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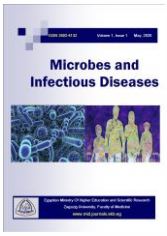

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**Original article**

# Antimicrobial activities of bioactive compounds isolated from *Acacia nilotica* against multi-drug resistant bacteria

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**ABSTRACT**

**Background:** The use of plants in the treatment of microbial diseases is increasing worldwide; especially with the increase of bacterial resistance to antibiotics and the development of new diseases that have no cure in modern medicines. **Aim:** This research was designed to determine the antimicrobial activities of bioactive compounds isolated from *Acacia nilotica (A. nilotica*) against multi-drug resistant bacteria. **Methods:** *Acacia nilotica* samples (*i.e*., leaves, pods, and back) were collected within Aliero local government area, in Nigeria. Metabolite extraction was performed through maceration. The obtained extract was tested *in vitro* against multi-drug resistant bacteria that cause diarrhea: *Salmonella typhimurium*, *S. paratyphi*, *Salmonella* sp, *Shigella dysenteriae*, *Shigella* sp, and *Shigella flexneri*, using the agar well diffusion assay. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the micro-plate serial dilution method. The synergistic (Pod + Leave Steam back) ethanolic crude extract was subjected to column and thin-layer chromatography (TLC) analyses. The obtained fractions were tested against multi-drugs resistance bacteria, and MIC and MBC of the fractions were also determined. **Results:** The results showed that the synergistic *A. nilotica* crude extract had the highest mean antibacterial activity recording inhibition zones that ranged from 15.0± 0.58 to 22.7± 0.33 mm. The MIC of ethanolic crude extracts ranged from 100 to 200 mg/ml, while the MBC ranged from 100 to > 400 mg/ ml. **Conclusion:** The antibacterial potential of the synergistic ethanolic fractions recorded higher activity that ranged from 16.7± 1.20 to 31.0±1.00 mm against all the tested bacteria, compared to the crude extracts.

## Introduction

*Salmonella and Shigella* spp is one of the bacterial pathogens that cause diarrhea worldwide [1]. Literature has shown that 1.7 billion cases of diarrhea occurred globally each year. About 200 people died in Africa due to diarrheal infection every hour [1]. In Nigeria, the comprehensive burden of diarrheal disease caused by *Salmonella* and *Shigella* spp is lacking due to the lack of an effective, well-coordinated surveillance system for diseases [2]. The infection was reported to be persistent due to increased urbanization, inadequate supplies of potable water, regional migration of

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large numbers of immigrant workers, inadequate facilities for processing human waste, overstretched healthcare delivery systems, and excessive use of antibiotics [3].

Multi-drug resistant *Salmonella* and *Shigella* spp are global public concerned [4]. Research on the antibacterial properties of herbal plants has been prompted due to the acceptance of traditional medicine as an alternative form of healthcare and the emergence of microbial resistance to existing antibiotics [5]. According to the World Health Organization (WHO), 80% of the world's population used herbal medicine to treat a variety of illnesses [5]. This move to herbal therapy can be linked to the low cost and widespread availability of herbal plants compared to conventional antibiotics.

*Acacia nilotica* (*A. nilotica)* is called Arabic gum tree, Babul acacia, and Egyptian mimosa in English, Garad in Arabic, and Bagaruwa in Hausa. *Acacia nilotica* is used in traditional formulation in Nigeria for the treatment of infectious diseases [6]. *Acacia nilotica* is widely distributed throughout tropical Africa, and it is documented to exhibit several ethno-medicinal uses in Nigeria which include the treatment of diarrhea, especially among the children [7].

The leaves and bark extracts of *A. nilotica* have been used for the determination of antimicrobial activity, but little information was documented on the effect of individual microbiologically bioactive compounds of *A. nilotica* on multi-drug resistant bacteria especially diarrhea causing bacterial pathogen. The worldwide emergence of resistant *Salmonella* and *Shigella* spp and many other ß-lactamase producers has become a major therapeutic problem worldwide [3]. Multi-drug resistant strains of *Salmonella* and *Shigella* spp are increasingly reported especially in developing countries [3]. Although research has been done on the antibacterial activities of *A. nilotica*, there is limited information on its antibacterial activity of synergistic crude extract fractions against multidrug resistance *Salmonella* and *Shigella* diarrhea-causing bacterial pathogens. Therefore, this research was designed to evaluate the antibacterial activities of *A. nilotica* in different crude extract fractions against multi-drug resistance *Salmonella* and *Shigella* spp-causing diarrheal pathogens with the hope to be a possible alternative for the treatment of diarrhea caused by these bacterial species.

## Materials and Methods

### Study area

Aliero local government is located in the South Eastern part of Kebbi State, Nigeria; on the higher way latitude and longitude 2°C'16°C' 42°C' N4' 27' 066E. It was created 1991 with a total land mass of 412 $Km^2$. The local government is bounded in the North-East by Gwandu local government area, in the South by Jega local government, in the East by Tambuwal local government area of Sokoto State, and in the North-West by Birnin Kebbi local government area. Kebbi state shares boundary with Sokoto state in the North Eastern axis, Zamfara state on the Eastern part, Niger state from Southern part, and Republic of Niger on Western part. According to NPC [8], Kebbi state has a total population of 3,238,628.

### Samples collection

Leaves, pods, and bark of *A. nilotica* were collected, identified, and authenticated by Prof. Dharmendra Singh at Department of Plant Science and Biotechnology, herbarium unit, Kebbi State University of Science and Technology, Aliero, Nigeria. These parts were selected based on the local used in the treatment of diarrheal especially among children. The selected plant parts were washed thoroughly under running tap water to remove the surface dirt, followed by rinsing with sterilized distilled water. The washed plant samples were dried under shade in an open air for 48 h, grounded using a mechanical grinder (Philips Co. Ltd., Shanghai, China), and finally were transformed into a fine powder by a pestle and mortar [6].

### Solvent extraction

Solvent extraction from each powdered part of *A. nilotica* was conducted following the method described by **Adwan et al.** [9]. About 50 g of a powdered sample was mixed individually with 250 ml of 75 % v/v of ethanol in conical flasks, and incubated at 25 ºC in a shaking incubator (Gallenkamp, UK) at 200 rpm for 48 h. In the same time, 50 g of mixed powder (leaves + pod +back) was also weighed in the ratio (1:1:1) and then extracted using 75 % ethanol. The extracts were filtered individually and then concentrated at 40ºC using a rotary evaporator (Büchi rotavapor R-144, Flawil, Switzerland). The dried crude extracts were then stored at 4ºC in refrigerator for further analysis.

**Isolation and identification of the test bacteria**

Several isolates of Gram negative multidrug resistance bacteria *(i.e., Salmonella* spp. and *Shigella* spp.) were isolated from stool samples collected from Sir Yahaya Memorial hospital Birnin Kebbi. The samples were inoculated on Salmonella Shigella agar and incubated at 37ºC 48h. The recorded isolates were Gram stained, identified using colony morphology, and identified biochemically using several assays, such as indole production, methyl red, Voges- Proskeur, citrate utilization test, oxidase, catalase, urease, and triple sugar iron agar. Moreover, sugar fermentation assays were carried out using several sugars, including glucose, lactose, mannitol, sucrose, and xylose [10].

**Antibiotic sensitivity assay**

The antibacterial resistance patterns of the test bacteria were determined using the disc diffusion assay, following the guidelines of CLSI. (2021). The following antibiotics were tested: Ampicillin (AMP) (10 µg), amoxicillin (AMOX) (10 µg), chloramphenicol (CHL) (30 µg), gentamicin (GEN) (10 µg), amikacin (AMK) (30 µg), streptomycin (S) (10 µg), tetracycline (TET) (30 µg), trimethoprim–sulfamethoxazole (SXT) (25 µg), ciprofloxacin (CIP) (5 µg), ceftriaxone (CRO) (30 µg), augmentin (AUG) (25/10 µg), meropenem (MER) (10 µg), and ceftazidime (CAZ) (30 µg) (Oxoid, UK). The 24 h cultures of the test bacteria were adjusted individually to 0.5 McFarland standard to get 1.5 $x10^8$ cfu/ml. The bacterial suspension was inoculated using the sterilized cotton swab on the surface of Mueller-Hinton Agar (MHA) (Himedia, India) Petri plates. The antibiotic discs were placed individually on the surface of the agar medium using a sterilized forceps and pressed gently. All the inoculated plates were incubated for 24 h at 37 ºC. After incubation, the diameter of the inhibition zones was measured using a calibrated ruler and the results were interpreted according to the guidelines of EUCAST and CLSI [12]. The *Salmonella* and *Shigella* isolates that showed resistance to more than two classes of antibiotics were considered as multidrug resistant bacteria, and were selected for further screening for the anti-diarrheal activity of the different *A. nilotica* crude extracts and their fractions.

**Screening for antibacterial potential of the *A. nilotica* extracts**

The antibacterial efficacy of *A. nilotica* leaves, bark, pod, and combined parts ethanol crude extracts were tested on Muller Hinton agar using the agar well diffusion technique according to the method described by **Agarry et al.** [12]. Using 10 % Di-Methyl Sulfoxide (DMSO) as a diluent, the dried ethanol crude extracts were reconstituted to different concentrations of 50, 100, 150, and 200 mg/ml, and their inhibitory activities were tested against the multi-drug resistant *Salmonella* and *Shigella* spp. The concentration of test bacteria cells was adjusted individually to 0.5 McFarland standard, and inoculated on to freshly prepared Muller Hinton agar. The inoculated plates with different concentrations of each extract were incubated at 37ºC for 24h. The presence of a zone of inhibition is evidence of the antibacterial activity of the tested extract. The assay was done in triplicates; along with a negative control (10% DMSO) and a positive control meropenem (30 µg/ ml), which was selected according to the obtained results of the previously mentioned antibiotics sensitivity assay.

**Determination of the minimum inhibitory concentration**

The minimum inhibitory concentration (MIC) of the different parts and combined parts of *A. nilotica* ethanolic crude extracts was determined using a two-fold serial micro-broth dilution method in micro-titre plates. The first concentration (400 mg/ ml) of the different ethanolic crude extracts was prepared by mixing 2 g of weighed dried extract into 4 ml of sterile DMSO (10 %) in a sterile glass beaker. Afterwards, 0.2 ml was pipetted from this prepared stock (400 mg/ ml) and serially diluted in wells of the micro-titre plates; each containing 0.2 ml of freshly prepared Muller Hinton broth (MHB), to obtain different concentrations of 200, 100, 50, 25, 12.5, and 6.25 mg/ ml. The test bacterial suspension prepared in sterile normal saline was adjusted to standard 0.5 McFarland equivalents to $1.5 \times 10^8$ cfu/ ml, which was further diluted to give a final cell density of $1.0 \times 10^6$ cfu/ ml. The diluted standardized bacterial suspension was added into each well that contain the serially diluted crude extract and was mixed to homogeneity to give a final inoculum of $5 \times 10^5$ cfu/ ml. The positive control wells contained broth and the test bacteria, while the negative control wells contained broth only. The inoculated micro-plates were incubated at 37°C for 24 h. After incubation, a blank for each well

concentration (extract and MHB only) was prepared, and followed by an examination for visible turbidity of the inoculated wells by measuring the optical density reading at 600 nm using a Beckman DU-70 UV-Vis spectrophotometer USA. The MIC of the extract was considered the lowest concentration that had an optical density equivalent to its respective blank well and thus had no visible bacterial growth. The test assays were prepared in triplicates [5].

**Determination of the minimum bactericidal concentration**

Using the MIC micro-titre plates, a loopfull of the mixture from each well that had no visible growth was cultured on freshly prepared Muller-Hinton agar petri plat using the streak plate method, and then the inoculated plates were incubated at 37°C for 24 h. After incubation, the plates were examined for any bacterial growth. The least concentration of the extract that had no visible colony growth was considered the minimum bactericidal concentration (MBC) [5].

**Isolation of the bioactive compounds**

**Column chromatography**

About 3 g of the combined parts ethanolic crude extract of *A. nilotica* was subjected to column chromatography to fractionate the extract into its constituent fractions. A 120 g Silica gel for column chromatography (Thomas Scientific, Chromatography Flex-Column®, Economy Column, 5/cs) (60-120 mesh) was used as the stationary phase. The extract was loaded on top of the packed column. Elution of the extract was carried out by the use of the solvent system that was composed of hexane: ethyl acetate: methanol (100:0:0 % v/v) to (0:80:20 % v/v); respectively, 100 % each. The eluent was collected into sterile sample bottles and then labeled accordingly [13].

**Thin layer chromatography**

Each eluent fraction ($F^1$, $F^2$ and $F^3$) was analysed using Thin layer chromatography (TLC) (Sheet L × W 20 cm × 20 cm, India) plates for homogeneity. Hexane: ethyl acetate: methanol (60:30:10 % v/v) was used as the motile phase. For optimum spot visibility, the developed chromatograms were sprayed with 10 % Sulphuric acid and then heated at 100$^o$C for 3 min [14]. Thereafter, the obtained fractions were combined based on their TLC profile or similarities **(Figure 1)**.

**Evaluation of antibacterial potency of the different fractions of *A. nilotica* crude extracts**

The different fractions obtained from column chromatography were allowed to stand at room temperature till dryness. The dried fractions were re-dissolved in 10 % DMSO and used for screening antibacterial activity against the selected multi-drug resistant pathogenic bacteria, according to the procedure described by **Agarry et al.** [12]. The antibacterial activity of the different fractions of combined parts of *A. nilotica* ethanolic crude extract (leaves, bark and pod extracts) were tested by agar well diffusion technique on Muller Hinton agar. The presence of a zone of inhibition (mm) is evidence of antibacterial activity. Each of the crude extract fractions of *A. nilotica* was tested against the Multidrug resistant *Salmonella* and *Shigell*a spp in triplicates along with negative control (10% DMSO) and positive control meropenem 30µg/ml due to the sensitivity of the tests bacteria against antibiotic during antibiotic sensitivity test .

**Determination of MIC of the separated fractions**

The MIC of the different fractions of synergistic *A. nilotica* ethanolic crude extracts were determined using a two-fold serial micro-broth dilution method in micro-titre plates. The first concentration (200mg/ml) of different fractions of synergistic ethanolic crude extracts of *A. nilotica* was prepared by mixing 2 g of dried fraction into 4 ml of sterile 10% DMSO contained in a sterile glass beaker) then, 0.2 ml was picked from this prepared stock (400 mg/ml) and serially diluted in wells of micro-titre plates each containing 0.2 ml of freshly prepared Muller Hinton broth (MHB) to obtain different concentrations ranging from 400, 200, 100, 50, 25, 12.5, 6.25 and 3.25 mg/ml. Test bacteria suspension was prepared in 0.85% sterile normal saline, and its turbidity was adjusted to standard 0.5 McFarland equivalent to $1.5 \times 10^8$ CFU/ml, this was further diluted by transferring 0.1 ml from this standardized bacteria suspension into a tube containing 9.9 ml of 0.85% sterile normal saline to give a final cell density of $1.0 \times 10^6$ CFU/ml which was used in the experiment). The diluted standardized bacterial suspension was added into each of the wells containing the serially diluted crude extract. ) this was mixed to homogeneity to give a final inoculum of $5 \times 10^5$ CFU/ml. Positive control wells containing broth and test organisms were used, while the others containing broth only aimed at checking the ability of the media to support

test bacterial growth (bacterial viability) and sterility of broth respectively, the fourth contained broth and crude extract aimed at ascertaining for any prior microbial contamination of the extract), the inoculated micro-plates were incubated at 37°C for 24 hours. After the incubation period, blanks for each well concentration (extract and MHB only) were prepared, and this was followed by an examination of inoculated wells for visible turbidity by optical density reading at 600nm with a Beckman DU-70 UV-Vis spectrophotometer. The MIC of the extract was considered the lowest concentration that had and optical density equivalent to its respective blank well and, thus, had no visible bacterial growth. The test experiments were prepared in triplicates [5].

**Determination of MBC of the separated fractions**

Using the MIC microtitre plates, a loop full of the mixture from each of the wells with no visible growth of bacteria after 24 hours of incubation was cultured on freshly prepared Muller-Hinton agar by the streak plate method. The plate was incubated at 37°C for 24 hours. The plates were examined for any colony growth. The least concentration of the extract which had no visible colony growth was considered as the minimum bactericidal concentration [5].

**Ethical consideration**

This research involved collection of samples from hospital, therefore ethical approval was obtained from Kebbi state Ministry of Health and the management of Sir Yahaya Memorial Hospital Birnin Kebbi.

**Statistical analysis**

All experiments were carried out in triplicates and results were expressed as mean values with standard deviation (± SD).

**Results and Discussion**

Plants have been described as one of the sources of natural products used to cure different diseases caused by bacterial pathogens due to the increase of resistance of these pathogens to the modern medicine. Plants products have also low cost and available to common man as the world is facing economic crises which make it difficult to a common man to purchase modern drugs. This research was carried out to determine antimicrobial activities of active compounds isolated from *A. nilotica* against multi-drug resistant bacteria. Dirrhaea was described as one of the major cause of death among children especially in African continent where only 500 million people out of 1.3 billion people have access to portable drinking water [15]. There is need to have chief and effective treatment to tackle this problem.

Different studies have reported the presence of different phytochemical constituents such as Alkaloids, Saponins, Tannins, Phenols, Flavonoids, Terpenoids, and Steroids from different extracts of *A. nilotica* [6, 16, 17]. There is a paucity of data on the synergistic effects of different parts of *A. nilotica* against multi-drug resistance bacteria causing Dirrhaeal pathogens. In this study, The *Salmonella* and *Shigella* spp isolated from stool samples were tested against different antibiotics classes and the isolates that showed resistance to ≥2 classes of antibiotics were used for this study **(Table 1)**.

The ethanolic crude extracts of leaves of *A. nilotica* showed the mean zone activities against tested bacterial pathogens ranged from 6.33±0.67 to 23.2±0.30 mm. the extract had no activity against *Shigella flexneri* at 50 mg/ml. It was observed that the mean zone of inhibition of ethanolic crude extracts of leaves of *A. nilotica* increases as the concentration of the crude extract increase. The positive control (meropenem 30µg/ml) showed a higher mean zone of activity against tested bacteria compared to the ethanolic crude extracts of leaves of *A. nilotica.* The negative control (10% DMSO) had no activity against all tested bacteria **(Table 2)**. The results obtained in this study was in line with finding of the **Manga et al.,** 2018 who reported 6.7±1.15 to 13.7 ±1.15mm mean zone of inhibition of aqueous crude extracts of leaves of *A. nilotica* against *S, aureus P. aeruginosa*. **Abubakar et al.** [16] reported a higher mean of antibacterial activity of methanol leaves crude extracts of *A. nilotica*, 25.67±2.08 and 33.00±0.45 mm against Gram-negative bacteria. This was lower than that of **Sadiq et al.** [18] who reported an 11.3 ± 1.53 to 17.7 ± 0.58 mean zone of inhibition of ethanolic leaves crude extracts against some foods and clinical *Salmonella* species from Pakistan. The variation could be due to the bacterial species, sources of bacterial species, solvents used for extraction and geographical location of the plant's sources which may contribute to the differences in phytochemical constituents. However, the bacterial spp used in this study were multidrug resistance Gram-negative bacterial clinical isolates.

The results of ethanolic pods crude extract of *A. nilotica* indicates the mean zone activity ranged from 5.0±0.58 to 18.0±1.15 mm **(Table 2**, **Figure1)**. The extract had a low effect against tested bacteria compared to the leaves crude extract. The extract had 0.0±0.00 mean zone activity against *Shigella flexneri* at 50 and 100mg/ml. The positive control (meropenem 30µg/ml) showed a higher mean zone of activity against tested bacteria compared to the ethanolic crude extracts of leaves of *A. nilotica.* It was observed that the negative control (10% DMSO) had no activity against all tested bacteria. The result of this finding was in line with **Sadiq et al.** [18] who reported a 7.7 ± 1.15 to 15.7 ± 1.53 mm mean zone of inhibition of ethanolic crude extract against food and clinical isolates of *Salmonella* species from Pakistan.

The results of ethanolic bark crude extract of *A. nilotica* against tested bacteria showed that the mean zone activity ranged from 5.0±0.62 to 15.3±0.33 mm. The positive control (meropenem 30µg/ml) showed a higher mean zone of activity against tested bacteria compared to the ethanolic crude extracts of leaves of *A. nilotica.* It was observed that the negative control (10% DMSO) had no activity against all tested bacteria. The finding of this research was in line with that of **Sadiq et al.** [18], who reported 5.3 ± 4.61 to 11.7 ± 0.58 mm mean zone of inhibition of ethanolic crude extract of *A. nilotica* against foods and clinical isolates of salmonella species from Pakistan. Similarly, **Jabaka et al.** [17], also reported a 3.67 ± 0.58 to 13.0 ± 0.00 mm mean zone of inhibition of ethanolic crude extract of the stem back of *A. nilotica* against *E. coli*.

The synergistic effect of different parts of the *A. nilotica* crude extract against tested bacteria showed that the mean zone of inhibition ranged from 15.0±0.58 to 22.7±0.33 mm. The synergistic crude extract showed higher activity compared to the single or individual crude extracts. However, the effect of synergistic crude extract against tested bacteria could be compared with the positive control (meropenem 30µg/ml). It was observed that the negative control (10% DMSO) had no activity against all tested bacteria.

The MIC and MBC of different ethanolic crude extracts of *A. nilotica* are presented in **Table 3.** The MIC of the *A. nilotica* crude extracts ranged from 100 to 200mg/ml. The MIC reported in this study was higher than that reported by Sadiq *et al*. [17], (1.56 to 6.25 mg/ml) against multidrug resistance *E. coli* and Salmonella spp. **Abubakar et al.** [16] also reported lower MIC of 5 to 10mg/ml against Gram positive and negative sensitive bacterial isolates. **Manga et al.** [6] also reported 7.81 to 25.25mg/ml MIC against Gram-positive and negative bacteria which is lower compared to this study. The differences in MIC could be due to bacterial strains and protocol used [17]. The MIC of the synergistic crude extract of *A. nilotica* was lower compared to that of single crude extracts. This could be due to the higher quantity of the different phytochemicals from different parts of the plants which accumulated in the extract. The MBC of the *A. nilotica* crude extracts ranged from 100 to >400mg/ml. **Sadiq et al.** [18] and **Abubakar et al.** [16] reported lower MBC compared to this study.

The results of antibacterial activity of different fractions of synergistic ethanolic crude extract of *A. nilotica* against multidrug resistance diarrhea causing bacteria showed that $F_1$ had the highest activity against *S. typhimurium, S. paratyphi* and *Shigella flexneri* with mean and SE zone of inhibition 22.7±0.33 mm and low activity against *Shigella dysenteriae* with mean and SE zone of inhibition 17.3±0.88mm **(Table 4).** Similarly, $F_2$ showed higher activity against *Shigella flexneri* with mean and SE 28.0±.58 and low activity against *Shigella* spp 16.7±1.20mm. Among all fractions tested against tested bacteria, $F_3$ had the highest activity against *S. paratyphi* with mean and SE zone of inhibition 31±1.00mm. Fractions of synergistic crude extract had higher mean and SE zone of inhibition compared the crude extract which is an indication the fractions could be good candidate for developing treatment against these bacterial. This was in line with a study done by **Jabaka et al.** [17], who reported antibacterial activity of different *A. nilotica* fractions against *E. coil* ranging from 11.67 ±0.58 to 15.67 ± 0.58mm. The differences in the mean zone of inhibition could be as results of the method used for extraction and testing for the activity. However, the fraction used in this research was from synergistic (composition of leaves, pod and back) crude extract.

It was observed that the positive control (meropenem 30µg/ml) had lower activity compared to the individual fraction while the negative control had no activity against all tested bacteria. The results of MIC of the 3 different fractions of the synergistic crude extracts of *A. nilotica* against multidrug resistance diarrhea-causing bacteria ranged from

12.5 to 50 mg/ml while the MBC ranged from 25 to 100 mg/ml. This was in line with the finding of **Jabaka et al.** [17], who reported MIC and MBC 15.625 to 62.5µg/ml and 12.5 62.5 µg/ml respectively.

**Table 1.** Antibiotics resistance profile of *Salmonella* and *Shigella* spp tested against different ethanolic crude extracts and fractions.

| Bacterial spp | Antibiotics (µg) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **AMP (10)** | **AMOX (10)** | **CHL (30)** | **GEN (10)** | **AMK (30)** | **S (10)** | **TET (30)** | **SXT (25)** | **CIP (5)** | **AUG (25/10)** | **MER (10)** | **CTZ (30)** |
| S. typhimurium | R | R | R | R | R | R | R | R | S | R | **S** | R |
| S. paratyphi | R | I | R | R | R | I | R | R | I | R | S | R |
| Salmonella spp | R | R | I | R | R | R | I | R | R | I | S | I |
| *Shigella dysenteriae* | R | R | R | R | I | R | R | R | I | R | S | S |
| *Shigella* spp | R | R | R | R | S | R | R | R | R | R | S | R |
| *Shigella flexneri* | R | R | R | R | R | R | R | R | R | R | S | R |

Key: AMP : ampicillin (10 µg), AMOX: amoxicillin (10 µg), CHL: chloramphenicol (30 µg), GEN: gentamicin (10 µg), AMK : amikacin (30 µg), S : streptomycin (10 µg), TET: tetracycline (30 µg), SXT: trimethoprim–sulfamethoxazole (25 µg), CIP: ciprofloxacin (5 µg), ceftriaxone (30 µg), AUG: amoxicillin and clavulanate (25/10 µg), MER: meropenem (10 µg) and CTZ: ceftazidime. R: resistance, I: intermediate, S: sensitive.

**Table 2.** Antibacterial activity of different ethanolic crude extracts of *A. nilotica* against multidrug resistance diarrhea causing bacteria.

| **Extract concentration (mg/ml)** | **Mean ± SE zone of inhibition (mm)** | | | | | |
|---|---|---|---|---|---|---|
| **ELCE** | ***S. typhimurium*** | ***S. paratyphi*** | ***Salmonella* spp** | ***Shigella dysenteriae*** | ***Shigella* spp** | ***Shigella flexneri*** |
| 50 | 16.2± 0.73 | 20.7 ±2.33 | 15.3±0.67 | 8.3 ±0.88 | 11.0±2.52 | 0±0.00 |
| 100 | 20.0±1.15 | 17 ±1.15 | 11.3±0.88 | 6.3±0.67 | 10.6±3.67 | 7.00±0.58 |
| 150 | 21.3±1.20 | 16.7±0.67 | 17.3±0.88 | 9.0±0.58 | 9.6±1.20 | 10.7±1.20 |
| 200 | 23.2±0.30 | 22.7± 1.45 | 20.3±0.88 | 10.7±0.67 | 17.0±1.53 | 14.7±1.20 |
| Meropenem 30µg/ml (+ve control) | 24.3±0.88 | 20.7± 0.67 | 20.7±1.76 | 14.0±2.08 | 14.3±2.40 | 21.3±1.86 |
| 10 % DMSO (-ve control) | 0.0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 |
| **EPCE** | | | | | | |
| 50 | 8.0±1.53 | 8.7±0.88 | 6.7± 1.20 | 5.0±0.58 | 9.7±0.89 | 0±0.00 |
| 100 | 9.3±0.67 | 9.0±0.58 | 12.7± 1.20 | 11.3±1.86 | 10.7±1.20 | 0±0.00 |
| 150 | 11.3±0.88 | 11.0±0.58 | 17.0± 1.52 | 14.0±1.45 | 13.7±0.58 | 7.3 ± 0.88 |
| 200 | 13.3± 0.88 | 18.0±1.15 | 16.7± 0.33 | 18.0±0.58 | 12.3±0.33 | 11.0 ± 0.58 |
| Meropenem 30µg/ml (+ve control) | 21.0±2.08 | 21.7±1.67 | 21.7± 1.67 | 21.3±1.86 | 20.3±1.20 | 23.3 ±0.67 |
| 10 % DMSO (-ve control) | 0.0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 |
| **EBCE** | | | | | | |
| 50 | 9.0 ±0.58 | 11.33±0.89 | 9.3±1.52 | 7.67±0.78 | 6.3±0.33 | 5.0±0.62 |
| 100 | 10.0±0.58 | 12.67±1.40 | 14.0±1.00 | 12.7± 0.58 | 9.0±0.58 | 9.0±.045 |
| 150 | 14.7±0.33 | 14.0±0.58 | 13.3±1.53 | 12.33333± | 9.3±0.33 | 9.3±0.45 |
| 200 | 13.0±0.58 | 15.3±0.33 | 13.7±1.52 | 13.66667± | 11.0±0.58 | 10.3±0.52 |
| Meropenem 30µg/ml (+ve control) | 21.7± 0.89 | 24.3±1.20 | 21.0 ± 1.00 | 20.3±0.33 | 20.3±0.33 | 20.0 ±0.32 |
| 10 % DMSO (-ve control) | 0.0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 |
| **ELPBCE** | | | | | | |
| 50 | 15.0±0.58 | 17.3±1.20 | 18.7±0.33 | 19.3±0.33 | 19.7±0.33 | 20.3±0.33 |
| 100 | 16.0±0.58 | 19.3±0.33 | 21.0±1.00 | 20.7±0.67 | 21.3±0.88 | 22.3±0.33 |
| 150 | 18.3±0.88 | 19.7±0.33 | 20.0±0.00 | 20.7±0.33 | 21.3±0.33 | 22.0±0.58 |
| 200 | 19.0±0.58 | 20.7±0.88 | 22.0±0.58 | 22.3±0.33 | 22.3±0.33 | 22.7±0.33 |
| Meropenem 30µg/ml (+ve control ) | 20.7±0.67 | 21.7±0.88 | 21.7±0.88 | 22.0±0.58 | 22.3±0.33 | 21.7±0.88 |
| 10 % DMSO (-ve control) | 0.0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 |

**Key**: **ELCE**: Ethanol leaves crude extract, **EPCE**: Ethanol Pods crude extract, **EBCE**: Ethanol bark crude extract, **ELPBCE**: Ethanolic leaves, pod and back crude extract, **DMSO**: Di- Methyl Sulfoxide

**Table 3.** Minimum inhibitory concentration and minimum bactericidal concentration of synergistic ethanolic crude extracts of *A. nilotica* against multidrug resistance diarrhea causing bacteria

| **Bacterial species** | **Minimum inhibitory concentration (mg/ml)** | | | | **Minimum bactericidal concentration (mg/ml)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **ELCE** | **EPCE** | **EBCE** | **ELPBCE** | **ELCE** | **EPCE** | **EBCE** | **ELPBCE** |
| *S. typhimurium* | 100 | 200 | 200 | 100 | 200 | 400 | >400 | 100 |
| *S. paratyphi* | 100 | 200 | 200 | 100 | 200 | 400 | >400 | 200 |
| *Salmonella* spp | 200 | 100 | 200 | 200 | 400 | 200 | 400 | 400 |
| *Shigella dysenteriae* | 200 | 200 | 200 | 100 | 400 | 400 | 400 | 200 |
| *Shigella* spp | 100 | 200 | 200 | 100 | 200 | 400 | 400 | 200 |
| *Shigella flexneri* | 200 | 100 | 200 | 100 | 400 | 400 | >400 | 200 |

**Key: ELCE**: Ethanol leaves crude extract, **EPCE**: Ethanol Pods crude extract, **EBCE**: Ethanol bark crude extract, **ELPBCE**: Ethanolic leaves, pod and back crude extract.

**Table 4.** Antibacterial activity of different fractions of synergistic ethanolic crude extracts of *A. nilotica* against multidrug resistance diarrhea causing bacteria

| **Extract fraction concentration (mg/ml)** | **Mean ± SE zone of inhibition (mm)** | | | | | |
|---|---|---|---|---|---|---|
| **$F^1$ ELPBCE** | ***S. typhimurium*** | ***S. paratyphi*** | ***Salmonella* spp.** | ***Shigella dysenteriae*** | ***Shigella* spp.** | ***Shigella flexneri*** |
| 25 | 22.7±3.18 | 20 .0± 0.58 | 20.0±0.58 | 17.3±0.88 | 21.0±1.00 | 21.0 ±1.00 |
| 50 | 22.3±3.38 | 21.3±1.33 | 21.0±1.53 | 17.0±0.58 | 21.3±1.33 | 21.0±0.58 |
| 75 | 21.0±0.58 | 21.0±0.58 | 20.7±0.33 | 20.3±0.89 | 21.0±0.58 | 22.7±0.67 |
| 100 | 22.7±0.33 | 22.7±0.67 | 19.7±0.67 | 17.0±0.58 | 21.0±0.58 | 22.7±0.67 |
| Meropenem 30µg/ml (+ve) | 20.3±0.33 | 21.0±0.58 | 21.0±0.58 | 19.3±0.67 | 21.0±0.58 | 22.3±1.20 |
| 10 % DMSO (-ve) | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| **$F^2$ ELPBCE** | | | | | | |
| 25 | 23.0±0.58 | 23.0±0.58 | 23.7±0.88 | 21.0±1.00 | 16.7±1.20 | 20.7±1.20 |
| 50 | 23.0±1.52 | 23.7±0.33 | 24.3±0.88 | 22.3±0.67 | 20.7±1.20 | 23.3±2.03 |
| 75 | 25.0±0.58 | 26.0±0.58 | 26.7±0.88 | 24.0±2.31 | 23.0±1.53 | 25.7±1.20 |
| 100 | 25.3±0.7 | 26.3±0.33 | 27.7±0.33 | 27.0±1.00 | 25.7±1.20 | 28.0±.58 |
| Meropenem 30µg/ml (+ve) | 21.7±0.88 | 22.0±1.00 | 21.7±0.88 | 21.0±0.58 | 21.0±0.58 | 21.0±0.58 |
| 10 % DMSO (-ve) | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| **$F^3$ ELPBCE** | | | | | | |
| 25 | 20.7±0.88 | 21.7±0.88 | 19.7±0.33 | 18.3±0.67 | 18.7±0.88 | 20.3±0.88 |
| 50 | 25.0±1.73 | 25.7±1.20 | 21.0±0.58 | 19.0±0.58 | 19.7±0.33 | 21.0±1.00 |
| 75 | 27.0±0.58 | 28.0±0.58 | 23.3±0.33 | 22.0±1.00 | 22.7±1.45 | 24.7±0.33 |
| 100 | 29.7±0.33 | 31.0±1.00 | 26.0±0.58 | 24.0±0.58 | 27.7±0.33 | 27.7±0.33 |
| Meropenem 30µg/ml(+ve) | 21.7±0.33 | 21.7±0.33 | 22.3±0.33 | 22.0±0.58 | 22.0±0.58 | 22.0±0.58 |
| 10 % DMSO (-ve) | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |

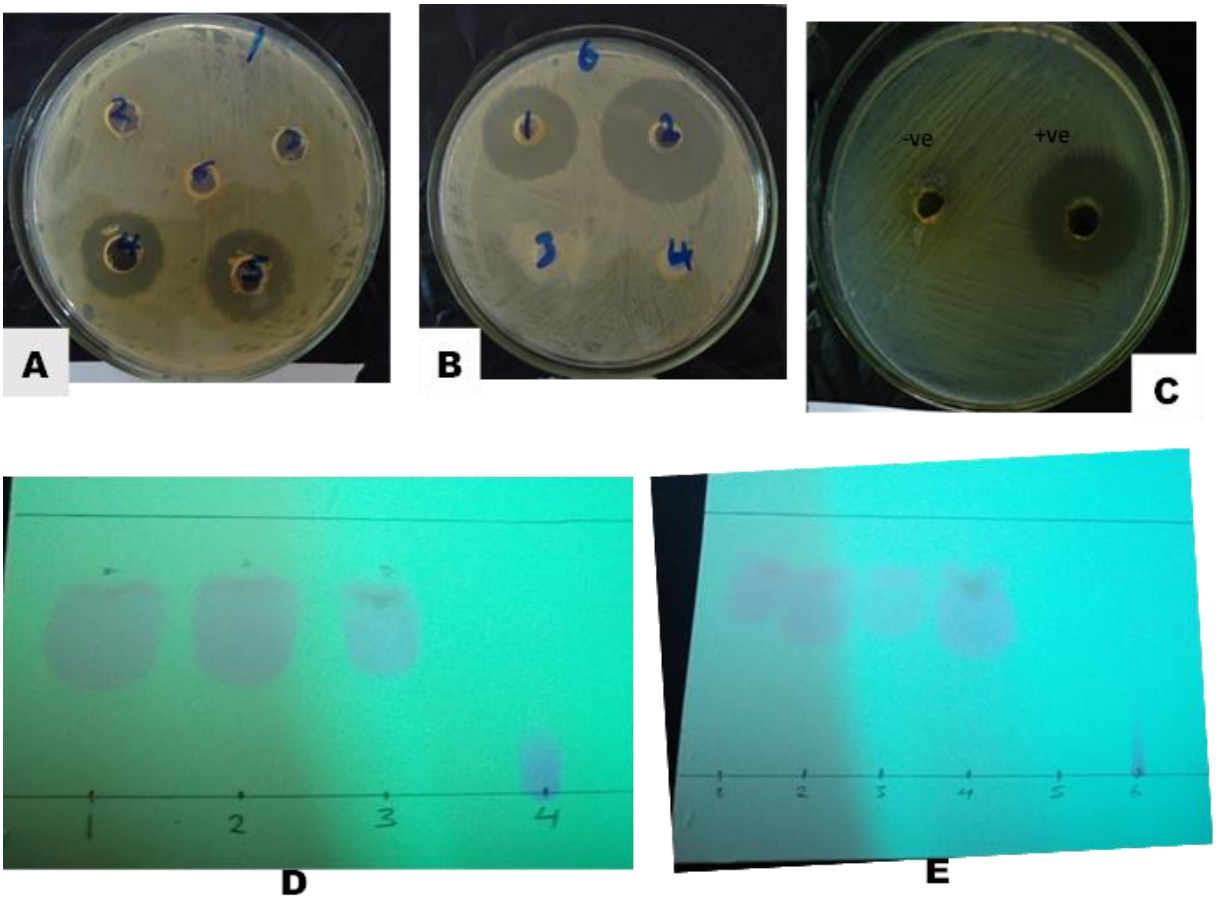
Key: ELPBCE: Ethanolic leaves, pod and back crude extract, $F^{1,2,3}$: Fraction 1, 2 and 3.

**Table 5.** Minimum inhibitory concentration and minimum bactericidal concentration of different fraction of synergistic ethanolic crude extract of *A. nilotica* against multidrug resistance diarrhea causing bacteria

| **Bacterial species** | **Minimum inhibitory concentration (mg/ml)** | | | **Minimum bactericidal concentration (mg/ml)** | | |
|---|---|---|---|---|---|---|
| | **$F^1$** | **$F^2$** | **$F^3$** | **$F^1$** | **$F^2$** | **$F^3$** |
| ***S. typhimurium*** | 12.5 | 12.5 | 12.5 | 25.0 | 25.0 | 50.0 |
| ***S. paratyphi*** | 25.0 | 12.5 | 25.0 | 50.0 | 25.0 | 100.0 |
| ***Salmonella spp*** | 25.0 | 25.0 | 50.0 | 50.0 | 50.0 | 50.0 |
| ***Shigella dysenteriae*** | 50.0 | 25.0 | 50.0 | 100.0 | 50.0 | 100.0 |
| ***Shigella spp*** | 12.5 | 50.0 | 25.0 | 25.0 | 50.0 | 50.0 |
| ***Shigella flexneri*** | 12.5 | 25.0 | 25.0 | 25.0 | 50.0 | 50.0 |

Key: MIC: Minimum inhibitory concentration and MBC: Minimum bactericidal concentration

**Figure 1. A,B.** Antibacterial activities of *Acacia nilotica* ethanolic Pods crude extract against Multidrug resistance *Shigella flexneri*. **C**: -ve control (10% DMSO), +ve control (Meropenem; 30 µg/ ml), **E,D**: Thin layer chromatography (TLC) of synergistic ethanolic crude extract.




## Conclusion

This research found that synergistic ethanolic crude extract of *A. nilotica* had higher antibacterial activity compared to pod, leaves and back ethanolic crude extracts against multi-drug resistance Diarrhea causing pathogens. The fractions of synergistic ethanolic crude extract of *A. nilotica.*

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