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Thermostability and *in-vitro* Antibacterial Activity of Aqueous Extracts of *Tetrapleura tetraptera* Pods on Multidrug Resistant Clinical Isolates

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Authors' contributions

This work was carried out in collaboration between both authors. Author OJA designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors OJA and AOO managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The thermostability of aqueous extracts of *Tetrapleura tetraptera* pods (AETTP) and its *in-vitro* antibacterial activities on multidrug-resistant (MDR) clinical isolates were carried out using standard bacteriological and disc diffusion techniques. Eleven genera comprising *Staphylococcus*, *Escherichia*, *Proteus*, *Streptococcus, Klebsiella, Pseudomonas, Citrobacter, Enterobacter, Salmonella*, *Enterococcus* and *Serratia* were obtained from the clinical samples. The results showed that between 19 /35 (54.3%) and 30 /35 (85.7%) faecal isolates were sensitive to all the antibiotics, while *Streptococcus pyogenes* isolated from wound samples were highly sensitive to gentamycin, nalidixic acid, ciprofloxacin and ofloxacin. Less than 31 (32.6%) of the bacteria showed resistance to multiple antibiotics with 19 (20%) showing resistance to ≥ 4 antibiotics. The phytochemical screening and qualitative estimations of AETTP revealed the presence of alkaloids,

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anthraquinones, tannins, flavonoids, steroids, reducing sugar, saponins, cardiac glycoside and protein in varied concentrations. The MDR bacteria were markedly inhibited by 40 $mgml^{-1}$ and 80 $mgml^{-1}$ AETTP kept at 26ºC with inhibitory zones ranging from 10.3 ± 1.0 mm to 17.4 ± 2.2 mm, while decrease in the inhibitory zones were observed when AETTP boiled to 50ºC, 75ºC and 100ºC were used. The AETTP boiled to 100^0C immensely lost its antibacterial activities, as ≥ 42.1% of the bacteria were resistant to AETTP. The MIC values of AETTP ranged from 5 to 40 $mgml^{-1}$, while the MBC values ranged from 20 to 160 $mgml^{-1}$. The highest MIC of 40 $mgml^{-1}$ and MBC of 160 $mgml^{-1}$ were obtained when the AETTP was tested against *S. aureus* (SAU5) and *Salmonella* spp (SSS2). Although, AETTP has antibacterial activities on both MDR Gram positive and Gram negative bacteria, could be used to develop a novel and broad-spectrum antibiotic for the treatment of MDR bacterial infections, its antibacterial activities could be markedly lost by high temperature.

Keywords: Thermostability; phytochemical; Tetrapleura tetraptera; bacteria; aqueous; multidrug-resistant.

1. INTRODUCTION

Resistance of microorganisms to antibiotics have been frequently reported in recent years from all over the world, predominantly in developing countries, due to indiscriminate use of commercial antimicrobial drugs in the treatment of infectious diseases [1,2,3]. The emergence of multi-drug resistant (MDR) bacteria is also of immense concern to both clinicians and the pharmaceutical industries, since it is a major cause of treatment failure in numerous infectious diseases and increase in mortality among the general population, particularly in developing countries [4,5,6,7].

Though, the resistance development by microbes to conventional antimicrobials cannot be stopped, appropriate action such as the use antibiotic resistant inhibitors of plant origin will significantly decrease the mortality and health care costs [8]. Scientists and pharmaceutical companies are increasingly rummaging around for novel and efficient antimicrobial substances; turning their attention to medicinal plants. Exploitation of plants, owing to its availability and affordability, as traditional remedies occupy a fundamental place in developing countries particularly among a large proportion of low income rural populace and many plants have shown to be highly helpful for treating sundry ailments [9].

Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world [10]. P lants produce and contain a diverse range of bioactive constituents such as alkaloids, flavonoids, cardiac glycoside and phenolics, consequently, purportedly provide excellent leads for new drug developments [11].

Tetrapleura tetraptera is one of the flowering, medicinal, deciduous plants which belongs to the family Fabaceae and usually found in the lowland forest of tropical Africa [12]. *T. tetraptera* is known as 'Aridan' in the South Western part of Nigeria; 'Uyayak' in Ibibio; 'Edeminang' in Efik; 'Ubukirihu' or Oshosho in Igbo; 'Dawo' in Hausa and 'Abogolo' among the Igalas [13]. The four winged pods (fruits) of *T. tetraptera* comprise woody shell, a freshy pulp and small brownish-black seeds with a pleasant and fragrant odour [12,14]. In tropical Africa, the pods of *T. tetraptera* are regularly and therapeutically useful in the management and / or control of convulsions, asthma, epilepsy, rheumatoid pains, schistosomiasis, hypertension, flatulence, jaundice, fevers. [13,15] and treatment of skin infections, leprosy and gastrointestinal related clinical problems [16,17]. The *Aridan* and *aridanin* isolated from the fruits, leaf-stalk, stem-bark and root-bark of *T. tetraptera* could effectively interrupt the life cycle of the schistosome by its strong molluscicidal properties [18]. The aqueous extract of the pods have been reported to possess hypoglycaemic properties, consequently, are used to manage and control adult-onset, type 2 diabetes mellitus in South-Western Nigeria [13].

In West Africa, particularly Southern and Eastern parts of Nigeria, *T. tetraptera* is also traditionally used as a seasoning spice and dietary supplement rich in multivitamins [19,20]. *T. tetraptera* fruits could be used to prepare soup for nursing mothers to avert post-partum contractions [15]. The antimicrobial activities of several plant metabolites have been reported to be unstable due to a number of factors such as enzymatic degradation, environmental factors (light, oxygen, metal ions), heat (temperature)

and duration of time between storage and usage [21,22].

Consequently, this study was designed to determine the preliminary phytochemical constituents of aqueous extracts of *T. tetraptera* pods (fruits), and its thermostability and activity on some MDR clinical isolates.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Sixty (60) clinical samples comprising mid stream urine (n =20), stool (n =20) and wound swab (n =20) were aseptically collected using sterile wide-mouth containers and swab sticks, between June and September, 2014, from patients in a tertiary institution health centre in Uyo, Akwa Ibom State. The samples were properly labelled indicating the source, date, time of collection and sex of the patients and were transported in cooler boxes to the Microbiology Laboratory, University of Uyo for bacteriological analysis.

2.2 Bacteriology of the Clinical Samples

Zero point one (0.1 ml) of each well mixed mid stream urine samples was inoculated onto plates of MacConkey agar, blood agar and cysteine lactose electrolyte deficient agar. For the wound samples, the swab end of the swab sticks used for the collection was cut, dropped into 9 ml of sterile distilled water and 0.1 ml was pipetted and inoculated onto plates of MacConkey agar, mannitol salt agar and blood agar. The stool samples were serially diluted and 0.1 ml of the aliquot inoculated onto plates of MacConkey agar, Eosin Methylene Blue agar and Salmonella- Shigella agar. All the plates were incubated for 24hr at 37ºC. After incubation, cultures with significant growth were further subcultured and incubated for 24 hr. Characterization and identification of the isolates were carried out using their colonial appearances, Gram staining technique and biochemical tests (catalase test, citrate utilization, coagulase, oxidase, Vogues-Proskauer, indole production , sucrose, maltose, lactose and mannitol) [23].

2.3 Antibiotic Susceptibility Testing

In vitro susceptibility of the clinical isolates to ten different antibiotics was determined using disc diffusion technique [24]. Zero point one (0.1) ml of each bacterial isolates prepared directly from an overnight agar plate, adjusted to 0.5 McFarland Standard, was inoculated using sterile pipette onto each of the plates of Mueller-Hinton Agar (MHA). The commercially available antibiotic discs containing Ampicillin (AMP, 10 µg), Tetracycline (TET, 30 µg), Amoxicillin (AMX,10 µg), Amoxy-clavulanic (AMO, 20 /10 µg), Ofloxacin (OFL,5 µg), Ceftriaxone (CTX, 30 µg), Nalidixic acid (NA, 30 µg), Ceftazidime (CTZ, 30 µg), Ciprofloxacin (CIP, 5 µg) and Gentamycin (GEN, 10 µg) (Oxoid, UK) were aseptically placed on the surfaces of the culture plates with a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37℃ for 18-24 hr; zones of inhibition were observed and measured in millimeters (mm) using a ruler. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative manual. Isolates that were resistant to three or more antibiotics were taken to be multiple antibiotic resistant [25].

2.4 Sources of Plant Pods, Preparation and Concentration of the Extracts

The *Tetrapleura tetraptera* pods were obtained in Uyo, Akwa Ibom State. The plant's pods were authenticated by a taxonomist in Department of Botany and Ecological Studies and later transferred to Pharmacognosy and Natural Medicine Laboratory, Faculty of Pharmacy, University of Uyo for processing. The pods were washed with distilled water, air-dried at room temperature for one month and the dried pods were pulverized using mortar and pestle into fine powdered. The aqueous extract was prepared by soaking 1 kg of the powdered pods into 1 litre of distilled water for 24 hrs with constant shaking at room temperature. It was then filtered using Whatman No 1 filter paper and the extracted liquid (filtrate) was evaporated to dryness with steam on water bath (40℃). The dried extract was weighed and stored. The graded concentrations (20 $mgml^{-1}$, 40 $mgml^{-1}$ and 80 $mgml^{-1}$) of the extracts were aseptically prepared using 100 ml of Dimethyl sulphoxide (DMSO), Aldrich, Milwaukee, WI, USA) and shaken vigorously to obtain a homogenous mixture.

2.5 Effect of Temperature on Stability of Aqueous Extracts of *T. tetraptera* pods (AETTP)

Thermostability of AETTP was determined by putting 100 ml each of the different

concentrations of the extracts in three different conical flasks and heating the extracts in a water bath at 50ºC, 75ºC and 100ºC for 15 min. After cooling, the extracts were tested for antibacterial activity.

2.6 Antibacterial Activity of AETTP kept at 26ºC and AETTP Boiled at Different Temperatures on MDR Bacterial Isolates

The antibacterial activities of AETTP kept at room temp. (26ºC) and AETTP boiled at different temps. (50ºC, 75ºC and 100ºC) were determined by disc diffusion method. Mueller – Hinton Agar (MHA, Difco, France) was sterilized, cooled to 45 – 50ºC and then poured into sterilized Petri dishes. Sterile filter paper discs (6 mm) were impregnated with AETTP solution of graded concentrations (20 $mgml^{-1}$, 40 $mgml^{-1}$ and 80 $mgml^{-1}$), carefully placed on to agar plates which had previously been inoculated with 0.1 ml of standardized inoculum suspension (10^6 CFU/ml of MDR-bacteria) using sterilized forceps and the plates were then incubated at 37ºC for 24 hr. Control experiments comprising Levofloxacin (5 µg / disc) and DMSO were also set up. Assays were performed in triplicate; the diameters of the inhibitory zones were measured in millimeters and reported as the mean ± standard deviation (SD).

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

The MIC of the AETTP was determined for each of the test MDR-bacterial isolates in test tubes using macro broth dilution techniques [26]. Eight (16 g) of the AETTP were weighed and dissolved into 100 ml of the DMSO to give a concentration of 160 $mgml^{-1}$. The stock solution (160 $mgml^{-1}$) was serially diluted by two-fold dilution with pipette to concentrations of 80, 40, 20, 10 and 5 $mgml^{-1}$. To 0.1 ml of varying concentrations of the AETTP (5, 10, 20, 40, 80 and 160 $mgml^{-1}$) in test tubes, nutrient broth (9 ml) was added and then a loopful of the MDR-bacteria. A tube containing nutrient broth was only inoculated with the MDR-bacteria to serve as control. The culture tubes were then incubated overnight at 37ºC. After incubation the tubes were then examined for turbidity (indication of microbial growth). The tube containing the lowest concentration of AETTP showing no visible sign of growth was considered as the MIC. To determine the MBC of the AETTP, 1ml of the broth was collected from the tubes that showed no growth and inoculated onto sterile nutrient agar. The plates were then incubated at 37ºC for 24 hrs. After incubation, the concentration that showed no visible growth was considered as the MBC.

2.8 Determination of Activity Index (A.I)

Activity Index (A.I) was calculated as the mean inhibitory zone of AETTP divided by the mean inhibitory zone of the standard drug used (Levofloxacin).

[Activity Index = (Mean inhibitory zone of AETTP/ Mean inhibitory zone of Levofloxacin)]

2.9 Phytochemical Screening

The phytochemical constituents of the AETTP were analyzed using the methods described by [27,28].

2.10 Test for Alkaloids

Half a gram (0.5 g) of the AETTP was dissolved in 5ml of 5% Hydrochloric acid (HCl) and then filtered. The filtrate was used to test for the presence of alkaloids as follows:

(i) Picric Test: One millilitre (1 ml) of the filtrate was treated with a few drops of saturated picric acid solution. Formation of a yellow coloured precipitate indicated the presence of alkaloids.

(ii) Mayer's Test: One millilitre (1 ml) of the filtrate was treated with a few drops of Mayer's reagent (Potassium mercuric iodide). Formation of a turbidity / yellow coloured precipitate indicated the presence of alkaloids.

(iii) Drangendorff's Test: One millilitre (1ml) of the filtrate was treated with a few drops of Drangendorff's reagent. Formation of a pink or red coloured precipitate with turbidity indicated the presence of alkaloids.

2.11 Test for Tannins

Half a gram (0.5 g) of the AETTP was dissolved in 5 ml of distilled water and then filtered. The filtrate was used to test for the presence of alkaloids as follows:

(i) Ferric Chloride Test: One millilitre (1 ml) of the filtrate was treated with a few drops of

5% ferric chloride ($FeCl_3$) reagent. Formation of a blue-green precipitate indicated the presence of tannins.

(ii) Bromine Water Test: Five (5) drops of the filtrate was mixed with the bromine water. Decolourization of bromine water was taken as evidence for the presence of tannins.

2.12 Test for Resins

Half a gram (0.5 g) of the AETTP was dissolved in acetone; thereafter, water was added and then shaken. Turbidity indicated the presence of resins.

2.13 Test for Saponins

(i) Frothing Test: Half a gram (0.5 g) of the AETTP was shaken vigorously with distilled water in a test tube. Frothing that persisted on warning was taken as preliminary evidence for the presence of saponins.

(ii) Sodium Bicarbonate Test: Half a gram (0.5 g) of the AETTP was added with 5% sodium bicarbonate and Fehling's solution A and B and boiled. Presence of brown precipitate indicated the presence of saponins.

2.14 Test for Cardiac Glycosides

(i) Salkowski's Test: Half a gram (0.5 g) of AETTP was dissolved in 2 ml of chloroform. Concentrated sulphuric acid was carefully added by running it down the side of the test tube. A reddish brown colour at the interphase showed the presence of glycon portion of cardiac.

(ii) Keller-Killiani's Test: Half a gram (0.5 g) of AETTP was dissolved in 2 ml glacial acetic acid. This was then underplayed with 1ml concentrated sulphuric acid. A brown ring obtained at the interphase, indicated the presence of deoxy-sugar, characteristic of cardiac glycoside. A violet ring appeared below the brown ring while in the acetic acid layer a greenish, ring formed just above the brown ring and gradually spread throughout this layer.

(iii) Lieberman's Test: Half a gram (0.5 g) of AETTP was added to 3mls of chloroform and filtered; 10 drops of acetic anhydride was added to the filtrate along with 2 drops of concentrated sulphuric acid. A pink colour at the interphase was a positive for terpenes, while a bluish colour was the steroids.

2.15 Test for Phlobatanins

Half a gram (0.5 g) of the AETTP was dissolved in 5 ml of distilled water and then filtered. Two millilitres (2 ml) of the filtrate was added to 2 ml of diluted HCl, thereafter boiled. Formation of a red precipitate indicated the presence of phlobatanins.

2.16 Test for Anthraquinones

(i) Borntrager's test for the free hydroxyanthraquinones: Half a gram (0.5 g) of AETTP was shaken with 10ml benzene and filtered. To the filtrate were added 10% ammonia (NH_3) solution and the mixture shaken. The presence of a pink, red or violet colour in the ammonical (lower) phase indicated the presence of free anthraquinones.

(ii) Borntrager's test for combined anthraquinones: Half a gram (0.5 g) of AETTP was boiled with 10 ml dilute sulphuric acid (H_2SO_4) and filtered. The filtrate was shaken with 5 ml benzene and 10% ammonia (NH_3) solution was added to the separate benzene layer. A pink red or violet colouration in the ammonia (lower) layer indicated the presence of anthraquinones derivative.

2.17 Test for Flavonoids

(i) Shinoda Reduction Test: A few fragments of magnesium metal were added to 5 ml of AETTP solution, followed by drop-wise addition of concentrated hydrochloric acid (HCl) to dissolve the extract. The formation of orange, red crimson or magenta colouration after few minutes indicated the presence of flavonoids.

(ii) Alkaline Reagent Test: The AETTP was treated with a few drops of sodium hydroxide (NaOH) solution. Formation of intense yellow colour, which became colourless on addition of diluted acid, indicated the presence of flavonoids.

2.18 Test for Reducing Sugar

Half a gram (0.5 g) of the AETTP was dissolved in 5 ml of distilled water and then filtered. The

filtrate was used to test for the presence of reducing sugar as follows:

(i) Benedict's test: One millilitre (1 ml) of the filtrate was treated with a few drops of Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.
(ii) Fehling's Test: One millilitre (1 ml) of the filtrate was hydrolysed with a few drops of diluted HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of an orange red precipitate indicated the presence of reducing sugars.

2.19 Test for Protein

(i) Xanthoproteic Test: Half a gram (0.5 g) of the AETTP was dissolved in a few drops of concentrated nitric acid solution. Formation of yellow colour indicated the presence of proteins
(ii) Biuret's Test: Half a gram (0.5 g) of the AETTP was dissolved in 1 ml of 10% sodium hydroxide (NaOH) solution and heated. Thereafter, a drop of 0.7% copper sulphate solution was added. Formation of purplish violet colour indicated the presence of proteins.

Fig. 1. *Tetrapleura tetraptera* pods

3. RESULTS

A total of ninety-five (95) bacterial isolates were obtained from the 60 clinical (faecal, mid-stream urine and wound) samples and their frequencies of occurrences are shown in Table 1. Thirty-five (35) enteric bacteria comprising *Escherichia coli* 12 (34.3%), *Proteus mirabilis* 8 (22.9%), *Enterobacter* spp 6 (17.1%), *Serratia marcescens* 3(8.6%), *Citrobacter freundii* 3(8.6%) and *Salmonella* spp 3(8.6%) were obtained from the faecal samples. *E. coli* was the most prevalent bacterial isolate obtained from the mid-stream urine samples, accounting for 30% of the isolates; followed by *Staphylococcus aureus* 6 (20.0%) and *P. mirabilis* 5 (16.7%), *Pseudomonas aureginosa* 4 (13.3%), while *Klebsiella pneumoniae* and *Enterococcus faecalis* had 3 (10.0%) each. Seven genera consisting of *Staphylococcus*, *Escherichia*, *Proteus*, *Streptococcus, Klebsiella, Pseudomonas* and *Enterococcus* were obtained from the wound samples (Table 1).

Table 2 indicates the detailed results of the antibiotic sensitivity of the bacteria isolated from the clinical samples. Of all the faecal bacterial isolates (n =35), between 19 (54.3%) and 30 (85.7%) isolates were sensitive to all the antibiotics tested. All the *S. marcescens* were sensitive to NA, CIP and OFL, while *C. freundii* and *Salmonella* spp were resistant to AMP. Antibiogram results of the urinary isolates showed CIP and GEN as the most active antibiotics with resistance level < 23.3%, while between 17(56.7%) and 26 (86.7%) of the isolates from the wound were sensitive to the antibiotics used. *S. pyogenes* isolated from wound samples were highly sensitive to TET, GEN, NA, CIP and OFL, and were equally highly resistant to AMP, AMX, AMO, CTZ, and CTX (Table 2).

Of the 95 bacteria isolated from wound, stool and urine, ≥ 31 (32.6%) of the isolates showed resistance to multiple antibiotics with 19 (20%) showing resistance to 4-8 antibioitcs. Interestingly, only the *E. coli* especially those with codes ECU5, ECW4 and ECS8 were resistant to ≥ 8 antibiotics. Varied resistant patterns of the clinical isolates are shown in Table 3. Although *S. aureus* (SAW7) and *S. aureus* (SAW2) were isolated from wound samples their antibiotic resistant patterns were completely different. The antibiotic resistant pattern of *S. aureus* (SAW7) was AMP-TET-AMO-CTZ, while *S. aureus* (SAW2) was AMO-CTZ-GEN-NA-OFL (Table 3).

The results of phytochemical screening and qualitative estimation of AETTP are shown in Table 4. The phytochemical screening revealed the presence of the active medicinal chemical constituents such as alkaloids, anthraquinones,

tannins, flavonoids, steroids, reducing sugar, saponins, cardiac glycoside and protein, while phlobatannins and resin were not detected. The qualitative estimations of AETTP showed alkaloids and tannins to be present in very high concentration; flavonoids and cardiac glycoside in moderately high concentration, while anthraquinones, reducing sugar and protein were present in low concentration (Table 4).

The results of antibacterial activities of AETTP kept at room temperature (26ºC) on MDR bacteria obtained from clinical samples are shown in Table 5. All the MDR bacteria were markedly inhibited by both 40 $mgml^{-1}$ and 80 $mgml^{-1}$ AETTP kept at room temperature (26ºC) with inhibitory zones ranging from 7.9 ± 1.5 mm to 16.0 ± 2.5 mm (Table 5). The narrowest inhibitory zone was observed against *S. aureus,* a Gram positive organism, while the widest zone of inhibition was observed against *P. aeruginosa*, a Gram-negative organism. Thus, indicating that AETTP has the potential of a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. Wider inhibitory zones were obtained when the antibacterial activities of 80 $mgml^{-1}$ AETTP kept at room temperature (26ºC) were evaluated on *E. coli* (ECW4), *P. aureginosa* (PAU6) and *C. freundii* (CFS2) than when antibiotic (levofloxacin) was used.

The effects of temperature (50ºC, 7ºC and 100ºC) on the stability of different concentrations (20 $mgml^{-1}$, 40 $mgml^{-1}$ and 80 $mgml^{-1}$) of AETTP are shown in Tables 6, 7 and 8. From the results obtained it was evident that temperature had marked effect on the antibacterial efficacies of the AETTP as decrease in the inhibitory activities of (20 $mgml^{-1}$, 40 $mgml^{-1}$ and 80 $mgml^{-1}$) AETTP boiled to 50ºC, 75ºC and 100ºC were observed. Thus, suggesting that the lower the temperature at which the AETTP was boiled the wider the inhibitory zones. *S. aureus* with codes (SAW2 and SAU5) were resistant to growth inhibition of 20 $mgml^{-1}$, 40 $mgml^{-1}$ and 80 $mgml^{-1}$ AETTP boiled to 50ºC (Table 6). Of the 19 MDR bacterial isolates used, *P. aureginosa* (PAU6) was most sensitive to 80 $mgml^{-1}$ AETTP boil ed at 75ºC with inhibitory zones of 13.5 ± 2.0 mm (Table 7). The AETTP boiled at 50ºC, 75ºC and 100ºC showed stronger growth inhibitory activity than the commercial antibiotic (levofloxacin) on *E.coli* (ECU5) isolated from urine sample with the mean inhibitory zones ranging from 8.3 ± 1.0 mm to 11.6 ± 1.0 mm. The AETTP boiled to 100ºC immensely lost its antibacterial activities, as ≥ 8 (42.1%) of the isolates were resistant to 20 $mgml^{-1}$ and 40 $mgml^{-1}$ of the AETTP (Table 8). The lowest zone of inhibition obtained when AETTP boiled to 100ºC was tested against the MDR bacteria was 7.3 ± 1.5 mm as observed in *E. coli* (ECS8) with the activities index of 0.51 (Table 8).

Table 1. Occurrence of bacteria isolated from clinical samples

Sample	Bacterial isolates	No of occurrence	% of occurrence
Stool (n=20)	*Escherichia coli*	12	34.3
	Proteus mirabilis	8	22.9
	Serratia marcescens	3	8.6
	Citrobacter freundii	3	8.6
	Enterobacter spp	6	17.1
	Salmonella spp	3	8.6
	Total	35	100
Urine (n=20)	*Escherichia coli*	9	30.0
	Proteus mirabilis	5	16.7
	Staphylococcus aureus	6	20.0
	Klebsiella pneumoniae	3	10.0
	Enterococcus faecalis	3	10.0
	Pseudomonas aeruginosa	4	13.3
	Total	30	100
Wound (n=20)	*Staphylococcus aureus*	8	26.7
	Streptococcus pyogenes	1	3.3
	Escherichia coli	4	13.3
	Klebsiella pneumoniae	2	6.7
	Pseudomonas aeruginosa	4	13.3
	Enterococcus faecalis	3	10.0
	Proteus mirabilis	8	26.7
	Total	30	100

Table 2. Antibiotic susceptibility of bacteria isolated from clinical samples

Sample	Bacterial isolates	No (%) sensitive									
		AMP	**AMX**	**TET**	**AMO**	**CTZ**	**CTX**	**GEN**	**NA**	**CIP**	**OFL**
Stool	*E. col*	6(50.0)	6(50.0)	8(66.5)	8(66.5)	7(58.3)	6(50.0)	10(83.3)	6(50.0)	11(91.6)	7(58.3)
	P. mirabilis	5(62.5)	6(75.0)	5(62.5)	6(75.0)	5(62.5)	6(75.0)	7(87.5)	5(62.5)	7(87.5)	7(87.5)
	S. marcescens	2(66.7)	2(66.7)	1(33.3)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	3(100)	3(100)	3(100)
	C. freundii	1(33.3)	1(33.3)	2(66.7)	2(66.7)	3(100)	3(100)	2(66.7)	2(66.7)	2(66.7)	2(66.7)
	Enterobacter sp	4(66.7)	3(50.0)	4(66.7)	4(66.7)	4(66.7)	5(83.3)	5(83.3)	4(66.7)	5(83.3)	6(100)
	Salmonella spp	1(33.3)	2(66.7)	2(66.7)	3(100)	2(66.7)	1(33.3)	2(66.7)	3(100)	2(66.7)	2(66.7)
	Total (%)	19(54.3)	20(57.1)	22(62.9)	25(71.4)	23(65.7)	23(65.7)	28(80.0)	23(65.7)	30(85.7)	27(77.1
Urine	*E. coli*	5(55.6)	4(44.4)	5(55.6)	6(66.7)	5(55.6)	7(77.8)	7(77.8)	6(66.7)	8(88.9)	6(66.7)
	P. mirabilis	3(60.0)	3(60.0)	3(60.0)	4(80.0)	4(80.0)	3(60.0)	4(80.0)	3(60.0)	5(100)	4(80.0)
	S. aureus	3(50.0)	5(83.3)	4(66.7)	4(66.7)	3(50.0)	4(66.7)	5(83.3)	4(66.7)	4(66.7)	4(66.7)
	K. pneumoniae	2(66.7)	2(66.7)	2(66.7)	2(66.7)	3(100)	2(66.7)	3(100)	2(66.7)	2(66.7)	2(66.7)
	E. faecalis	2(66.7)	1(33.3)	1(33.3)	1(33.3)	2(66.7)	3(100)	2(66.7)	1(33.3)	2(66.7)	2(66.7)
	P. aeruginosa	2(50.0)	3(75.0)	3(75.0)	3(75.0)	2(50.0)	2(50.0)	2(50.0)	3(75.0)	3(75.0)	3(75.0)
	Total (%)	17(56.7)	18(60.0)	18(60.0)	20(66.7)	19(63.3)	21(70.0)	23(76.7)	19(63.3)	24(80.0)	21(70.0)
Wound	*S. aureus*	5(62.5)	7(87.5)	6(75.0)	6(75.0)	5(62.5)	7(87.5)	7(87.5)	6(75.0)	8(100)	7(87.5)
	S. pyogenes	0(0.0)	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)	1(100)	1(100)	1(100)	1(100)
	E. coli	2(50.0)	3(75.0)	1(25.0)	3(75.0)	2(50.0)	3(75.0)	2(50.0)	2(50.0)	3(75.0)	3(75.0)
	K. pneumoniae	1(50.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	2(100)	2(100)	2(100)	2(100)	2(100)
	P. aeruginosa	3(75.0)	2(50.0)	2(50.0)	2(50.0)	2(50.0)	3(75.0)	3(75.0)	2(50.0)	3(75.0)	3(75.0)
	E. faecalis	2(66.7)	2(66.7)	1(33.3)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	2(66.7)
	P. mirabilis	6(75.0)	5(62.5)	6(75.0)	6(75.0)	5(62.5)	6(75.0)	6(75.0)	6(75.0)	7(87.5)	6(75.0)
	Total (%)	19 (63.3)	20 (66.7)	18 (60.0)	20 (66.7)	17 (56.7)	23 (76.7)	23 (76.7)	21 (70.0)	26 (86.7)	24 (80.0)

Keys: AMP: Ampicillin; AMX: Amocillin; TET: Tetracycline; AMO: Amoxy-clavulanic; CTZ: Ceftazidime; CTX: Ceftriaxone; GEN: Gentamycin; NA: Nalidixic Acid; CIP: Ciprofloxacin; OFL: Ofloxacin.

Table 3. Antibiotic resistant patterns of some clinical isolates

Bacterial isolates	Codes	Antibiotic resistant pattern
E. coli	ECS4	TET-AMO-CTZ-CTX-GEN-NA-OFL
E. coli	ECU5	AMX-TET-AMO-CTZ-CTX-GEN-NA-OFL
E. coli	ECW4	AMP-AMX-TET-AMO-CTZ-CTX-GEN-NA
S. aureus	SAU5	CTZ-CTX-NA-CIP-OFL
S. aureus	SAW7	AMP-TET-AMO-CTZ
S. marcescens	SMS4	TET-AMO-CTZ-CTX-GEN
K. pneumoniae	KPU6	TET-AMO-CTZ-NA
P. aeruginosa	PAU6	AMP-AMX- TET-AMO-CTZ-CTX-NA
S. pyogenes	SPW3	TET-GEN-NA- CIP-OFL
E. faecalis	EFW9	AMP-TET-GEN-OFL
E. faecalis	EFW7	AMX-TET-AMO-CTZ-NA
K. pneumoniae	KPW8	AMP-TET-AMO-CTZ
Salmonella spp	SSS2	AMP-AMX-CTZ-CTX-NA-CIP
P. mirabilis	PMS7	AMP-TET-CTZ-CTX-GEN-NA-CIP
S. aureus	SAU1	CTZ-CTX-GEN-NA-CIP-OFL
P. mirabilis	PMS2	AMX-TET-CTZ-CTX-NA-OFL
E. coli	ECS8	AMP-AMO-CTZ-CTX-GEN-NA-CIP-OFL
S. aureus	SAW2	AMO-CTZ-GEN-NA-OFL
C. freundii	CFS2	AMX-TET-AMO-GEN-OFL

Table 4. Phytochemical constituents of aqueous extracts of *T. tetraptera* pods

Bioactive constituents	Test	Occurence
	Picric test	+++
Alkaloids	Drangendorff's test	+++
	Mayer's test	++
Tannins	Ferric Chloride test	++
	Bromine Water test	+++
Saponins	Frothing test	++
	Sodium Bio-carbonate test	+
Flavonoids	Shinoda Reduction test	++
	Alkaline Reagent test	++
Cardiac glycoside	Salkowski's test	+
	Lieberman's test	++
	Keller-killiani's test	++
Anthraquinones	Borntrager's test for Free Anthraquinones	+
	Borntrager's test for Combined Anthraquinones	+
Reducing Sugar	Benedict's test	+
	Fehling's test	+
Phlobatannins	General test	ND
Steroids	General test	++
Protein	Xanthoproteic test	+
	Biuret's test	+
Resin	Acetone-water test	ND

Keys: +++: Present in very high concentration; ++: Present in moderately high Concentration; +: Present in very low concentration; ND: Not detected.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the AETTP on the MDR bacteria are shown in Fig. 2. The MIC values of AETTP ranged from 5 to 40 $mgml^{-1}$, while the MBC values ranged from 20 to 160 $mgml^{-1}$. The MIC of the AETTP against the *E. coli* (ECS4), *E. coli* (ECS8) and *E. coli* (ECW4) were 10 $mgml^{-1}$, 20 $mgml^{-1}$ and 5 $mgml^{-1}$, respectively. The highest MIC of 40 $mgml^{-1}$ and MBC of 160 $mgml^{-1}$ were obtained when the AETTP was tested against *S. aureus* (SAU5), *Salmonella* spp (SSS2) and *S. aureus* (SAW2).

Table 5. Antibacterial activity of aqueous extracts of *T. tetraptera* pods kept at the room temp (26ºC) on MDR-bacteria

Bacteria isolates	Source	Code	Z. I. (mm± S.D) 20 mg/ml	A.I	Z. I. (mm± S.D) 40 mg/ml	A.I	Z. I. (mm± S.D) 80 mg/ml	A.I	Control	
									Levofloxacin	DMSO
E. coli	Stool	ECS4	9.5 ± 1.0[a]	0.56	11.4 ±1.0[a]	0.67	14.1 ±1.5[b]	0.83	17.0 ± 0.5[c]	NZ
E. coli	Urine	ECU5	9.9 ±1.5[a]	-	11.9 ±2.5[a]	-	12.9 ±1.0[a]	-	NZ	NZ
E. coli	Wound	ECW4	11.8 ±1.0[b]	0.79	14.1 ±1.0[b]	0.94	14.9 ± 0.5[b]	0.99	15.0 ±1.0[b]	NZ
S. aureus	Urine	SAU5	NZ	-	7.9 ± 1.5[a]	0.78	9.4 ± 0.5[a]	0.93	10.1 ±1.5[a]	NZ
S. aureus	Wound	SAW7	9.6 ± 1.0[a]	0.67	10.7 ±1.0[a]	0.74	12.0 ±1.0[a]	0.83	14.4 ±1.5[b]	NZ
S. marcescens	Stool	SMS4	11.0 ±0.5[b]	0.69	11.2 ±1.0[a]	0.70	11.9 ±1.0[a]	0.75	15.9 ± 0.5[b]	NZ
K. pneumoniae	Urine	KPU6	9.3 ±1.0[a]	0.60	9.7 ± 1.5[a]	0.63	10.3 ±1.0[a]	0.66	15.5 ±1.2[b]	NZ
P. aeruginosa	Urine	PAU6	12.3 ±1.0[b]	0.91	15.2 ±1.0[b]	1.13	16.0 ±2.5[c]	1.19	13.5 ±1.2[a]	NZ
S. pyogenes	Wound	SPW3	8.6 ± 1.5[a]	0.53	10.7 ±0.5[a]	0.66	12.6 ±1.0[a]	0.78	16.1 ±1.0[b]	NZ
E. faecalis	Wound	EFW9	8.0 ± 1.5[a]	0.61	8.5 ± 1.4[a]	0.65	10.2 ± 0.6[a]	0.78	13.1 ± 0.5[a]	NZ
E. faecalis	Wound	EFW7	8.4 ± 1.5[a]	0.59	10.2 ±1.0[a]	0.71	12.0 ±1.0[a]	0.84	14.3 ± 1.0[b]	NZ
K. pneumoniae	Wound	KPW8	10.6 ±1.0[b]	0.74	11.9 ±1.5[a]	0.83	13.1 ±0.5[b]	0.91	14.4 ±1.0[b]	NZ
Salmonella spp	Stool	SSS2	NZ	-	8.3 ±2.0[a]	0.67	9.6 ±1.0[a]	0.78	12.3 ±1.3[a]	NZ
P. mirabilis	Stool	PMS7	9.7 ±1.0[a]	0.66	12.2 ±1.0[a]	0.83	14.2 ±2.0[b]	0.97	14.7 ±0.8[b]	NZ
S. aureus	Urine	SAU1	NZ	-	9.9 ±1.2[a]	0.79	11.0 ±1.5[a]	0.87	12.6 ±1.0[a]	NZ
P. mirabilis	Stool	PMS2	9.6 ±2.0[a]	0.57	11.0 ±0.7[a]	0.66	13.4 ±1.0[b]	0.80	16.7 ± 0.5[b]	NZ
E. coli	Stool	ECS8	8.8 ±2.5[a]	0.61	10.1 ±1.1[a]	0.70	11.7 ±1.0[a]	0.81	14.4 ± 1.5[b]	NZ
S. aureus	Wound	SAW2	NZ	-	8.4 ± 2.0[a]	-	9.0 ± 2.0[a]	-	NZ	NZ
C. freundii	Stool	CFS2	9.4 ±1.0[a]	0.67	12.8 ±0.5[a]	0.91	15.1 ±1.5[b]	1.08	14.0 ±2.2[b]	NZ

Keys: Each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test (P <0.05). Z. I: Zone of Inhibition. A.I.: Activity Index.

Table 6. Effect of temperature (50ºC) on the stability and activity of aqueous extracts of *T. tetraptera* pods on MDR-bacteria

Bacteria isolates	Source	Code	Z. I. (mm± S.D) 20 mg/ml	A.I	Z. I. (mm± S.D) 40 mg/ml	A.I	Z. I. (mm± S.D) 80 mg/ml	A.I	Control	
									Levofloxacin	DMSO
E. coli	Stool	ECS4	8.8 ± 0.6[a]	0.52	10.5 ±1.2[a]	0.62	13.0 ±1.5[b]	0.76	17.0 ± 0.5[c]	NZ
E. coli	Urine	ECU5	9.2 ±1.0[a]	-	11.0 ±2.0[a]	-	11.6 ±1.0[a]	-	NZ	NZ
E. coli	Wound	ECW4	11.0 ±1.5[b]	0.73	13.0 ±1.5[b]	0.87	13.5 ± 0.5[b]	0.90	15.0 ±1.0[b]	NZ
S. aureus	Urine	SAU5	NZ	-	NZ	-	NZ	-	10.1 ±1.5[a]	NZ
S. aureus	Wound	SAW7	9.1 ± 0.8[a]	0.63	9.9 ± 1.0[a]	0.69	10.5 ±1.0[a]	0.73	14.4 ±1.5[b]	NZ

Bacteria isolates	Source	Code	Z. I. (mm± S.D) 20 mg/ml	A.I	Z. I. (mm± S.D) 40 mg/ml	A.I	Z. I. (mm± S.D) 80 mg/ml	A.I	Control	
									Levofloxacin	DMSO
S. marcescens	Stool	SMS4	10.3 ±1.2[a]	0.65	10.5 ± 1.5[a]	0.66	11.2 ±1.0[a]	0.70	15.9 ± 0.5[b]	NZ
K. pneumoniae	Urine	KPU6	8.7 ± 1.5[a]	0.56	9.2 ± 0.5[a]	0.59	9.8 ±1.0[a]	0.63	15.5 ±1.2[b]	NZ
P. aeruginosa	Urine	PAU6	11.8 ±1.0[b]	0.87	14.8 ±1.1[b]	1.09	15.2 ±0.5[b]	1.13	13.5 ±1.2[a]	NZ
S. pyogenes	Wound	SPW3	NZ	-	9.4 ±1.5[a]	0.58	11.4 ± 1.0[a]	0.70	16.1 ±1.0[b]	NZ
E. faecalis	Wound	EFW9	NZ	-	NZ	-	9.7 ± 0.6[a]	0.74	13.1 ± 0.5[a]	NZ
E. faecalis	Wound	EFW7	NZ	-	9.1 ±1.2[a]	0.64	11.7 ±1.0[a]	0.82	14.3 ± 1.0[b]	NZ
K. pneumoniae	Wound	KPW8	9.6 ±1.0[a]	0.67	10.9 ±1.4[a]	0.76	11.2 ±0.5[a]	0.78	14.4 ±1.0[b]	NZ
Salmonella spp	Stool	SSS2	NZ	-	NZ	-	9.0 ±1.0[a]	0.73	12.3 ±1.3[a]	NZ
P. mirabilis	Stool	PMS7	9.0 ±1.0[a]	0.61	11.5 ±1.8[a]	0.78	13.0 ±2.0[b]	0.88	14.7 ±0.8[b]	NZ
S. aureus	Urine	SAU1	NZ	-	9.3 ± 0.6[a]	0.74	10.5 ±1.5[a]	0.83	12.6 ±1.0[a]	NZ
P. mirabilis	Stool	PMS2	8.6 ±2.0[a]	0.51	10.2 ±1.0[a]	0.61	12.5 ±1.0[a]	0.75	16.7 ± 0.5[b]	NZ
E. coli	Stool	ECS8	7.9 ±2.5[a]	0.55	9.1 ±1.5[a]	0.63	11.0 ±1.0[a]	0.76	14.4 ± 1.5[b]	NZ
S. aureus	Wound	SAW2	NZ	-	NZ	-	NZ	-	NZ	NZ
C. freundii	Stool	CFS2	9.2 ±1.0[a]	0.66	12.2 ±1.5[a]	0.87	14.8 ±1.5[b]	1.06	14.0 ±2.2[b]	NZ

Keys: Each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test (P <0.05). Z. I: Zone of Inhibition. A.I.: Activity Index

Table 7. Effect of temperature (75ºC) on the stability and activity of aqueous extracts of *T. tetraptera* pods on MDR-bacteria

Bacteria isolates	Source	Code	Z. I. (mm± S.D) 20 mg/ ml	A.I	Z. I. (mm± S.D) 40 mg/ml	A.I	Z. I. (mm± S.D) 80 mg/ml	A.I	Control	
									Levofloxacin	DMSO
E. coli	Stool	ECS4	8.4 ±1.0[a]	0.49	10.0 ±1.5[a]	0.71	12.0 ±1.5[b]	0.86	17.0 ± 0.5[c]	NZ
E. coli	Urine	ECU5	8.9 ±0.5[a]	-	10.4 ±1.0[a]	-	11.0 ±1.0[a]	-	NZ	NZ
E. coli	Wound	ECW4	10.6 ±1.0[b]	0.71	11.2 ±1.0[b]	0.75	11.8 ± 0.5[a]	0.79	15.0 ±1.0[b]	NZ
S. aureus	Urine	SAU5	NZ	-	NZ	-	NZ	-	10.1 ±1.5[a]	NZ
S. aureus	Wound	SAW7	9.0 ±1.2[a]	0.63	9.4 ± 1.0[a]	0.65	10.0 ±1.0[a]	0.69	14.4 ±1.5[b]	NZ
S. marcescens	Stool	SMS4	9.0 ±1.0[a]	0.57	9.2 ± 1.5[a]	0.58	10.1 ±1.0[a]	0.64	15.9 ± 0.5[b]	NZ
K. pneumoniae	Urine	KPU6	8.6 ±1.5[a]	0.54	8.6 ± 2.0[a]	0.54	9.0 ±1.0[a]	0.58	15.5 ±1.2[b]	NZ
P. aeruginosa	Urine	PAU6	10.9 ±0.5[b]	0.81	11.8±1.5[b]	0.87	12.3 ±2.2[b]	0.91	13.5 ±1.2[a]	NZ
S. pyogenes	Wound	SPW3	NZ	-	8.7±2.0[a]	0.54	9.4 ± 1.0[a]	0.58	16.1 ±1.0[b]	NZ
E. faecalis	Wound	EFW9	NZ	-	NZ	-	8.2 ± 0.6[a]	0.63	13.1 ± 0.5[a]	NZ
E. faecalis	Wound	EFW7	NZ	-	9.4 ±1.2[a]	0.66	10.2 ±1.0[a]	0.71	14.3 ± 1.0[b]	NZ
K. pneumoniae	Wound	KPW8	9.4 ±1.0[a]	0.65	10.2 ±1.0[a]	0.71	10.4 ±0.5[a]	0.72	14.4 ±1.0[b]	NZ
Salmonella spp	Stool	SSS2	NZ	-	NZ	-	8.9 ±1.0[a]	0.72	12.3 ±1.3[a]	NZ

Bacteria isolates	Source	Code	Z. I. (mm± S.D) 20 mg/ ml	A.I	Z. I. (mm± S.D) 40 mg/ml	A.I	Z. I. (mm± S.D) 80 mg/ml	A.I	Control	
									Levofloxacin	DMSO
P. mirabilis	Stool	PMS7	9.0 ±1.0[a]	0.61	11.0 ±0.7[b]	0.75	12.3 ±2.0[b]	0.84	14.7 ±0.8[b]	NZ
S. aureus	Urine	SAU1	NZ	-	9.4 ±1.2[a]	0.75	9.8 ±1.5[a]	0.77	12.6 ±1.0[a]	NZ
P. mirabilis	Stool	PMS2	NZ	-	8.6 ±1.0[a]	0.51	10.3 ±1.0[a]	0.62	16.7 ± 0.5[b]	NZ
E. coli	Stool	ECS8	7.5 ±2.5[a]	0.52	8.1 ±1.0[a]	0.56	8.7 ±1.0[a]	0.60	14.4 ±1.5[b]	NZ
S. aureus	Wound	SAW2	NZ	-	NZ	-	NZ	-	NZ	NZ
C. freundii	Stool	CFS2	9.2 ±1.0[a]	0.66	10.9 ±0.5[a]	0.78	13.2 ±1.5[b]	0.94	14.0 ±2.2[b]	NZ

Keys: Each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test (P <0.05). Z. I: Zone of Inhibition. A.I.: Activity Index.

Table 8. Effect of temperature (100ºC) on the stability and activity of aqueous extracts of *T. tetraptera* pods on MDR-bacteria

Bacteria isolates	Source	Code	Z. I. (mm± S.D) 20 mg/ml	A.I	Z. I. (mm± S.D) 40 mg/ml	A.I	Z. I. (mm± S.D) 80 mg/ml	A.I	Control	
									Levofloxacin	DMSO
E. coli	Stool	ECS4	8.0 ±1.0[a]	0.47	9.4 ±2.0[a]	0.55	11.4 ±1.5[b]	0.67	17.0 ± 0.5[c]	NZ
E. coli	Urine	ECU5	8.3 ±1.0[a]	-	10.0 ±1.0[a]	-	10.4 ±1.5[a]	-	NZ	NZ
E. coli	Wound	ECW4	9.8 ±0.8[a]	0.65	10.6 ±1.2[a]	0.71	11.2 ± 0.5[b]	0.75	15.0 ±1.0[b]	NZ
S. aureus	Urine	SAU5	NZ	-	NZ	-	NZ	-	10.1 ±1.5[a]	NZ
S. aureus	Wound	SAW7	8.4 ±1.1[a]	0.58	9.0 ± 2.0[a]	0.63	9.7 ±1.5[a]	0.67	14.4 ±1.5[b]	NZ
S. marcescens	Stool	SMS4	8.7 ±1.5[a]	0.55	9.0 ± 1.4[a]	0.57	9.3 ±1.0[a]	0.58	15.9 ± 0.5[b]	NZ
K. pneumoniae	Urine	KPU6	8.3 ±1.0[a]	0.54	8.5 ± 1.0[a]	0.55	9.0 ±0.7[a]	0.58	15.5 ±1.2[b]	NZ
P. aeruginosa	Urine	PAU6	8.6 ±1.5[b]	0.64	9.5 ±1.0[a]	0.70	11.4 ±1.0[b]	0.84	13.5 ±1.2[a]	NZ
S. pyogenes	Wound	SPW3	NZ	-	8.5±0.8[a]	0.53	9.2 ± 1.5[a]	0.57	16.1 ±1.0[b]	NZ
E. faecalis	Wound	EFW9	NZ	-	NZ	-	8.0 ± 2.5[a]	0.61	13.1 ± 0.5[a]	NZ
E. faecalis	Wound	EFW7	NZ	-	9.2 ±1.0[a]	0.64	9.9 ± 2.0[a]	0.69	14.3 ± 1.0[b]	NZ
K. pneumoniae	Wound	KPW8	8.9 ±2.0[a]	0.62	9.8 ±2.0[a]	0.68	10.1 ±1.5[a]	0.70	14.4 ±1.0[b]	NZ
Salmonella spp	Stool	SSS2	NZ	-	NZ	-	8.3 ±1.5[a]	0.67	12.3 ±1.3[a]	NZ
P. mirabilis	Stool	PMS7	8.4 ±1.5[a]	0.57	9.4 ±0.7[a]	0.64	11.4 ±1.0[b]	0.78	14.7 ±0.8[b]	NZ
S. aureus	Urine	SAU1	NZ	-	8.5 ±1.0[a]	0.67	9.5 ±1.2[a]	0.75	12.6 ±1.0[a]	NZ
P. mirabilis	Stool	PMS2	NZ	-	8.5 ±1.2[a]	0.51	9.7 ±1.0[a]	0.58	16.7 ± 0.5[b]	NZ
E. coli	Stool	ECS8	7.3 ±1.5[a]	0.51	8.0 ±2.0[a]	0.56	8.5 ±1.5[a]	0.59	14.4 ±1.5[b]	NZ
S. aureus	Wound	SAW2	NZ	-	NZ	-	NZ	-	NZ	NZ
C. freundii	Stool	CFS2	9.0 ±1.0[a]	0.64	10.3 ±1.5[a]	0.74	12.8 ±2.5[b]	0.91	14.0 ±2.2[b]	NZ

Keys: Each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test (P <0.05). Z. I: Zone of Inhibition. A.I.: Activity Index

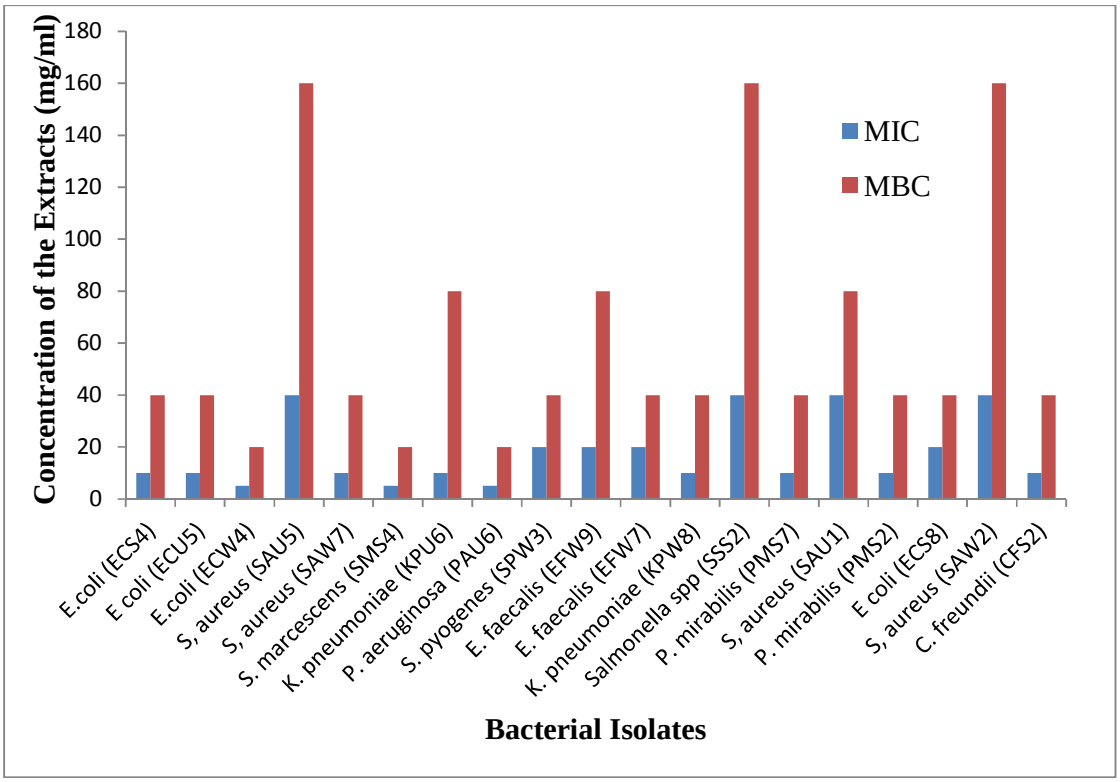

Fig. 2. Minimum Inhibitory concentration and minimum bacteriocidal concentration of AETTP

4. DISCUSSION

The increasingly resistance of microorganisms to scores of drugs and enormous increase in the death rate attributable to infectious diseases have necessitated scientists all over the world to continue to seek and develop new antimicrobial agents [2]. In this study, the MDR bacteria isolated from the clinical samples (stool, urine and wounds) were noticeably inhibited by 80 mg/ml AETTP kept at room temperature (26ºC) with the widest inhibitory zone of 16.0 ± 2.0 mm, while decrease in activities were observed when the temperature was raised to 50℃, 75℃ and 100℃, with widest inhibitory zones of 15.2 ± 0.5 mm, 13.2 ± 1.5 mm and 12.8 ± 2.5 mm, respectively. Thus, increase in temperature (50℃ to 100℃) markedly affected the antibacterial activities of the AETTP on MDR bacteria and this corroborates the previous reports that volatilization, physical and / or chemical changes that occurred during heating of natural products (plants) might result in loss of antibacterial activity of the plants [22]. Although, [29] reported the worldwide increase in the resistance of *P. aeruginosa* to many antimicrobial agents, nonetheless, the results obtained in this research showed the growth inhibition of *P. aeruginosa* by AETTP. The widest inhibitory zone obtained when AETTP was tested against *P. aeruginosa* in this study concurs with the results of [30] and this suggests that AETTP possesses substances which when extracted and purified could be used for therapeutic management of *P. aeruginosa* infections. The antibacterial activities of the AETTP on MDR *S. aureus* isolated from wound samples and MDR *E. coli* isolated from stool samples imply its potential use for the treatment of wound infections and diarrhoea caused by these organisms.

The AETTP boiled to 100ºC immensely lost its stability, antibacterial activities and this could be an indication that the bioactive components present in AETTP are labile and may be destroyed, deteriorated or become ineffective when boiled at a high very temperature for prolong periods. The deterioration of some thermolabile products as a result of rapid rate of chemical reaction when normal range of temperature is exceeded has been reported [21]. The loss of stability and antibacterial activity as temperature increases observed in this study is in dissimilarity with the previous reports that at various temperature ranges, the antibacterial

activities of *B. aegiptica* and *M. oleifera* extracts remained relatively unaffected [31]. However, our findings substantiates the earlier results of [32] who reported the complete destruction of the antibacterial activity of garlic extracts treated at 80℃ to 90℃ for 5 minutes. The observed low MIC of less than or equal to 10 mgml and MBC of less than or equal to 40 mg.ml against *E. coli*, *P. aeruginosa*, *S. pyogenes* and *S. marcescens* signify that the AETTP has the potential to treat ailments associated with these bacterial pathogens effectively.

The phytochemical screening and qualitative estimations of AETTP revealed the presence of alkaloids, anthraquinones, tannins, flavonoids, steroids, reducing sugar, saponins, cardiac glycoside and protein in varied concentrations. The detection of tannin, cardiac glycoside and protein in AETTP is in conformity with results of [33]. The presence of secondary metabolites in AETTP could provide a synergistic effect which modifies the bioavailability, effectiveness of the active components and might be responsible for its antibacterial activities. The activity of flavonoids has been traced to its ability to complex with bacterial cell walls and disruption of the microbial membrane [2]. Tannins have the ability to inactivate microbial adhesion, enzyme, cells envelop and transport proteins. It has also been reported that tannins can precipitate the proteins covering the surface of the cell or tissue, which acts as a barrier between tissue and irritants, and the underlying tissue is therefore soothed and protected from further damage, so that healing of wound can take place [34]. Consequently, the detection of tannins in AETTP and the antibacterial activities of AETTP on MDR bacteria isolated from wound infections denote its possible potential to enhance healing of wounds.

5. CONCLUSION

Conclusively, this study shows that AETTP possess broad spectrum activity against the MDR bacteria from clinical samples, justifies its ethno-pharmacological uses in the treatment of microbial infections and equally revealed that high temperature could adversely affect its antimicrobial efficacies.

CONSENT

The authors declared that written informed consent was obtained from the patients for publication of this paper.

ETHICAL APPROVAL

It is not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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