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IN VITRO ANTIBACTERIAL ACTIVITIES OF SELECTED MEDICINAL PLANTS AGAINST DIARRHOEA CAUSING PATHOGENS

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Abstract

Diarrhea has been a major cause of death, especially among children in developing countries. Traditional preparations of medicinal plants with antimicrobial activities have been extensively used in West African regions. This study was conducted to determine the antibacterial activities of selected medicinal plants against pathogens that cause diarrhea. Fresh leaves of three medicinal plants, namely Anogeissus leiocarpus, Vernonia amygdalina, and Piliostigma thonningii, were collected in Minna, Niger State. Pathogens causing diarrhea (Vibrio cholerae, Klebsiella pneumoniae, and Salmonella enterica serovar) were isolated and identified from stool samples of individuals suffering from diarrhea. The plant material was extracted using the maceration technique with methanol. The antibacterial effectiveness of the selected plant extracts was evaluated using the agar well diffusion method on Muller Hinton agar. The minimum inhibitory concentration was determined using the Broth tube dilution method, while the minimum bactericidal concentration was determined by plating out on nutrient agar plates with no visible growth. The results indicated that all plant extracts effectively suppressed the microbial growth of diarrhea-causing bacteria with varying potency. Among them, the extract of Anogeissus leiocarpus consistently exhibited the largest inhibitory zone diameters across all three bacterial strains at a concentration of 300 mg/mL (Vibrio cholerae: 32.33 mm, Klebsiella pneumoniae: 34.00 mm, and Salmonella enterica serovar Kentucky: 36.33 mm). This extract can be considered the most active. For Anogeissus leiocarpus, the MIC values ranged from 0.19 to 0.96 mg/mL, and the MBC values ranged from 0.20 to 1.09 mg/mL. For Vernonia amygdalina, the MIC values ranged from 0.96 to 1.80 mg/mL, and the MBC values ranged from 1.20 to 1.920 mg/mL. The results of this study validate the traditional use of these plants in medicine. However, further studies, including the isolation and identification of active compounds

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would be necessary to fully understand the mechanisms behind the observed antimicrobial effects.

Keywords: Diarrhea; Medicinal Plants; Maceration Technique; Agar Well Diffusion Method; Broth Tube Dilution Method.

INTRODUCTION

Diarrhea is characterized by the frequent passage of loose or watery stools, occurring at least three times a day (Sweetser 2012). It can also involve more frequent bowel movements with a total volume exceeding 200 mL or 200 g within 24 hours. Despite being preventable, diarrhea affects a significant portion of the global population and contributes to 5% of health-related issues and 4% of total global deaths (Blaser, Deane, and Fruhwald 2015). Annually, approximately 2.2 million people lose their lives to diarrhea, with a major portion of these deaths attributed to bacterial infections. Notable bacterial culprits like *Vibrio cholerae, Clostridium difficile*, various Shigella species, *Escherichia coli, Pseudomonas aeruginosa*, and Salmonella species are responsible for causing different types of diarrhea (Shane et al. 2017).Diarrhea is one of the leading causes of morbidity and mortality worldwide, especially in developing countries in sub-Saharan Africa and South East Asia (Boschi-Pinto, Velebit, and Shibuya 2008). Most diarrhea-associated morbidities and mortalities occur in low-income and medium-income countries, usually in rural areas as well as in the suburbs and slums of urban areas (Mohammed and Tamiru 2014). In these settings, the incidence is further fueled by the vicious cycles of poverty, ignorance, malnutrition, and endemic infectious diseases. Issues directly or remotely connected to socio-environmental factors such as sanitation and quality of water, unhygienic feeding practices (including hand hygiene), suboptimum breastfeeding, zinc deficiency, and barriers to appropriate and affordable health care exist as catalysts for diarrheal disease burden among under-five in these parts of the world (Omona et al. 2020; Walker et al. 2013).

Large numbers of plant species have been documented for the treatment of various ailments and serve as remedies for human diseases because they contain chemical components of therapeutic value (Shakya 2016). Nigeria is blessed with a large number of plant species such as *Acacia nitolitica, Carica papaya, Khaya senegalenses, Ficus sycomorus, and Piliostigma thonningii*, some of which have been in use for centuries to diagnose, prevent and treat various ailments. The exploration of newer antimicrobial agents in plants brings about a different approach to minimizing antibiotic resistance and thus offers potential benefits.

The medicine quest focuses on the drug of the future that will be derived from natural products. The search for unfamiliar plants in the wild with potential value as human and animal food, as well as curative medicine, is gathering momentum (Anand et al. 2019). The derivatives of these plants are claimed to have several medicinal and other desirable properties (Vaou et al. 2021). Furthermore, the nontoxic nature of most chemicals in plants, positive healthy properties, consumer perception, and acceptance of their use have been well demonstrated. There are estimated 250,000–500,000 species of plants on Earth. A relatively small percentage (1–10%) of these is consumed as food by both humans and animal species. A greater number may be used for medicinal purposes. (Rates 2001). People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants. Currently, antimicrobial plant extracts are of special interest to chemists and microbiologists due to growing public awareness of the negative effects of the overuse of antibiotics and disinfectants. Hence, the present study was conducted to evaluate the in vitro antibacterial activities and synergistic effect of the bioactive compounds of selected medicinal plants against diarrhea-causing pathogens.

METHODS

Study Area

The study was carried out in General Hospital Minna, Niger State, Nigeria. Which is the major hospital attended by the populace. Minna is a city in Middle Belt Nigeria, consisting of 2 Major ethnic groups: Nupe and Gwari. Minna Lies between Latitude 9.58360 N and Longitude 6.54630 E at an altitude of 256m above sea level and has a land area of about 88 km2 (www.minna.climatetemps.com/map.php).

Sources of Media, Solvents, and Reagents

All chemicals and reagents used for the analysis were of analytical grade (alanar) manufactured by British drug house limited. The media were oxoid products.

Plant Sample collection and identification

Fresh leaves of three medicinal plants *Anogeissus leiocarpus*, *Vernonia amygdalina*, and *Piliostigma thonningii* was collected in Minna, Niger State. The plants were identified at the Department of Plant Biology School of Life Sciences, Federal University of Technology Minna, Niger State.

Table 1: Three Medicinal Plants

Botanical name	Voucher	Common name
Anogeissus leiocarpus	(KSUSTA/PSB/H/84DC)	Marke
Vernonia amygdalina	(KSUSTA/PSB/H/42B)	Shuwaka
Piliostigma thonningii	(KSUSTA/PSB/H/109L)	Kalgo



Figure 1: *Anogeissus leiocarpus leaves*



Figure 2: *Vernonia amygdalina leaves*



Figure 3: *Piliostigma thonningii leaves*

Isolation and identification of Diarrhoea causing Pathogens

Sterile sample bottles were employed to collect stool samples from individuals suffering from diarrhea. These samples were then carefully packed with ice packs to maintain their

integrity during transportation to the microbiology laboratory at the Center for Genetic Engineering and Biotechnology, Federal University of Technology, Minna. Upon arrival, the collected samples were inoculated onto MacConkey agar, Eosin Methylene Blue (EMB) agar, and Thiosulfate Citrate Bile-Salts Sucrose (TCBS) agar. Incubation at a temperature of 37°C was carried out for a duration of 24 hours. Subsequently, distinct colonies that developed were subjected to repeated sub-culturing to obtain pure cultures of the pathogens responsible for diarrhea. These pure cultures were preserved on nutrient agar slants for future use. The suspected bacterial pathogens associated with causing diarrhea were identified using various conventional methods, including assessing colony morphology and performing biochemical tests. These tests encompassed procedures such as Gram staining, Oxidase test, Voges-Proskauer test, Indole test, Methyl Red test, Citrate test, Catalase test, Urease test, and Motility test.

Extraction of the Crude Extracts

The process of extracting compounds from the plant followed the procedure outlined in the AOAC method of 2016. Various parts of the plant were air-dried in the shade at room temperature for a minimum of 7 days. After drying, these parts were separated and then crushed into coarse powder using a mechanical grinder. A quantity of 100g of this powder was precisely measured and soaked in 500 ml of methanol in a 1:5 (weight-to-volume) ratio. The mixture was allowed to macerate for 72 hours. Subsequently, the resulting liquid portion was filtered using Whatman No. 1 filter paper, and the filtrate was then subjected to evaporation under reduced pressure (204 bar) at a temperature of 40°C until it became dry.

In vitro Antimicrobial Assay

The antibacterial effectiveness of the chosen plant extracts was evaluated using the agar well diffusion method on Muller Hinton agar. To initiate the experiment, a suspension of the target microorganisms was prepared in peptone water until it reached a turbidity equivalent to 0.5 McFarland standard. This suspension was then inoculated onto Mueller Hinton agar. Utilizing a sterile cork borer, agar wells measuring 8mm in diameter were created under aseptic conditions. Into each of these wells, the extract solutions were introduced at their designated concentrations (50, 70, and 100mg/ml). Following this, the agar plates were incubated at a temperature of 37°C for a duration of 24 hours. The

assessment of the extracts' antimicrobial potential was carried out following the guidelines set by the Association of Official Analytical Chemists (AOAC) in 2010.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Based on the results of the antibacterial testing, the most efficient extract was chosen to identify its MIC and MBC. The Broth tube dilution method as described by (Srikacha and Ratananikom 2020) was used. Serial dilution was adopted to prepare various concentrations of the selected extracts in test tubes, which were inoculated with the standardized number of organisms and incubated at 370C for 24 hours. The lowest concentration (highest dilution) of the extract with no visible growth of the test organism was considered to be the MIC. The MBC was determined by plating the contents of the MIC tubes onto nutrient agar plates. The lowest concentration of extract with minimum growth of the test organism on the agar plate was considered the MBC.

(CLIS 2020).

Statistical Analysis

The data was evaluated using analysis of variance (ANOVA) and was presented as the mean value ± SEM (standard error of the mean). Differences among the means for the groups were assessed using Duncan's Multiple Range Test to determine which mean values were significantly different at $p<0.05$.

RESULTS

Yields of plant extract

Table 2 illustrated the extraction yields of plant extracts in grams (g) obtained during the study. The plant species studied are *Anogeissus leiocarpus, Vernonia amygdalina*, and *Poliostigma thoningii*. *Anogeissus leiocarpus* has the highest extraction yield (22.87 g), *while Poliostigma thoningii* has the lowest extraction yield (22.80 g) among the three species.

Table 2: Yield of Extract from Plants used in this study

Plants	Extraction Yield (g)
Anogeissus leiocarpus	22.87
Vernonia amygdalina	22.86
Poliostigma thoningii	22.80

Minimum Inhibitory and Minimum Bactericidal Concentrations of effective plant extract

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of plant extracts against different bacterial strains are given in table 3. For *Anogeissus leiocarpus* MIC values range from 0.19 to 0.96 mg/mL and MBC values range from 0.20 to 1.09 mg/mL. For *Vernonia amygdalina* MIC values range from 0.96 to 1.80 mg/mL and MBC values range from 1.20 to 1.920 mg/mL.

Table 3: Minimum Inhibitory and Minimum Bactericidal Concentrations of Most Active Extract

Plant Extract	MIC (mg/mL)			MBC (mg/mL)		
	Vibrio cholerae	*Klebsiella pneumoniae*	*Salmonella enterica serovar Kentucky*	*Vibrio choleae*	*Klebsiela Pneumonia*	*Salmonella enterica serovar Kentucky*
Anogeissus leiocarpus	0.96±0.03	0.19±0.00	0.96±0.06	1.09±0.87	0.20±0.05	1.04±0.17
Vernonia amygdalina	0.96±0.04	0.96±0.05	1.80±0.17	1.20±0.12	1.40±0.29	1.920±0.23
Control	0.005	0.005	0.020	0.005	0.005	0.020

Values are presented as mean ± standard error of the mean (SEM) of triplicates

MIC = Minimum Inhibitory; MBC = Minimum Bactericidal Concentrations

Inhibitory Effects of Methanolic Crude Extracts Against Different Bacteria (mm).

Table 4 presents crucial experimental data on the inhibitory effects of methanolic crude extracts from various plant species against different bacterial strains using different concentrations (200 mg/mL, 250 mg/mL, and 300 mg/mL).

Table 4: Inhibitory Effects of Methanolic Crude Extracts Against Bacteria (mm).

Extract	Concentration (mg/mL)	*Vibrio choleraee*	*Klebsiella pneumoniaee*	*Salmonella enterica serovar Kentucky*
Vernonia amygdalina	200	24.00±1.15^{c}	16.33±0.60^{cd}	12.00±0.58^{bc}
	250	28.00±0.58^{d}	18.00±1.15^{d}	14.33±0.60^{cd}
	300	29.00±0.15^{d}	24.00±1.44^{e}	16.50±0.29^{de}
Anogeissus leiocarpus	200	28.00±0.58^{d}	18.00±1.15^{d}	28.17±0.60^{h}
	250	32.00±1.15^{e}	22.00±0.58^{e}	32.00±1.73^{i}
	300	32.33±0.73^{e}	34.00±1.15^{f}	36.33±1.17^{j}
Poliostigma thoningii	200	0.00±0.00^{a}	12.50±0.29^{b}	8.50±0.29^{a}
	250	0.00±0.00^{a}	14.00±0.58^{bc}	10.00±0.29^{ab}
	300	0.00±0.00^{a}	24.00±1.15^{e}	12.00±0.58^{bc}
Control	8mg/ml	24.21±0.82	12.03±13	32.07±1.43

Keys: Values are presented as mean ± standard error of the mean (SEM) of triplicates

Values with different superscripts in a column are significantly different at $p < 0.05$.

DISCUSSION

Three plant species were investigated to evaluate their antibacterial activity against diarrhea causing bacteria including *Vibrio cholerae, Klebsiella pneumoniae*, and *Salmonella enterica serovar Kentucky*. Diarrhea is characterized by rapid and frequent passage of semisolid or liquid

fecal material. It involves decreased absorption of fluid, increased motility of the intestinal tract, and increased secretions. In addition, diarrhea leads to loss of electrolytes, particularly sodium (Na+) and water, and finally, end up with dehydration and death (Elmi and Dioso 2017; Sarin and Bafna 2012).

The extraction yields from the plants used in the study show relatively similar values, with *Anogeissus leiocarpus* and *Poliostigma thoningii* yielding slightly higher amounts compared to *Vernonia amygdalina*. Extraction is a very important first step in the analysis of medicinal plant properties because the choice of solvent influences the types of compounds that can be extracted and ultimately, the biological activities imparted by the extracted compounds (Masoko et al. 2008). Methanol was used as a macerating solvent for plant extraction and it was similar to other laboratory works done by (Kefe et al. 2016; Saadu and Shamsudeen 2022; Taye et al. 2011).

The results revealed that all plant extracts were effective in suppressing microbial growth of diarrhea-causing bacteria with variable potency. *Anogeissus leiocarpus* extract consistently demonstrates the largest inhibitory zone diameters across all three bacterial strains and various concentrations. It can be considered the most active extract. This finding was in line with a study done on the leaf extract of *Anogeissus leiocarpus* on some bacteria associated with diarrhea (Zumbes, Belenu, and Onwuliri 2007). Also in another study, the ethanol extract of stem bark of *Anogeissus leiocarpus* inhibited the growth of standard strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* (Kubmarawa et al. 2007). The inhibitory zone diameters generally increase with higher concentrations of the plant extracts. This suggests a concentration-dependent relationship between extract concentration and bacterial growth inhibition. *Anogeissus leiocarpus* extract consistently demonstrates the largest inhibitory zone diameters across all concentrations and bacterial strains tested, indicating its strong antimicrobial potential. The potency of the extracts varies depending on the bacterial strain. For example, *Anogeissus leiocarpus* is more effective against *Salmonella enterica serovar Kentucky* compared to other strains.

Anogeissus leiocarpus generally demonstrates lower MIC and MBC values compared to *Vernonia amygdalina* across the tested bacterial strains. The relatively higher MIC and MBC values for *Vernonia amygdalina* could indicate that this extract might require higher concentrations to achieve the same level of inhibition and bactericidal activity compared to *Anogeissus leiocarpus*. *Anogeissus leiocarpus* extract appears to be more effective against the

tested bacterial strains, based on the lower MIC and MBC values. *Vibrio cholerae* seems to be more sensitive to the extracts, as indicated by relatively lower MIC and MBC values compared to the other strains. The differences observed in the MIC and MBC values documented in these research studies might stem from variations in the phytochemical makeup of their individual extracts. This discrepancy is typically influenced by the polarity of the solvents employed during the extraction process. The type of solvent used tends to impact the type of bioactive compounds liberated from the plant materials, potentially leading to these variations(Altemimi et al. 2017).

CONCLUSION

From this study, we can conclude that exhibits varying antimicrobial activity of different plant extracts against diarrhea causing bacterial strains. *Anogeissus leiocarpus* extract shows promising antimicrobial activity, demonstrating the largest inhibitory zone diameters and lower MIC/MBC values compared to other plant extracts. The results of this study validate the use of these plants in traditional medicine in the treatment of gastrointestinal disorders such as dysentery and diarrhea and suggest that at least part of their action is due to their antibacterial property. However, further studies, including isolation and identification of active compounds, would be necessary to fully understand the mechanisms behind the observed antimicrobial effects.

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