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 (Bagarawa), Carica papaya (Gwandi) 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°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. cholerae. The MIC of the plant crude extracts against V. 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±0.23mg/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 nilotica and K. senegalensis can be recommended for diarrhoeal therapy in accordance with the traditional uses.

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 cholerae, 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 diarrhoeal 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., 2024).

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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 km² (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°C 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 senegalensis (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°C.

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°C 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°C 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), Asia nitida (18.67g) and the lowest yield was observed in Khaya senegalensis (13.94g).

https://scientifica.umyu.edu.ng/
Table 1: Identity of test organisms

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Isolate code</th>
<th>Scientific Name</th>
<th>E value</th>
<th>Per. Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KP</td>
<td>Klebsiella pneumoniae strain IAUK 8738</td>
<td>9e-171</td>
<td>98.57%</td>
<td>MK571203.1</td>
</tr>
<tr>
<td>2</td>
<td>ST</td>
<td>Salmonella enterica subsp. enterica serovar Kentucky strain Medellin Colombian</td>
<td>0</td>
<td>97.40%</td>
<td>MH445517.1</td>
</tr>
<tr>
<td>3</td>
<td>VC</td>
<td>Vibrio cholera strain RP01</td>
<td>0</td>
<td>100.00%</td>
<td>MH174981.1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plants</th>
<th>Extraction Yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acasia nolitica</td>
<td>18.67</td>
</tr>
<tr>
<td>Carica papaya</td>
<td>19.05</td>
</tr>
<tr>
<td>Khaya senegalensis</td>
<td>13.94</td>
</tr>
</tbody>
</table>

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

The MIC and MBC of the crude extracts of Acasia nolitica and Khaya senegalensis are shown in Table 3. The Minimum Inhibitory Concentration (MIC) of Acasia nolitica 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

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>MIC</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V. cholera</td>
<td>K. pneumoniae</td>
</tr>
<tr>
<td>C. papaya K. senegalensis</td>
<td>0.96±0.03</td>
<td>0.19±0.00</td>
</tr>
<tr>
<td>Control</td>
<td>0.005</td>
<td>0.005</td>
</tr>
</tbody>
</table>

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 and Salmonella enterica serovar Kentucky is shown in Table 4 below.

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).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (mg/mL)</th>
<th>Vibrio cholera</th>
<th>Klebsiella pneumoniae</th>
<th>Salmonella enterica serovar Kentucky</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acasia nolitica</td>
<td>200</td>
<td>11.00±0.58b</td>
<td>22.00±0.87c</td>
<td>14.00±0.58c</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>25.33±0.60c</td>
<td>31.50±0.87f</td>
<td>11.00±0.58b</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>29.33±1.17d</td>
<td>31.50±0.29f</td>
<td>9.00±1.15a</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.00±0.00a</td>
<td>9.00±1.15a</td>
<td>18.00±0.58f</td>
</tr>
<tr>
<td>Carica papaya</td>
<td>250</td>
<td>0.00±0.00a</td>
<td>17.00±1.15d</td>
<td>26.33±0.60b</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.00±0.00a</td>
<td>24.00±0.58</td>
<td>28.33±1.17d</td>
</tr>
<tr>
<td>Khaya senegalensis</td>
<td>200</td>
<td>29.00±0.87d</td>
<td>12.00±0.58b</td>
<td>20.00±0.29%</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>32.50±0.29e</td>
<td>16.20±1.27d</td>
<td>20.00±1.15c</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>36.00±0.81f</td>
<td>19.00±0.98d</td>
<td>22.00±0.69c</td>
</tr>
<tr>
<td>Control</td>
<td>8mg/ml</td>
<td>24.21±0.82</td>
<td>12.03±13</td>
<td>32.07±1.43</td>
</tr>
</tbody>
</table>

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 nilotica and Khaya senegalensis are well-known medicinal plants with health benefits against many ailments (Doughhari 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 Doughhari 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 pneumoniae 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 Polystigma tondingii 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 caby (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 of 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 pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Streptococcus faecalis and Corynebacterium alvei. In Chomini et al. (2021).
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 nilotica and Carica papaya being the most active.

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