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**Original Research Article**

# Phytochemical and Antibacterial Studies of *Ensete gilletii* (E.A.J. De Wildman) Stem Extract and Fractions

**[1]Tanko, E., [1]Dauda, B.E.N., [1]Mann, A., [2]Oyeleke, S.B., [1]Fadipe, L. A. and *[3]Mathew, J.T.**

[1]Department of Chemistry, Federal University of Technology, Minna, Nigeria.
[2]Department of Microbiology, Federal University of Technology Minna, Nigeria.
[3]Department of Chemistry, Ibrahim Badamasi Babangida University Lapai, Nigeria.
*johntsadom@gmail.com; ezekieltanko@gmail.com

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**ABSTRACT**

*Ensete gilletii (family Musaceae) is ethnomedicinally used for the treatment of diarrhoea, dysentery, stomach ache, digestive disorder and other bacterial infections were investigated for their phytochemical constituents and antibacterial potentials. The phytochemical screening of the stem fractions (S1 – S4) revealed the presence of alkaloids, carboxylic acids, flavonoids, phenolic compounds, phlobatannins, reducing sugar, tannins and terpenes. Antibacterial screening of crude ethanol stem extract against Gram-positive (B. subtilis, S. aureus, S. Pyogenes) and Gram-negative (E. coli, K. pneumoniae, S. typhi and S. dysentriae) at different concentration (40, 80, 120 and 160 mg/cm$^3$). Test bacteria isolates in ethanolic stem crude extracts revealed a broad spectrum of activity in dose dependent manner. The zone of inhibition ranged from 16 to 29 mm and 16 to 31 mm of stems, compared to standard antibiotic (Ampiclox) (40 mg/cm$^3$). S. aureus, S. pyogenes and K. pneumoniae were resistant against the stem extract. The Antibacterial susceptibility test of the stem sub-fractions displayed a wider zone of inhibition in all test bacterial isolates with concentration (200 mg/cm$^3$) than the crude stem extracts with concentration (40 to 160 mg/cm$^3$), with zone of inhibition ranging (18 to 24 mm) respectively. The MIC ranged (25 to 100 mg/cm$^3$), while the MBC ranging from (50 to 100 mg/cm$^3$) in all the susceptible organisms in the stem extracts. The broader spectrum of activity displayed than the standard antibiotic drugs (Ampiclox) suggest that the stem parts of the plant could be used as pharmaceutically important agents in drug formulation in the treatment of numerous diseases.*

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## 1. INTRODUCTION

The term ‘medicinal plants’ include various types of plants used in herbals, and some of these plants can be used for medicinal or therapeutic purposes (Jamshidi-kia *et al*., 2018). These plants have played a vital role

for centuries in the development of human cultures around the globe (Welz *et al*., 2018). Various parts of the plants (leaves, seeds. flowers, fruits, stems, barks and roots) are essential parts for medicinal purposes and provide numerous bioactive compounds useful for therapeutic purposes (Inoue and Craker, 2014). The prospects for the development of antibacterial drugs from medicinal plants are in high interest as they can mitigate the adverse effects that are often associated with synthetic antibiotics (Pooja and Tannaz, 2017). Therefore, exploration of novel chemical classes derived from medicinal plants is one of the practical approaches that attract researchers in the quest for new chemotherapeutics (Shehla *et al*., 2018). Therefore, the search for new drugs against the emergence of the new infectious diseases through conventional approaches is still imperative (Weng *et al*, 2018).

*Ensete gilletii* is a wild banana which has a wide distribution range in West Africa. This specie grows in different habitat (grassland and savanna woodlands), but it seems to prefer forest edges on steep slopes, which is quite inaccessible areas, making the specie less susceptible to human interference (Scott, 2013). *E. gilletii* is described as large monocarpic flowering plant with unbranched pseudo-stem of concentric layers of fleshy leaf-petioles rising to 1.5 meters high from swollen base. *E. gilletii* resembles the banana plants, but has a long, paddle-shaped leaves with crimson midribs (Tesfaye *et al*., 2016). The fruits are similar in appearance to those of banana, but *E. gilletii* are dry, seedy plant. The seeds are dark-brown with a conspicuous white, powdery endosperm.

In Nigeria, information from local sources regarding the plant revealed that the plant has been used by rural people for the treatment of various ailments (Yemataw *et al*., 2016). For example, it was reported that, the leaves, stems and roots are used to treat various ailments, such as; dysentery, diarrhoea, stomach ache, digestive disorder, cholera, fever, urinary infection, gonorrhoea, malaria, menstrual pains, typhoid fever and insect bites as well as scorpion bites (Afolayan *et al.* 2014). Extensive literature review of the isolation and characterization of isolated compound from *E. gilletii* is scanty. However, phytochemical screening of the hexane, ethyl acetate and ethanol extracts of *E. gilletii* seeds revealed the presence of various phytoconstituents, such as; alkaloids, balsam, carbohydrates, phenolic compounds (flavonoids, tannins and phlobatannins), and steroidal compounds. Proximate composition of the seed extracts revealed the presence of moisture content, ash, fats, crude fibre and protein (Afolayan *et al*., 2014).

*Ensete gilletii* stem is ethnomedicinally used for the treatment of diarrhoea, dysentery and other bacterial infections. However, this claim based on literature review has not been investigated; hence, the growing need to explore the stem of this plant for its active metabolites and natural therapy against not only stomach disorder but other infections caused by bacteria. Therefore, the aim of this present research is to investigate the phytochemical and antibacterial potentials of the stem of *Ensete gilletii* obtained from one of the farm land in Munya Local Government Area, Niger State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1. Extraction Procedures

Fresh stems of *Ensete gilletii* were collected from a farmland in Sarkin Pawa area in Munya Local Government Area, Niger State, Nigeria in December, 2016. stems were identified and authenticated at the National Institute of Pharmaceutical Research and Development (NIPRD) Idu, Abuja Nigeria and a voucher specimen was deposited (Voucher no. NIPRD/H/6991).

### 2.2. Extraction *E. gilletii* Stem Extract

Air-dried and pulverized stem (1 kg) of *E. gilletii* was exhaustively extracted with 70 % ethanol by maceration until the extractant became colourless. The resulting solution was decanted, filtered and then

concentrated using a rotary evaporator and further dried over a water bath to afford ethanol stem extract of *E.gilletii* coded as S.

### 2.3. Phytochemical Screening

#### 2.3.1. Preliminary test for phytochemicals

The crude ethanol extracts of the stem (S) of *E. gilletii* was screened qualitatively using standard methods (Sofowora, 1993; Harbone, 1998; Trease and Evans, 2002; AOAC, 2010).

### 2.4. Fractionation of Crude Ethanol Stem Extracts of *Ensete gilletii*

#### 2.4.1. VLC of crude ethanol stem extracts (S) of *E. gilletii*

Crude ethanol stem extract (S) (20 g) of *E. gilletii* was fractionated using vacuum – liquid chromatography (VLC). The column in the glass Buchner funnel was packed with 300 g of silica gel (60 – 120 mesh) and chloroform (400 $cm^3$) was poured onto the surface of the silica gel, and suction applied with the aid of vacuum pump. The extract was solubilized with few drops of chloroform and was gently introduced on to the surface of the packing after which elution commenced with chloroform. Elutions continued in gradient form with varying proportions of increasing polarity of chloroform ($CHCl_3$): methanol (MeOH). The resultant eluates were collected in column fractions of 100 $cm^3$ each and identical fractions pooled, based on TLC profile in various solvent systems ($CHCl_3$: MeOH) (100: 0 – 0: 100) to afford four major column fractions coded as S1 – S4.

### 2.5. Antibacterial Studies

#### 2.5.1 Materials and test organisms

##### 2.5.1.1. Source of test organisms and standard drug for reference

Ampiclox capsule (500 mg) (Sam-Ace Ltd, Pharmaceutical Manufacturer Division, Nigeria) was used as the standard drug for reference. Gram – positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and*Streptococcus pyogenes*) and Gram – negative bacteria (*Escherichia coli*, *Salmonella typhi, Klebsiella pneumoniae and Shigella dysentriae*) were used for the antibacterial studies. Pure isolates were obtained from Microbiology laboratory, Federal University of Technology, Minna, Niger State, Nigeria.

##### 2.5.1.2. Preparation of inoculum

Twenty four hours (24 h) pure culture of the test organisms were sub-cultured in 20 $cm^3$ sterile nutrient agar plates and incubated at 37 $^{\circ}$C for 24 h. After the period of incubation, the test organisms were standardized for antibacterial activity (Tsado *et al.,* 2018).

### 2.6. Antibacterial Assay

#### 2.6.1. Standardization of the test organisms

Exactly 0.2 $cm^3$ of overnight cultures each organism was transferred into 20 $cm^3$ of sterile nutrient broth and incubated for 3 – 5 h to standardize the culture to $10^6$ colony-forming unit (cfu)/$cm^3$). A loopful of the standard cultures was used for the antibacterial assay (Collins *et al.*, 1995).

#### 2.6.2. Screening for antibacterial activity of the stem extract

Exactly 0.2 g of the stem extracts was reconstituted in 5.0 $cm^3$ sterile distilled water. The resulting solution was vortexed to achieve homogeneity. Exactly 1.0 $cm^3$ of the reconstituted extract was added to petri dishes after the molten nutrient agar has been sterile to afford the concentration of 200 $mg/cm^3$. The plates were prepared in duplicates which were kept at room temperature. A loopful of each of the standardized culture of test organisms was streaked on the solidified medium and incubated for 24 h at 37 °C. Control plates comprising extracts without inoculums and inoculums with extracts were made parallel. The procedure was repeated using a standard drug (Ampiclox) as a positive reference. The zones of inhibition were measured and recorded in millimeters (mm) (Collins *et al.*, 1995).

#### 2.6.3. Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined according the method of the Clinical and Laboratory Standard Institute (CLSI, 2012). Exactly 2 $cm^3$ of nutrient broth were dispensed into pre-sterilized test tubes and 2 $cm^3$ of reconstituted extract was added and serial diluted. To a test tube containing 2 $cm^3$ of nutrient broth Ampiclox was added. All the test tubes were incubated at 37 °C for 24 hours. The first test tube with no visible growth of organism is refers to as MIC.

#### 2.6.4. Determination of the minimum bactericidal concentration (MBC)

The MBC of the extract was determined using the method of Clinical and Laboratory Standard Institute (CLSI, 2012). The MIC test tube was sub-cultured on fresh sterile nutrient agar plate and incubated at 37 °C for 24 h. Presence of growth after the period of incubation was interpreted as bacteriostatic, while; the absence of growth was considered bactericidal.

### 2.7. Data Analysis

The diameter of zone of inhibition around each hole was measured when the incubation period was completed. The average of duplicate independent readings for each test organism was recorded. The data were represented as mean $\pm$ standard error of the mean (SEM). Comparison between groups were carried out using analysis of variance (ANOVA) coupled with Duncan multiple range test where significance was observed. Analysis was taken based on the significant at $< 0.05$ levels of significance. All analysis was carried out using SPSS software.

## 3. RESULTS AND DISCUSSION

Preliminary phytochemical screening of the crude ethanol stem extracts of *E. gilletii* and their fractions using standard methods are presented in Table 1. Preliminary phytochemical screening of the crude ethanol stem extract (S) as shown in Table 1 revealed the presence of alkaloids, carboxylic acids, flavonoids, phenolic compounds, reducing sugars, tannins and terpenes; while anthraquinones, cardiac glycosides, phlobatannins, saponins and steroidal compounds were not detected. Fractionation of the crude ethanol stem extract yielded 4 fractions (S1 – S4) which revealed the presence of carboxylic acids and phenolic compounds in all fractions.

Table 1: Phytochemical constituent of the crude ethanol stem extract and the fractions of the extract

| Phytochemicals | Test | Observation | S | S1 | S2 | S3 | S4 |
|---|---|---|---|---|---|---|---|
| Alkaloids | Dragendorff's | Orange precipitate | + | - | - | - | - |
| Anthraquinones | Borntrager's test | Pink- colouration | - | - | - | - | - |
| Carboxylic acids | Sodium bicarbonate test | Effervescence gas evolved | + | + | + | + | + |
| Cardiac glycosides | Keller Killani's test | Reddish-brown precipitate | - | - | - | - | - |
| Flavonoids | NaOH reagent test | Intense yellow colouration | + | + | + | - | - |
| Phenolic compounds | $FeCl_3$ test | Red precipitate | + | + | + | + | + |
| Phlobatannins | HCl reagent test | Brick-red precipitate | + | + | - | - | - |
| Reducing sugars | Fehling's test | Brick-red precipitate | + | + | - | - | - |
| Saponins | Frothing test | Persistent froth | - | - | - | - | - |
| Steroidal compounds | Libermann Buchard's test | Violet colouration | - | - | - | - | - |
| Tannins | $FeCl_3$ test | Bluish-black colouration | + | + | - | - | - |
| Terpenoids | Salkwiski's test | Reddish-brown interphase | + | + | + | - | - |

S = crude ethanol stem extract, S1 – S4 = VLC column fractions of the stems extract

Flavonoids were detected in S1 – S3; while phlobatannins, reducing sugars and tannins were all present in S1. Terpenes was present in fractions S1 and S2. Afolayan *et al.* (2014) reported the presence of similar phytochemicals in hexane, ethyl acetate and ethanol seed extracts of *E. gilletii*. The curative properties of plants are due to the presence of various phytochemical constituents such as alkaloids, carboxylic acids, phenolic compounds, saponins, reducing sugars, glycosides and other phytoconstituents (Kumar and Navaratnam, 2013; Ahmad *et al.,* 2016).

Antibacterial susceptibility test of the ethanol crude stem extracts of *E. gilltii* (Figure 1) against *B. Subtilis, S. aureus*, *S. pyogenes*, *E. coli, K. Pneumoniae, S. dysenteriea* and *S. typhi* at different concentrations (40, 60, 80, 120, and 160 mg/cm$^3$) revealed a potent activity in concentration dependent manner. The zone of inhibition ranged from 16 to 31 mm which is significantly different from the standard antibiotics (Ampiclox) at concentration (40 mg/cm$^3$). *S. aureus, S. pyogenes* and *K. pneumoniae* were all resistant against the stem crude extracts. Microorganisms vary broadly in their degree of susceptibility to antimicrobial agent; hence, the potent activity displayed by extract may depend on the concentration of the extracts (Wanda, 2018).

The MIC and MBC of crude stem extract are shown in Figures 2 and 3 respectively. The crude stem extract had the lowest MIC (80 mg/cm$^3$) for *B. Subtilis* and *S. typhi*. (Figure 2), and lowest MBC (120 mg/cm$^3$) for *E. coli* and *S. typhi* (Figure 3). The relatively high MIC (Figure 2) and MBC (Figure 3) exhibited by the crude extract against *B. Subtilis*, *E. coli*, *S. typhi*, and *S. dysentriae*, might be contributed to their cell wall properties. Gram-negative bacteria are usually protected by lipopolysaccharide layer (LPS) which hinders the direct exposure of the inner membrane layer to antimicrobial activities (Tuan *et al.*, 2018).

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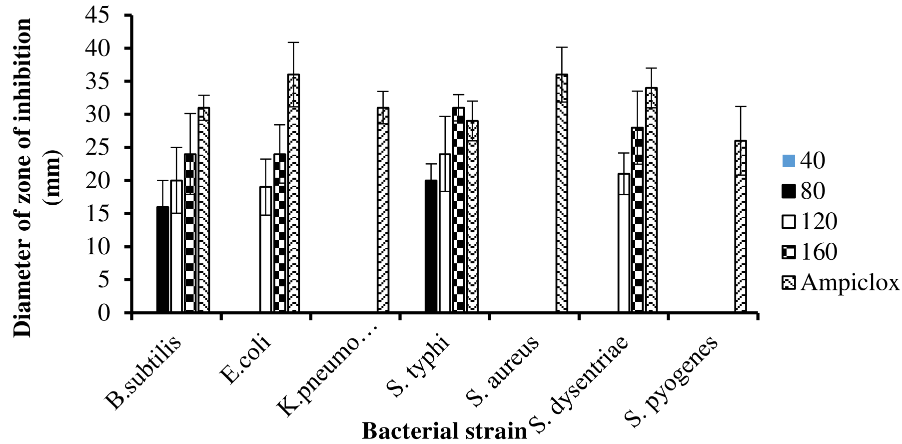


Figure 1: Susceptibility test for the crude ethanol stem extract (S) of *E. gilletii* at different concentration (40 – 160 mg/cm$^3$) comparison with Ampiclox (40 mg/cm$^3$)

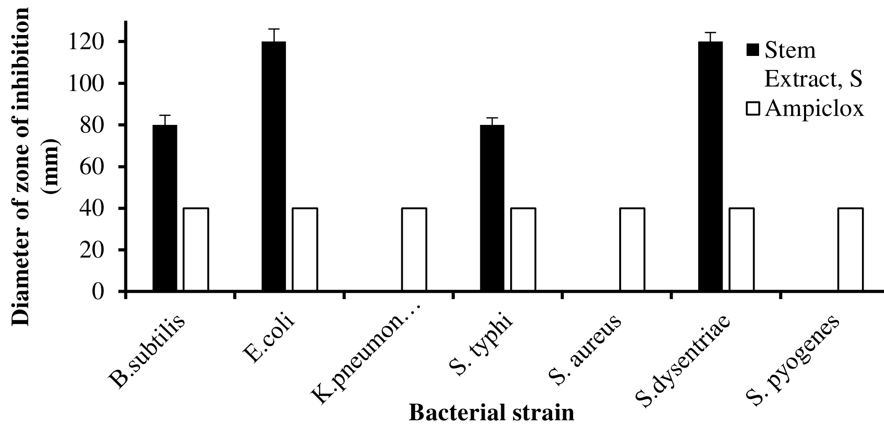


Figure 2: Minimum inhibitory concentration of the ethanol crude stem extracts of *E. gilletii* at different concentration (40 – 160 mg/cm$^3$) in comparison with Ampiclox (40 mg/cm$^3$)

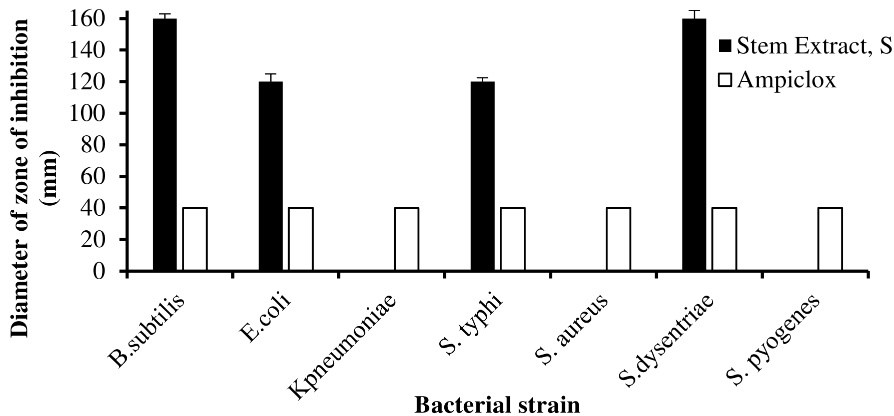


Figure 3: Minimum bactericidal concentration of the ethanol crude stem extract of *E. gilletii* at different concentration (40 - 160 mg/cm$^3$) in comparison with Ampiclox (40 mg/cm$^3$)

Susceptibility test for the stem fractions (S1 – S4) obtained from fractions of crude ethanol stem extract of *E. gilletii* is shown in Figure 4 while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are presented in Figure 5 and 6 respectively.

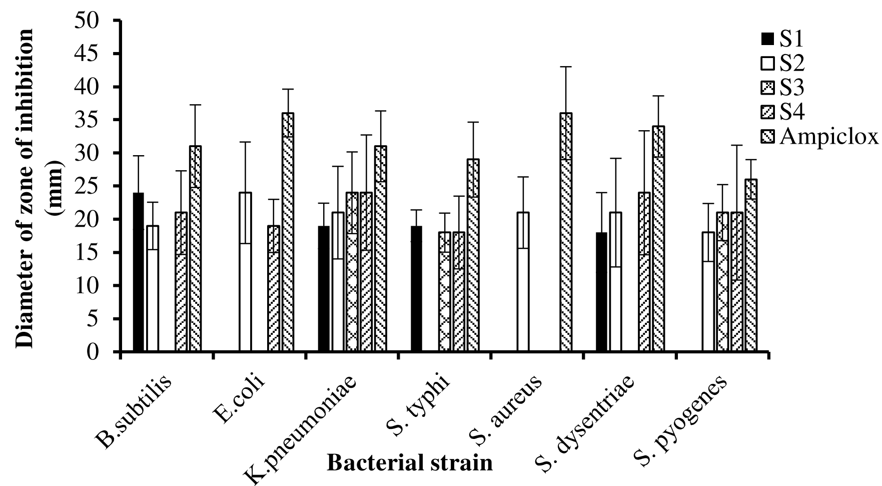


Figure 4: Susceptibility test for stems fractions (S1- S4) at concentration (200 mg/cm$^3$) of *E. gilletii* in comparison with Ampiclox (40 mg/cm$^3$)

Antibacterial susceptibility of the stem fractions (Figure 4), displayed a wide zone of inhibition in all the test bacteria isolates at (200 mg/cm$^3$) than that observed in the crude extracts with a zone of inhibition ranging from 18 to 24 mm for fractions 1 to 4 (S1-S4). *S. pyogenes* and *E. coli* were resistant against fraction S1. *B. Subtilis* and *S. aureus* were resistant against fractions S3 and S4. *S. typhi* was resistant against S2 and *S. dysentriae* was resistant against fraction S3.

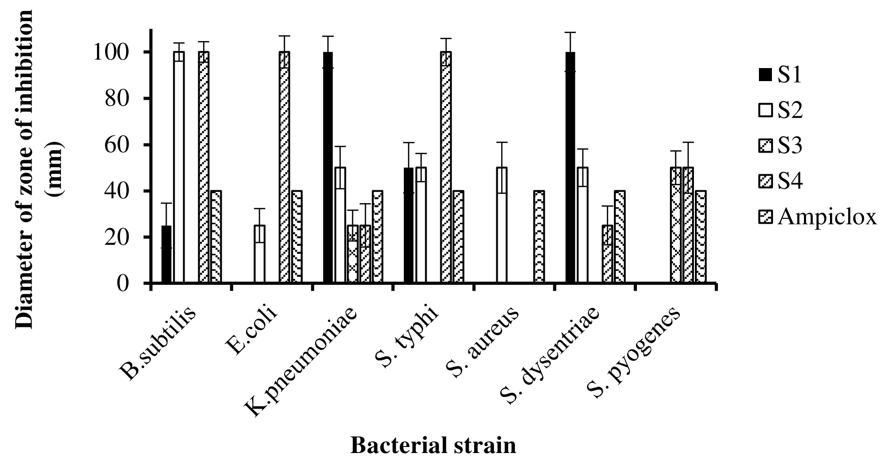


Figure 5 Minimum inhibitory concentration of the stem fractions (S1 – S4) of *E. gilletii* at concentration (200 mg/cm$^3$) in comparison with Ampiclox (40 mg/cm$^3$)

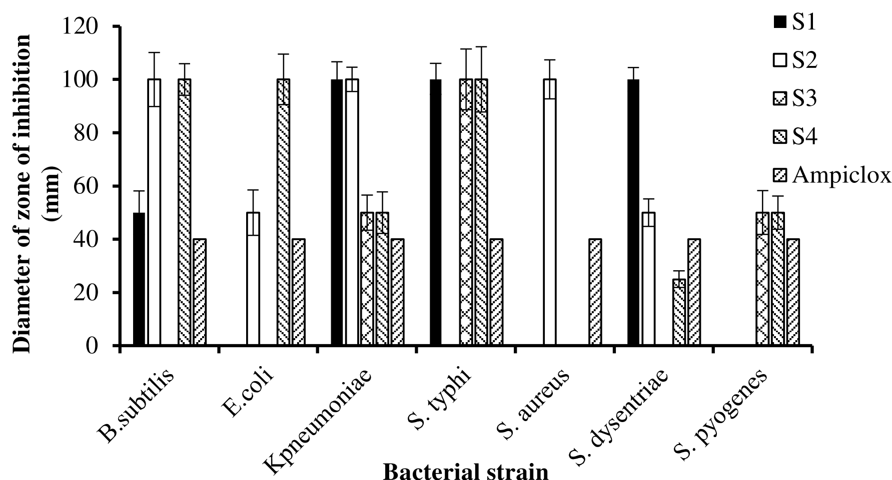


Figure 6: Minimum bactericidal concentration of the stem fractions (S – S4) of *E. gilletii* at concentration (200 mg/cm$^3$) in comparison with Ampiclox (40 mg/cm$^3$)

The MIC and MBC of crude stem extracts are shown in Figure 5 and 6 respectively. MIC and MBC of the fractions were also found do be lowered when compared with the crude stem extracts. This suggests that the crude extracts might contain compounds that inhibited the antibacterial activity (Bipul *et al.*, 2013; Tuan *et al.*, 2018). The MIC ranged from 25 to 100 mg/cm$^3$, while the MBC ranged from 50 to 100 mg/cm$^3$ in the entire susceptible test.

## 4. CONCLUSION

The broader spectrum of activity displayed compared to the standard antibiotic drug (Ampiclox) suggests that stem of the plant could make a good candidate for the treatment of infections caused by these organisms. It is suggested that further purification, characterization and structural elucidation should be carried out in order to isolate the active compounds responsible for the activities observed in the stem extracts of the plant.

## 5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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