

References

- Chaachouay N, Zidane L: Plant-derived natural products: A source for drug discovery and development. *Drugs Drug Candidates*, 3: 184-207. 2024.
- Cowan MM: Plant products as antimicrobial agents. *Clin Microbiol Rev*, 12: 564-582. 1999.
- Damtie D: Review of medicinal plants traditionally used to treat diarrhea by the people in the Amhara region of Ethiopia. *Evid Based Complement Alternat Med*, 2023: 8173543. 2023.
- Edeoga HO, Okwu DE, Mbaebie BO: Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol*, 4: 685-688. 2005.
- Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR: Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front Microbiol*, 9: 1639. 2018.
- Guerrant RL, Van Gilder T, Steiner TS, Thielman NM, Slutsker L, Tauxe RV: Practice guidelines for the management of infectious diarrhea. *Clin Infect Dis*, 32: 331-351. 2001.
- Ilahy R, Hdider C, Lenucci MS, Tili I, Dalessandro G: Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. *J Food Compos Anal*, 24: 588-595. 2011.
- Kanarek P, Breza-Boruta B, Stocki M: Antimicrobial activity and phytochemical profiling of natural plant extracts for biological control of wash water in the agri-food industry. *Appl Sci*, 15: 5199. 2025.
- Lamuella-Raventós RM: Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. In: *Measurement of Antioxidant Activity and Capacity: Recent Trends and Applications*. Apak R, Capanoglu E, Shahidi F (eds.) John Wiley & Sons, New York, pp. 107-115. 2018.
- Latif R, Nawaz T: Medicinal plants and human health: A comprehensive review of bioactive compounds, therapeutic effects, and applications. *Phytochem Rev*, 25: 2299-2342. 2025.
- LeDuc JW, Hughes JM: Surveillance for emerging infectious diseases. In: *Tropical Infectious Diseases: Principles, Pathogens and Practice*. Guerrant RL, Walker DH, Weller PF (eds.) Churchill Livingstone, Philadelphia, pp. 251-260. 1999.
- Maikere-Faniyo R, Van Puyvelde L, Mutwewingabo A, Habiyaremye FX: Study of Rwandese medicinal plants used in the treatment of diarrhea I. *J Ethnopharmacol*, 26: 101-115. 1989.
- Makkar HP, Siddhuraju P, Becker K: Plant secondary metabolites. Humana Press, Totowa, pp. 1-130. 2007.
- NCCLS: Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A2. Clinical and Laboratory Standards Institute, Wayne. 2002.
- Ogueke CC, Ogbulie JN, Njoku HO: Antimicrobial properties and preliminary phytochemical analysis of ethanolic extracts of *Alstonia boonei*. *Niger J Microbiol*, 20: 896-899. 2006.
- Omidiwura BRO: Qualitative and quantitative analysis of pawpaw (*Carica papaya*) leaf extract and its antimicrobial effect in animal production. *Niger J Anim Prod*, 44: 78-83. 2017.
- Ouedraogo A, Nikiema PA, Nikiema MEM, Yameogo GJ, Sourabie PB, Bassave BRH, Nikiema O, Gombri WBF, Barro N: Phytochemical properties and antimicrobial activities of *Carica papaya* and *Balanites aegyptiaca* seed aqueous extracts. *J Drug Deliv Ther*, 14: 46-52. 2024.
- Sagayaraj MI, Britto SJ, Arulappan MT, Krishnakumar J, Thomas S, George M: Antimicrobial studies and phytochemical screening of the leaves of *Stephania japonica* and *Cocculus hirsutus*. *Eur J Biomed Pharm Sci*, 2: 201-210. 2015.
- Shoba FG, Thomas M: Study of antidiarrheal activity of four medicinal plants in castor oil induced diarrhea. *J Ethnopharmacol*, 76: 73-76. 2001.
- Suresh K, Deepa P, Harisaranraj R, Vaira Achudhan V: Antimicrobial and phytochemical investigation of selected medicinal plants including *Psidium guajava*. *Ethnobot Leaflets*, 12: 1184-1191. 2008.
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H: Phytochemical screening and extraction: A review. *Int Pharm Sci*, 1: 98-106. 2011.