Phytochemical and Antibacterial Studies of *Ensete gilletii* Leaf Extract and Fraction

[1]Tanko, E., [2]Dauda, B. E. N., [3]Mann, A. A., [4]Oyeleke, S. O., [5]Fadipe, L. A.,
[1,2,3,5]Department of Chemistry, [5]Department of Microbiology, Federal University of Technology, Minna, P. M. B, 65,
Minna, Niger State, Nigeria
Email: ezekieltanko@gmail.com

ABSTRACT

Ensete gilletii (family *musaceae*) is ethnomedicinally used for the treatment of diarrhoea, dysentrieae, stomach ache, digestive disorder, cholera, fever, urinary infection and gonorrhoea. The quantitative determination of the phytochemicals constituents revealed the presence of alkaloids, flavonoids, phenolic compounds, Saponins and tannins with the values (3.970, 3.900, 12.712, 0.419 and 18.857 mg/cm^3, respectively).The preliminary phytochemical screening of the Leaf extracts revealed the presence alkaloids, flavonoids, phlobatannins, reducing sugar, saponins, tannins and terpenoidal compounds; While the preliminary phytochemical screening for the VLC fraction (L1 –L4) revealed the presence of flavonoids, plobatannins, reducing sugar, tannins and terpenoidal compounds. The thin layer chromatography (TLC) sprayed with chromogenic reagents ($FeCl_3$) also revealed the present of phenolic compounds. Antibacterial susceptibility test of crude ethanol extract of the leaf of *Ensete gelletii* against Gram-positve (*B. subtilis, S. aureus, S. Pyogenes*) and Gram-negative (*E. coli, K. pneumonaie, S. Typh* and *S. dysentriea*) at (40, 80, 120 and 160 mg/cm^3) test bacteria isolates revealed a broad spectrum of activity in dose dependent manner. The zone of inhibition ranged from (16 to 29 mm) in the leaf extract (16 to 31 mm) which is significantly different from the standard antibiotics (Ampiclox) at (40 mg/cm^3). *S. pyogenes* and *S. dysentriea* were resistant against the leaf extract. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concetration (MBC) of the fractions were also observed to be lower when compared with the leaf crude extract. The MIC ranged (25 to 100 mg/cm^3) while the MBC ranged from (50 to 100 mg/cm^3) in all the susceptible organisms. The broad spectrum activity displayed than the standard antibiotic drugs (Ampiclox) suggest that the leaf part of the plant could be used as pharmaceutically important agents in drug formulation in the treatment of numerous diseases.

INTRODUCTION

Nature has endowed man with abundant natural resources, especially plants and their medicinal potentials. Over the years, man has exploited these medicinal plants, some for therapeutic purposes. Because of the unique medicinal potentials discovered from these plants, people have been using these plants for their health care needs (Tee *et al.*, 2015). Traditional medicines have been universally employed for treatment of various ailments and as basic template for many pharmaceutical drugs (Abbott, 2014). It has been reported that 80 % of the world's

population relies on medicinal plants for their primary health care needs (World health Organization (WHO, 2014).

Therefore, continuing exploration for novel chemical classes derived from medicinal plants is one of the practical and promising approaches that attract researchers in the quest for new chemotherapeutics (Daniels and Malomo, 2014). *Ensete gilletii* is a wild banana (Family *Musaceae*) commonly called wild banana (English), Ayaƃar dāji (Hausa), *Uhia unune* (Igbo), *Egbo ogede* (Yoruba), *Ayabaladde* (Fulani) and *Gbmamiyai* (Gbagyi) has a wide distribution range (West Tropical Africa to Malawi, Nigeria, Benin, Cote d'ivore, Sierra Leon, Togo, Central Republic of Africa, Cameroun and Democratic Republic of Congo) (Scott, 2013). *E. gilletii* is a large monocarpic flowering plants and one of the three genera in the banana family, *Musaceae*, with unbranched pseudo-stem of concentric layers of fleshy leaf-petioles arising to 1.5 meters high from a swollen base, not suckering like *Musa* specie, and dying after flowering. It resemble banana plants (Tesfaye *et al*., 2016), but they have a long, paddle-shaped leaves with crimson midribs. The fruits are similar in appearance to those of banana, but they are dry, seedy plant. The seeds coats are black, ovoid, and have about 0.4 – 0.5 mm diameter and 0.5 – 0.7 mm long with a conspicuous white, powdery endosperm. The entire plant dies after fruiting and is widely used for household, domestic and personal uses (Afolayan *et al*., 2014, Tesfaye *et al*., 2016).

Ensete gilletii is used by rural people for the treatment of various ailments. In Nigeria, information from local sources revealed that the roots have reportedly been used to treat various ailments such as dysentery, diarrhoea, stomach ache, digestive disorder, cholera, fever, urinary infection, gonorrhea, malaria, menstrual pains, typhoid fever, insect bites, skin infections, treatment of wounds and general weakness. The flower, stem and leaves and roots of *musacea* family have reportedly been used in the treatment of diabetics, diarrhoea, dysentery and easy delivery among pregnant women (Sethiya, 2016). Although much work have been carried out

on the other species, *Ensete superbum, Ensete ventricosum* among others. Literature on *Ensete gilletii* however, is scanty, although it has been reported that the ethyl acetate and ethanol seed extracts of the plant have a promising antibacterial and fungal activities (Afolayan *et al.*, 2014). A review of literature reveals no work has been carried out on the leaf extracts and the fractions of *Ensete* plant. This work therefore presents the results of the phytochemical and antibacterial potentials of the extracts and fractions of both the leaf of *E. gilletii* against selected bacteria in comparison with standard antibiotic drug.

Materials and Methods

Collection of Plant

Fresh leaves of *Ensete gilletii* were collected from a farmland in Sarkin Pawa in Munya Local Government Area, Niger State, Nigeria in the month of December, 2018. The plant was duly authenticated by the herbarium at National Institute of Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria, and a voucher specimen deposited (Voucher no. NIPRD/H/6991). The fresh leaf collected was cut into pieces, air-dried at room temperature. The dried sample was then pulverized.

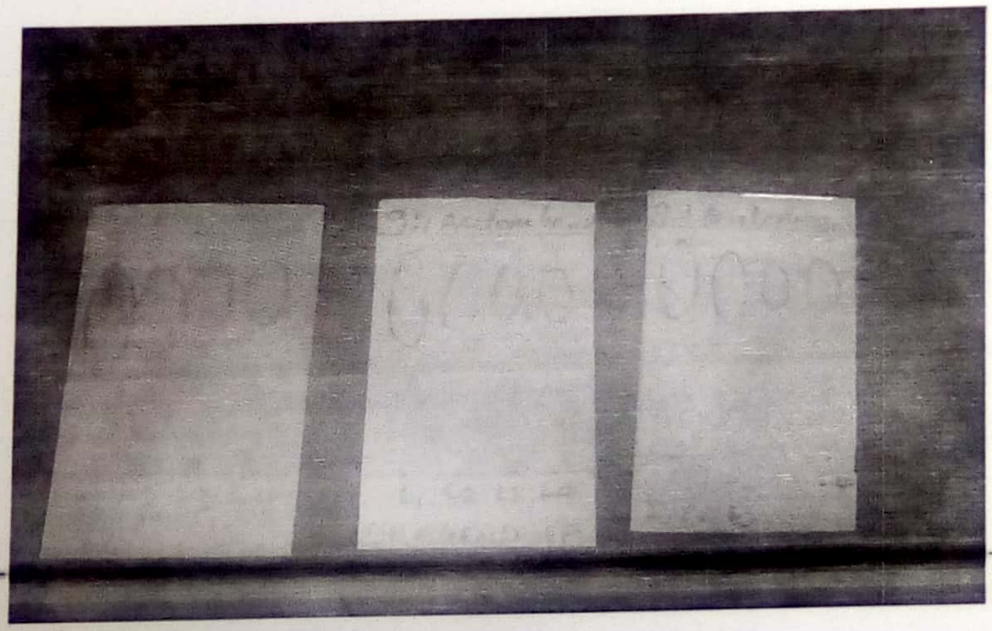
Extraction Procedures

Pulverized leaves (1 kg) of *Ensete gilletii* was exhaustively extracted with 70 % ethanol by hot maceration continuously at water bath (temperature, 70°C) for several days until the extractant became colorless. The resulting solution was decanted, filtered and then concentrated in *vacuo* and finally dried over a water bath to afford a deep brown pasty mass coded ethanol leaf extracts (L) (109.6 g, 10.96 % recovery).

Fractionation of Crude Extracts

Vacuum – Liquid Chromatography (VLC) of Crude Extracts L

Ethanol leaf extract (L) (pasty brown mass, 20 g) was fractionated using vacuum – liquid chromatography (400 g silica gel, mesh 60-120, increasing polarity of $CHCl_3$:MeOH (100-0: 0-100). The resultant eluates were collected in factions of 100 cm^3 each and identical fractions pooled based on TLC profile in various solvent systems to give four major fractions coded L1-L4. The pooled fractions were sprayed using chromogenic reagents: $FeCl_3$ (test for phenolic compounds), Dragendorff's (test for Alkaloids) and LiebermannBuchard's reagents reagents (test for steroidal compound). The chromatogram is shown on palate I.



A B C

Plate I: Chromatograms of the TLC (solvent system: Me_2CO:MeOH (9:1) of the leaf fractions (L1-L4) sprayed with Chromogenic reagents $FeCl_3$ (A) (Bluish-black, Dragendorff's (B) (no colour) and LiebermannBuchard's (C) reagents (no colour)

Qualitative and Quantitative Screening

The various classes of the phytochemical present in the ethanol leaf extract of *E. gilletii* detected using various standard methods (Sofowora, 1993; Harbone, 1998; Trease and Evans, 2002; AOAC, 2010).

Antibacterial Assay

Source of organisms and bacterial culture

Crude ethanol leaf extract (L) and Fractions (L1-L4) tested against overnight cultures of three Gram – positive (*Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus pyogens*) and four Gram – negative (*Escherichia coli*, *Salmonella typhi, Klebsiella pneumoniae and Shigella dysentriae*) bacterial strains. All obtained from the Department of Microbiology, Federal University of Technology Minna, Niger State, Nigeria.

The Antimicrobial screening of the crude ethanol leaf (L) extracts and the fractions (L1-L4) were carried out using agar dilution method as described by Sofowora, 1993 and Trease and Evans (2002).

Results and Discussion
Preliminary Phytochemical Screening

Results of preliminary phytochemical screening (qualitative) of crude leaf extracts of *E. gilletii* is presented in Table 1 and 2.

Table 1: Quantitative phytochemical Screening of Ethanol Leaf (L) Extract of *Ensete gilletii*

	Concentation (mg/g) Phytoconstituents				
Extract	Alkaloids	Flavonoids	Phenolic compounds	Saponins	Tannins
L	3.97±0.00	3.548±0.33	12.712±1.32	0.419±0.89	18.857±0.19

Table 2.: Phytochemical Constituents of the Crude Leaf Ethanol Extracts and the various Fractions of *Ensete gilletii*.

Phytochemicals	Test	Observation	L	L1	L2	L3	L4

Alkaloids	Dragendorff's reagent	Orange recipitate	+	-	-	-	-
Anthraquinones	Borntrager's test	Pink redclour	-	-	-	-	-
Cardiac glycosides	Keller Killani's testtest	Reddish-brown precipitate	-	-	-	-	-
Flavonoids	NaOH reagent	Intense yellow colour	++	++	+	+	+
Phlobatannins	Borntrager's test	Red precipitate	++	++	+	+	+
Reducing sugar	Fehling's test	Brick red precipitate	++	+	+	+	+
Saponins	Frothing Test	Persistent froth	+	-	-	-	-
Steroidal compound	Libermann Burchard's test	Greenish colour	-	-	-	-	-
Tannins	$FeCl_3$ test	Bluish-black colour	++	++	++	+	+
Terpenes	Salkwiski's test	Layer of reddish brown colour	+	+	+	-	-

Key: + = present, ++ = moderately present

Table 3: Susceptibility Test for Ethanol Crude Leaf Extracts of *E. gilletti*

Test Organisms	Concentration (mg/cm^3)				Standard drug
	40	80	120	160	40
Bacillus subtilis	NA	NA	17	21	31
Escherichia coli	NA	16	22	28	36
Klebsilla pneumonieae	NA	NA	21	27	31
Salmonella typhi	17	21	23	29	29
Staphyloccus aureus	NA	22	24	29	36
Shigella dysentriae	NA	NA	NA	NA	34
Streptococcus pyogenes	NA	NA	NA	NA	26

NA= No activity

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethanol Crude Leaf Extracts of *E.gilletii*.

Test Organisms	MIC	MBC	Ampiclox
Bacillus subtilis	120	120	40
Escherichia coli	80	120	40
Klebsiella pneumoniea	120	120	40
Salmonella typhi	40	80	40
Staphyloccus aureus	80	120	40
Shigella dysentriae	NA	NA	40
Streptococcus pyogenes	NA	NA	40

NA= No activity

Table 4: Susceptibility Test for Crude Ethanol Leaf (L) Fractions of *E. gilletii*

Organisms	Fractions (Concentration 200 mg/cm^3)				Standard drug
	L1	L2	L3	L4	Ampiclox
Bacillus subtilis	24	NA	NA	18	31
Escherichia coli	NA	19	18	21	36
Klebsiella pneumonia	20	18	23	17	31
Salmonella typhi	21	17	NA	21	29
Staphyloccus aureus	18	NA	NA	17	36
Shigella dysentriae	19	22	20	20	34
Streptococcus pyogenes	NA	18	NA	NA	26

NA=No activity

Table 5: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethanol Leaf (L) Fractions of *E.gilletii*

	(Concentration 200 mg/cm^3)
	MIC MBC

Test Organisms	L1	L2	L3	L4	L1	L2	L3	L4	Ampiclox
Bacillus subtilis	25	NA	NA	100	50	NA	NA	100	40
Escherichia coli	NA	100	100	50	NA	100	100	50	40
Klebsiella pneumoniea	50	100	50	100	100	100	50	100	40
Salmonella typhi	50	100	NA	50	100	100	NA	50	40
Staphyloccus aureus	100	NA	NA	100	100	NA	NA	100	40
Shigella dysentriae	100	50	50	50	100	50	100	50	40
Streptococcus pyogenes	NA	100	50	NA	NA	100	50	NA	40

NA= No activity

The quantitative phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compound, saponins and tannins (3.97±0.00, 3.548±0.33, 0.419±0.89, 12.712±1.32, and 18.857±0.19 mg/g, respectively) (Table 1).

Qualitative phytochemical screening of the crude leaf extract and fractions also revealed the presence of alkaloids, flavonoids, phlobatannins, reducing sugar, saponins, Tannin and terpenes (Table 2). Fractions L1- L4 also contains flavonoids, phlobatannins, reducing sugar, saponins and Tannin. Afolayan *et al.*, 2014 reported the presence of similar phytochemicals in hexane, ethyl acetate and ethanol extract of *E. gilletii*. The used of chromogenic reagents ($FeCl_3$) revealed the presence of Phenolic compounds (Plate 1).

Antibacterial susceptibility test of crude ethanol leaf (L) extract of *E. gelletii* against *B. subtilis, S. aureus, S. pyogenes, E. coli, K. pneumonaie, S. dysentriea* and *S. typhi* at 40, 80, 120 and 160 mg/cm^3 revealed a broad spectrum of activity in dose dependent manner. The zone of inhibition ranged from 16 to 29 mm in the leaf extract which is significantly different from the

standard antibiotics (Ampiclox) at 40 mg/cm^3. *S. pyogenes* and *S. dysentriea* were resistant against the leaf extract (Table 3.).

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) which is the minimum concentration required inhibiting the growth of the microorganism or completely kill the microorganism respectively were also recorded. *S. typhi* have the lowest MIC and MBC (40 and 80 mg/cm^3 respectively), *S. aureus* and *E. coli* have an MIC of 80 mg/cm^3 and an MBC of 120 mg/cm^3 while *B. subtilis* and *K. pneumoniae* have the highest MIC and MBC (120 mg/cm^3 each) in the leaf extract.

Antibacterial susceptibility test of fractions have a wider zone of inhibition in all the test bacteria isolates at 200 mg/cm^3 than the crude extract with zone of inhibition ranging from 18 to 24 mm in fraction 1 to 4 of the leaf. *S. pyogenes* and *E. coli* were resistant against fraction L1; *B. subtilis* and *S. aureus* against fraction L2, L3; *S. typhi* against fraction L3 (Table 4). MIC and MBC of the fractions were also lowered when compared with the crude leaf extract. The MIC ranged from 25 to 100 mg/cm^3 while the MBC ranged from 50 to 100 in all the susceptible organisms (Table 5).

The curative properties of plants are due to the presence of various phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, saponins, steroidal compounds, tannins and terpenoidal compounds (Kumar *et al.*, 2013). These phytochemicals are known to have *in vitro* antimicrobial/antibacterial activity (Mann *et al.*, 2008; Doughari *et al.*, 2010; Ahmad and Wudil, 2013; Adesina *et al.*, 2013).

2013). Therefore, the presence of this phytochemicals in the crude and fractions of the extract may have contributed the observed spectrum of activity.

Conclusion

The studies revealed that the pathogens used in this study were susceptible to both the leaf and the stem of *E. Gilletii*, thus suggesting the usefulness of this plant as pharmaceutically active

agent in drug formulation in the treatment of numerous diseases. The fractions of the extracts obtained from this plant could provide a basis for the isolation of active compounds of broad spectrum antimicrobials.

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