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ORIGINAL RESEARCH ARTICLE

Antibacterial Activities of Three (3) Selected Medicinal Plants against Diarrhea Causing Pathogens.

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ABSTRACT
Diarrhoea is one of the leading causes of morbidity and mortality worldwide, especially in developing countries in sub-Saharan Africa and Southeast Asia. Extracts from *Acacia nilotica (Bagaruwa), Carica papaya* (Gwanda) and *Khaya senegalensis* (Marke) were prepared using the cold maceration method. Well diffusion method was used on Muller Hinton agar and thiosulfate citrate bile salt agar (TCBS), and suspension of the test organisms was prepared in peptone water to match 0.5 McFarland standard, each extract solution at 200, 250 and 300mg/ml, was introduced into respective wells, incubated at 37^{o}C for 24 hours. Ciprofloxacin, at 8 mg/ml, was used as a control. Plant crude extracts of *C. papaya* yielded (19.05g), *A. nilotica* (18.67g) and *K. senegalensis* (13.94g), respectively. The extracts exhibited varying degrees of inhibition against *V. cholerae, K. pneumoniae* and *S. enterica* serovar. *Khaya senegalensis* had the highest significant antibacterial activity at 300mg/ml (36.00±0.81f, 19.00±0.98, 22.00±0.69) and was the most active among the three plant extracts, followed by *C. papaya* at 300mg/ml (29.33±1.17, 31.50±0.29, 9.00±1.15) then *A. nilotica* at 200mg/ml, 29.00±0.87, 12.00±0.58, 20.00±0.29. Only *C. papaya* showed no activity at all concentration against *V. cholera*. The MIC of the plant crude extracts against *Vibrio cholerae, Klebsiella pneumoniae* and *Salmonella enterica* serovar *Kentucky* was 0.96±0.04, 0.96±0.05 and 1.80±0.17mg/mL, respectively, while MBC was 1.02±0.12, 1.04±0.29 and 1.92±0.23 mg/mL. In conclusion, the three plant crude extracts of (*K. senegalensis*, *A. nilotica* and *C. papaya)* showed varying degrees of antimicrobial activities against the test organisms. Therefore, the leaves of the plants *Carica papaya*, *Acasia nolitica* and *K. senegalensis* can be recommended for diarrhoeal therapy in accordance with the traditional uses.

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Diarrhea Pathogens, plant extracts, Antibacterial activity.

INTRODUCTION

In developing countries, especially in Sub-Saharan Africa, pathogenic diarrhoea is one of the leading causes of morbidity and mortality (Cohen *et al.*, 2022). Diarrhoea-associated bacterial pathogens include *Escherichia coli, Vibrio cholera,* Species of *Shigella, Salmonella, Proteus, Yersinia, and Campylobacter* (WHO, 2015). Most diarrhoea-associated morbidities and mortalities occur in low to medium-income countries, usually in rural areas. The incidence is fuelled by the vicious cycles of poverty, ignorance, malnutrition, and endemic infections (Mohammed *et al.*, 2014). Herbal preparations are an important traditional medicine therapy that were still used by approximately 80% of the world's population, particularly in developing countries (Kamboj, 2000). A large number of plant species have been documented for the treatment of various ailments and serve as remedies for human disease (Ibrahim *et al.,* 2016). Furthermore, research has consistently shown that most compounds found in plants have beneficial qualities with little adverse effects, and their derivatives have several medicinal and other desirable properties (Farouk *et al*., 2008).

In Nigeria, diarrhoeal diseases are the third leading cause of death among the populace, with children under five years more affected (WHO, 2015). In 2017, about 1.7 million people died from diarrheal diseases globally (Akpan *et al.*, 2012). Conventional diarrhoeal antibiotics are not quite affordable, and adverse effects that accompany their intake cannot be ignored (Ramos *et al.*,

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2008) Spread of drug-resistant microbes remains a major challenge to healthcare providers, particularly in developing nations like Nigeria (Seddik *et al*., 2005;), and most of the plants used have limited potentials of the most bacteria causing diarrhoea, the need to search for an alternative antimicrobial agent of plant origin cannot be overemphasized. Therefore, this research aims to determine the antibacterial Activities of Three (3) Selected Medicinal Plants against Diarrhea Causing Pathogens.

MATERIALS AND 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 two 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 km^2 (http://www.minna.climatetemps.com/map.php).

Sample collection

A stool sample from diarrhoea patients was collected in sterile sample bottles, packaged in icepacks and transported to the Center for Genetic Engineering and Biotechnology Laboratory, Federal University of Technology, Minna.

Isolation of diarrhoea-causing pathogens

Samples collected were inoculated onto MacConkey agar, Eosine methylene blue (EMB) agar and Thiosulfate citrate bile-salts sucrose (TCBS) agar and incubated at 37^oC for 24 hours. Distinct colonies were sub-cultured repeatedly to obtain pure cultures of diarrhoea-causing pathogens, which were stored on nutrient agar slant for further use.

Identification of isolated pathogens

Suspected bacterial diarrhoea-causing pathogens obtained were identified using colony morphology and conventional biochemical tests, including Gram staining, Oxidase test, Voges proskauer test, Indole test, Methyl red, Citrate test, Catalase, Urease and Motility test.

Gram staining

A thin smear of the pure 24-hour-old culture was prepared on a clean, grease-free slide; it was allowed to dry and fixed by passing over a gentle flame. The smear was then stained by adding 2 drops of crystal violet (Primary stain) solution for 60 seconds and then rinsed with water. The smear was again flooded with Lugol's iodine (Mordant) for 30 seconds and rinsed with water before decolourizing with 70% alcohol. The smear was then counter-stained with Safranin for 60 seconds before rinsing with water and then air drying. The smear was mounted on a microscope and observed under an oil immersion objective lens (x100). Gram-negative cells appeared pink or red, while gram-positive organisms appeared purple.

Plant Sample collection and identification

Fresh leaves of five medicinal *Carica papaya* (KSUSTA/PSB/H/109C), *Khaya senegalenses* (KSUSTA/PSB/H/61A) *Acacia nilotica* Wild. (KSUSTA/PSB/H/284 was collected in Minna, Niger state. The plants were identified at the Department of Plant Biology School of Life Sciences, Kebbi State University of Science and Technology Aliero Kebbi State.

Extraction of the crude extracts

The plant extraction procedure was carried out according to the method described by AOAC (2010). The plant leaves were dried under shade at room temperature for at least 7 days, segregated and pulverized by a mechanical grinder, forming a coarse powder. 100g of powdered samples were weighed and macerated into 500ml of n-hexane, ethyl acetate and methanol for 72 h in a 1:5 (w/v) ratio, respectively. The supernatants obtained were filtered using Whatman No 1 filter paper and evaporated until dried under reduced pressure (204 mbar) at 40 ^oC.

Antimicrobial assay

The antibacterial activity of the selected plant extracts was tested using the agar well diffusion technique on Muller Hinton agar according to the method described by Agarry *et al*. (2005)*. Suspension of the test organisms was prepared in peptone water to match turbidity of 0.5 Macfarland standard and inoculated onto Mueller Hinton agar. Agar wells with a diameter of 8mm were punched aseptically using a sterile cork borer. Each extract solution at the desired concentration (50, 70 and 100mg/ml) was introduced into each well and then incubated at 37^oC for 24 hours. The extracts' antimicrobial activity was measured according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002)*.

Minimum Inhibitory Concentration (MIC) and Maximum Bactericidal Concentration (MBC)

Based on the results of the antibacterial testing, the most efficient extracts were chosen to identify its MIC and MBC. The Broth tube dilution method was used as described by Srikacha and Ratananikom, (2020)*. Serial dilution was adopted to prepare various concentrations of the selected extracts in test tubes, which were inoculated with a standardized number of organisms and incubated at 37^0C for 18-24 hours. The lowest concentration (highest dilution) of the extract with no visible growth of the test organism was considered the MIC. The MBC was determined by plating the contents of the MIC tubes onto nutrient agar plates. The lowest extract concentration with minimum growth of the test organism on the agar plate was considered the MBC.

RESULTS

Extraction Yields of Plants

The extraction yield of a plant used in this study is shown in Table 2: *Carica papaya* (19.05g), *Acasia nolitica* (18.67g) and the lowest yield was observed in *Khaya senegalensis* (13.94g).

Table 1: Identity of test organisms

Sample ID	Isolate code	Scientific Name	E value	Per. Ident	Accession
1	KP	*Klebsiella pneumoniae* strain IAUK 8738	9e-171	98.57%	MK571203.1
2	ST	*Salmonella enterica* subsp. enterica serovar Kentucky strain Medellin Colombian	0	97.40%	MH445517.1
3	VC	*Vibrio cholerae* strain RP01	0	100.00%	MH174981.1

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

Plants	Extraction Yield (g)
Acasia nolitica	18.67
Carica papaya	19.05
Khaya senegalensis	13.94

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Crude Extracts

The MIC and MBC of the crude extracts of *Acacia nilotica* and *Khaya senegalensis* are shown in Table 3. The Minimum Inhibitory Concentration (MIC) of *Acacia nilotica* against *Vibrio cholera, Klebsiella pneumoniae* and *Salmonella enterica* serovar *Kentucky* was observed at 0.96±0.03, 0.19±0.00 and 0.96±0.06mg/mL respectively, while the minimum bactericidal concentration (MBC) was observed at 24.00±0.87, 0.96±0.05 and 4.80±0.17mg/mL respectively. For *Khaya senegalensis,* MIC was observed at 0.96±0.04, 0.96±0.05 and 4.80±0.17 while MBC was recorded at 4.80±0.12, 4.80±0.29 and 4.80±0.23. (0.64g) followed by aqueous (0.57g) and then ethyl-acetate (0.25g). Similarly, n-hexane had the highest yield (0.46g) in *Khaya senegalensis,* followed by ethyl-acetate (0.28g) and then aqueous (0.25g).

Table 3: Minimum Inhibitory and Maximum Bactericidal Concentrations

	MIC			MBC		
Plant Extract	*V. cholerae*	*K. pneumoniae*	*S. enterica serovar Kentucky*	*V. cholerae*	*K. pneumoniae*	*S. enterica serovar Kentucky*
C. papaya	0.96±0.03	0.19±0.00	0.96±0.06	1.09±0.87	0.20±0.05	1.04±0.17
K. senegalensis	0.96±0.04	0.96±0.05	1.80±0.17	1.02±0.12	1.04±0.29	1.92±0.23
Control	0.005	0.005	0.020	0,005	0.005	0.020

Values are presented as mean ± standard error of triplicates' mean (SEM).
MIC = Minimum Inhibitory; MBC = Minimum Bactericidal Concentrations

Antibacterial Activity of Crude Plant Extracts against Test Organisms

The Antibacterial activity of three different plant extracts used in this study against *Vibrio cholera, Klebsiella pneumoniae* and *Salmonella enterica* serovar *Kentucky* is shown in Table 4 below.

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

Extract	Concentration (mg/mL)	*Vibrio cholerae*	*Klebsiella pneumoniae*	*Salmonella enterica serovar Kentucky*
	200	11.00±0.58^{b}	22.00±0.87^{e}	14.00±0.58^{c}
Acasia nolitica	250	25.33±0.60^{c}	31.50±0.87^{f}	11.00±0.58^{ab}
	300	29.33±1.17^{d}	31.50±0.29^{f}	9.00±1.15^{a}
	200	0.00±0.00^{a}	9.00±1.15^{a}	18.00±0.58^{ef}
Carica papaya	250	0.00±0.00^{a}	17.00±1.15^{d}	26.33±0.60^{h}
	300	0.00±0.00^{a}	24.00±0.58	28.33±1.17^{h}
Khaya senegalensis	200	29.00±0.87^{d}	12.00±0.58^{b}	20.00±0.29^{fg}
	250	32.50±0.29^{e}	16.20±1.27^{cd}	20.00±1.15^{fg}
	300	36.00±0.81^{f}	19.00±0.98^{d}	22.00±0.69^{g}
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

Antibacterial Activity of Crude Plant Extracts against Test Organisms

Carica papaya, *Acasia nolitica* and *Khaya senegalensis* are well-known medicinal plants with health benefits against many ailments (Doughari *et al*., 2007; Ahmad and Wudil, 2013*; Ibrahim *et al*., 2013; Galalain *et al*., 2019; Namadina *et al*., (2021). Extracts from these plants used in this study exhibited varying degrees of antibacterial activity against *Vibrio cholera, Klebsiella pneumoniae* and *Salmonella enterica* serovar *Kentucky*. *Carica papaya* and *Khaya senegalensis* were noted to be the most active among the three plant extracts tested, as they showed significantly higher zones of inhibition at all concentrations against all the test organisms.

The efficacy of the plant extracts evaluated was concentration-dependent, as the antimicrobial activity increases with increasing concentration of the plant extracts, indicated by the increasing diameter of each inhibition zone. The observed inhibitory activity of the plant extracts against these diarrhoea-causing pathogens validates the use of the plant for treating diarrhoea in traditional medicine. This result is consistent with findings reported by Doughari *et al.* (2007), Mann *et al.* (2014), Kuta *et al.* (2015), Abdallah *et al.* (2016), Ali *et al.* (2017) Sadiku *et al.* (2020) with few exceptions. They all reported varying ranges of inhibitory effects of these plant extracts against various bacterial pathogens at various concentrations. According to Kuta *et al.* (2015), leaf and stem extracts of *Khaya senegalensis* showed inhibitory activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia* and *Escherichia coli* at concentrations ranging from 400 to 1000mg/ml,

Similarly, Abdallah *et al.* (2016) reported leaf and stem extracts of *Khaya senegalensis* to have inhibitory activity against diarrheal/stool isolates (*E. coli*, *Shigella* sp. and *Salmonella* sp.). Musah (2019) observed that matured leaf extract of *Poliostigma thoningii* showed antibacterial activity against *E. coli* and *S. enterica Typhi* at 10 and 5mg/cm3 concentrations but had no activity against *P. aeruginosa*. From the study of Evbuomwan *et al.* (2018),* *Vernonia amygdalina* leaf extract showed significant antibacterial activity against multidrug-resistant bacterial isolated, namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at various concentrations ranging from 25 to 200mg/mL.

Mann *et al.* (2014) observed that stem bark extract of *Anogeissus Leiocarpus* showed an inhibitory effect against multidrug-resistant *Staphylococcus aureus* and food pathogen of *Hibiscus sabdariffa calxy* (Zobo) drink, respectively. The antimicrobial activity of these plant extracts is usually attributed to their phytochemical constituent. Although the presence and roles of phytochemicals were not investigated in this study, previous studies have shown Tannins, Flavonoids, Saponins and Alkaloids, which are usually reported to be present in these plants, to have antibacterial properties (Mann *et al.*, 2014; Abalaka *et al.*, 2016;).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of Crude Extracts of *Carica papaya* and *Khaya senegalensis* against test isolates

The minimum inhibitory concentration (MIC) is the smallest concentration that visibly inhibits growth. The MIC is useful in determining the smallest effective dosage of a substance against an organism (Kuta *et al.*, 2015). The MIC of *Carica papaya* against *Vibrio cholera, Klebsiella pneumoniae* and *Salmonella enterica* serovar *Kentucky* was observed at 0.96±0.03, 0.19±0.00 and 0.96±0.06mg/mL, respectively, while the minimum bactericidal concentration (MBC) was observed at 1.09±0.04, 0.20±0.05 and 1.04±0.17mg/mL respectively. For *Khaya senegalensis,* MIC was detected at 0.96±0.04, 0.96±0.05 and 1.80±0.17 mg/mL concentration, while MBC was recorded at 1.02±0.12, 1.04±0.29 and 1.92±0.23 mg/mL concentration. Plant extracts are considered to have a good inhibitory activity if they present MIC value ≤ 100mg/mL, a moderate inhibitory activity when MIC ranges between 100 to 500mg/mL, a weak inhibitory activity if MIC value ranges between 500 to 1000mg/mL and no inhibitory activity when > 1000mg/mL (Jafari *et al*., 2008; Mann *et al*., 2014).

Considering this report, the MIC and MBC values recorded from the antibacterial activity of the present study showed good inhibitory activity. The current findings lend credence to the traditional use of this plant as a medicine for infectious diseases, particularly those caused by the test organisms susceptible to the extracts. However, this result is contrary to the findings of Kuta *et al.* (2015), Abdallah *et al.* (2016), Ali *et al.* (2017), Usman *et al.* (2020) and Amadou *et al.* (2020) who all reported much higher MIC and MBC values against various bacterial pathogens. Ugoh *et al.* (2014) observed methanolic and ethanolic stem bark extracts of *Khaya senegalensis* with a MIC value of 250 and 200mg/mL against *Salmonella enterica* subsp. enterica serovar Typhi. MIC value of 25mg/ml was reported by Shinde *et al.* (2020) for methanolic stem bark extract of *Khaya senegalensis* against both *Klebsiella pneumonia* and a carbapenem-resistant strain of *Escherichia coli.*

In Kuta *et al.* (2015) studies, the aqueous and ethanolic leaf extract of *Khaya senegalensis* had MIC values of 400 and 200mg/mL, respectively, against *Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Escherichia coli*. The study also reported MBC values 0f 800 and 400mg/mL for aqueous and ethanolic leaf extract of *Khaya senegalensis* against the same organisms, respectively. Ali *et al.* (2017) studied the antimicrobial activity of methanolic stems bark extracts of *Anogeissus leiocarpus* against several bacterial isolates; the study reported MIC and MBC values of 10 and 40 mg/mL for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus feacalis* and *Corynebacterium ulcerans.* In Chomini *et al.* (2021)

For the aqueous extract, MIC was detected at 100, 200 and 100 mg/mL, while the ethanolic extract had MIC values at 200, 400 and 200mg/mL against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus mutans* respectively. While the MBC was detected at 400mg/mL for both aqueous and ethanolic extract against all the isolates. The variation in the MIC and MBC values reported in these studies could be due to the phytochemical composition of their respective extracts, which is usually dependent on the polarity of the solvents used for extraction, as solvent type tends to influence the kind of bioactive compound released from the plant materials. Also, variation in results could be attributed to the genetic makeup of each test organism used. Different organisms have been shown to respond differently to different and same concentrations of a specific antimicrobial substance (Sadiku *et al.,* 2020).

CONCLUSION

Various diarrhoea-causing pathogens were isolated from stool samples of patients attending General Hospital, Minna. They include *Salmonella enterica* serovar *Kentucky* strain Medellin Colombian, *Klebsiella pneumoniae* strain IAUK 8738 and *Vibrio cholerae* strain RP01. Crude extract of the three (3) plants showed varying degrees of antimicrobial activity against the test organisms, with *Khaya senegalensis, Acacia nitolytica and Carica papaya* being the most active.

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