

1 **FAGARAMIDE AND PELLITORINE FROM THE STEM BARK OF *Zanthoxylum zanthoxyloides***
 2 **AND THEIR ANTIMICROBIAL ACTIVITIES.**

3
 4 **Abstract**

5 **Aim:** *Zanthoxylum zanthoxyloides* is a plant of the family Rutaceae used for treating different
 6 ailments such as malaria, sickle cell anaemia, tuberculosis, paralysis and intestinal disorder due
 7 to the presence of some bioactive constituents... The present study was aimed at identifying and
 8 characterizing some of the active principles from the stem bark of the plant.

9 **Places and Duration of study:** The isolation and characterization of the compounds was carried
 10 out at the Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of
 11 Strathclyde, Glasgow, United Kingdom between October, 2018 to February, 2019 while the
 12 bioassay analysis was done at Ahmadu Bello University, Zaria, Nigeria.

13 **Methodology:** The stem bark powder was subjected to Soxhlet extraction with hexane to obtain
 14 the crude extract, which was fractionated on column using hexane, and ethyl acetate in
 15 increasing ratios. The isolated components were tested for their antimicrobial activities against
 16 some plants and animal pathogens at Ahmadu Bello University, Zaria, Nigeria.

17 **Results:** white crystals were obtained which on spectra analysis (IR, ¹H-NMR, ¹³C-NMR, ²D-
 18 NMR) were identified as Fagaramide and Pellitorine. The isolated compounds exhibited
 19 appreciable antimicrobial activities against some microbes, thus confirming the many
 20 ethnomedical uses of the plant.

21 **Conclusion:** The compounds isolated were identified as fagaramide and pellitorine. They showed
 22 moderate sensitivity towards the pathogens tested in the study.

23
 24 **KEYWORDS:** *Zanthoxylum zanthoxyloides*, fagaramide, pellitorine, zone of inhibition, MIC,
 25 MBC, MFC and pathogen.

26 **Introduction**

27 A great number of modern drugs have their origin from natural medicinal agents making
 28 traditional medicine an important tool that are potentially useful components for the development
 29 of chemotherapeutics (Jiri, 2003). However, the future of modern medicine and therapeutics
 30 could be linked to primitive medicinal practices as majority of new drugs have been inspired by
 31 natural products as well as compounds derived from natural products. Plants contain several
 32 chemical components including lipids, flavours, pigments, metabolites and so on which account
 33 for their uses as medicines and also as precursors for the development of modern drugs
 34 (Hosseinzadeh *et al.*, 2015).

35 *Zanthoxylum zanthoxyloides* is a shrub, spiny and more or less scandent, up to 6-8 m tall, with
 36 straight, often short bole and rounded and quiet dense crown; bark grey to beige, rough, with fine
 37 vertical fissures, often with woody prickle-bearing protuberances. It is of the family Rutaceae in
 38 the tribe *Zanthoxyloideae* containing two taxa namely; *Fagara* L and *Zanthoxylum*. The major
 39 characteristics of the *Zanthoxylum* family is that the trunks, branches, branchlets, leaf stalks and
 40 inflorescence axes of all these species are covered by prickles or what is also called spines
 41 (Waterman, 1986). The plant is popularly known as Faschuari in Hausa, Rapeko in Moore, Gozo
 42 Ngua in Bambara, Aminpee in Ogoni. The plant is considered analgesic, antiseptic and
 43 diaphoretic (Wouatsa, 2013). The stem bark aqueous extracts are widely used for the treatment of
 44 malaria, fever, sickle cell anaemia, tuberculosis, paralysis, oedema and general body weakness in

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45 Cameroon (Wouatsa, 2013). The root is used in the treatment of intestinal problems including
 46 colic, dysentery, intestinal worms, gonorrhoea, urethritis as well as stimulants. In southern
 47 Nigeria, a decoction of the stem bark and roots is used to treat cancer, swellings, wounds, leprosy
 48 and syphilitic sores as well as rheumatic and arthritic pain and hernia (Nacoulma, 1996). The
 49 fruits of the plant have been reported to contain α -pinene trans- β -ocimene, citronellol, geraniol,
 50 limonene and β -myrcene (Ngassoum *et al.*, 2003). The present study investigated the stem bark
 51 of the plant for its chemical contents that could be responsible for the reported medicinal
 52 potencies of the plant as well as its antimicrobial activity against some clinical and plants
 53 pathogens.

54 **Materials and method**

55 The bark of *Zanthoxylum Zanthoxyloides* was collected from Bunu Tai, Tai LGA, in Rivers state.
 56 The plant material was identified at the Department of Forestry and Environmental studies,
 57 Rivers State University, Port Harcourt. The sample was assigned voucher specimen number
 58 RSU/2017/ZZ-56 and deposited in their herbarium. The stem bark was air dried and ground to
 59 powder using mortar and pistol.

60 **Extraction**

61 The pulverised *Zanthoxylum Zanthoxyloides* stem bark (1 kg) was extracted by soxhlet apparatus
 62 using hexane for 72hours. The extract was evaporated to dryness in rotary evaporated at 40 °C.

63 **Isolation and characterisation.**

64 The crude hexane extract (10 g) was dissolved in hexane (30 mL) and absorbed on to silica gel (8
 65 g) then the solvent was allowed to evaporate completely to form a slurry. The slurry was applied as
 66 a concentrated band onto a gravity column and eluted gradient wise, starting with hexane (200
 67 mL) followed by mixtures of hexane; ethyl acetate 90:10, 85:15, 80:20 (200 mL each) (Nande
 68 and Igoli, 2017). Fractions were collected in 20mL vials and allowed to stand until the solvents
 69 evaporated to dryness at room temperature. Similar column fractions were combined after TLC
 70 analysis (Hostellmon *et al.*; 1998). Fractions 29,30 and 31 eluted with hexane; ethyl acetate gave
 71 similar TLC profile single greenish spots when charred with concentrated sulphuric acid, RF
 72 values 0.63. Similarly, fractions 11,12,13 and 14 eluted with hexane; ethyl acetate gave similar
 73 TLC profile (brown spots when charred), RF values 0.61. The combined fractions 29-31 and 11-
 74 14 were recrystallized in ethyl acetate to yield compounds 1 and 2 labelled PJZS-29 and PJZS-13
 75 respectively. The compounds were subjected to spectroscopic analysis (NMR Spectroscopy and
 76 mass spectrometry), their melting points were also determined as well as bioassay to ascertain
 77 their biological activities against some pathogens

78 **Antimicrobial Screening**

79 The antimicrobial activities of the isolated compounds from the plant under investigation were
 80 determined using some animal and plant pathogens. The animal pathogens were obtained from
 81 the Department of Medical Microbiology Ahmadu Bello University teaching hospital, Zaria. The
 82 plant pathogens were obtained from I.A.R A.B.U. Zaria. The animal pathogens (bacteria) used
 83 for the antimicrobial assay include; *Methicillin Resist staph aureus*, *Vancomycin resist*
 84 *enterococci*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella tryphi*, *Pseudomonas*
 85 *aeruginosa*. The plant pathogens (fungi) used in this study were, *Aspergillus flavus*, *Aspergillus*

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86 *fumigatus*, *Aspargillus nigre*, *Coniophora puteana*, *Fibrophoria vaillentii*, *Fomitopis pinicola*,
 87 *Fusarium oxysporum*, *Fusarium proliferatum*, *Rhizopus sp*, *Sclerotium rofsii* and *Serpula*
 88 *lacrymans*. All micro-organisms were checked for purity and maintained in slants for agar
 89 (Usman *et al.*, 2007).

Comment [AP11]: Write *Aspergillus niger*

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90 Cultivation and standardization of test organism.

91 A loop full of test organisms were taken from the agar slant and sub culture into test tubes
 92 containing sterile nutrient agar for bacterian and subourand dextrose medium (20 ml) for for
 93 fungi, the test tubes were incubated for 48 hrs at 37 °C. The broth cultures were standardized
 94 using sterile normal saline to obtain a density of 106 cfu/mL for bacteria. A sporulated test fungal
 95 spore was harvested with 0.05 % Tween80 in sterile normal and standardized to 106 spores/mL.

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96 Antimicrobial profile (Sensitivity test)

97 0.002 mg of the compound was weighed and dissolved in 10mls of DMSO obtain a concentration
 98 of 20 µg/ml. Diffusion method was used for screening the compound. Mueller Hinton agar
 99 sabouraud dextrose agar were the media used as the growth media for the microbes. The media
 100 were prepared according to the manufacturer's instructions sterilized at 121 °C for 15 minutes,
 101 poured into sterile petri dishes and were allowed to cool and solidify. The Mueller Hinton agar
 102 was seeded with 0.1 ml of the standard inoculum of the test bacteria while the sabouraud dextrose
 103 agar was seeded with 0.1 ml of the test fungi. The inoculum was spread evenly over the surface
 104 of the medium by the use of a sterile swab. By the use of a standard sterile cork borer of 6mm in
 105 diameters, well was cut at the centre of each inoculated media. 0.1 ml of the solution of the
 106 compound of the concentration of 20 µg/ml was then introduced into the well on the inoculated
 107 media. Incubation was made at 37 °C for 24hrs for the bacteria and at 30 °C for 1-7 days for the
 108 fungi after which the plates of the media were observed for the zone of inhibition of growth, the
 109 zone was measured with a transparent ruler and the result recorded in millimetres (Nna *et al.*,
 110 2019)

Comment [AP16]: The concentration is 2 rather than 20 µg/ml

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111 Determination of minimum inhibitory concentration (MIC)

112 The minimum inhibition concentration of the compounds was determined using the broth dilution
 113 method. Mueller Hinton broth and sabouraud dextrose broth were prepared, 10 mL of the broth
 114 was dispensed into test tubes and were sterilized at 121°C for 15 minutes, the broth was allowed
 115 to cool. Mc-farland's turbidity standard scale number 0.5 was prepared to give turbid solution.
 116 Normal saline was prepared, 10 mL was dispensed into sterile test tube and the test microbe was
 117 inoculated and incubated at 37 °C for 6hrs. Dilution of the test microbe was done in normal saline
 118 until. The turbidity marched that of Mc-farland's scale by visual comparison at this point the test
 119 microbe has a concentration of 1.5×10^8 cfu/ml. Two-fold serial dilution of the compound was
 120 done in the sterile broth to obtain the concentrations of 20 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml
 121 and 1.25 µg/ml. the initial concentration was obtained by dissolving 0.002 mg of the compound
 122 in 10mls of the sterile broth. Having obtained the different concentrations of the compound in the
 123 sterile broth, 0.1 ml of the test microbe in the normal saline was then inoculated into the different
 124 concentrations incubation was made at 37 °C for 24 hours for the bacteria and at 30 °C for 1-7
 125 days for the fungi, after which the test tubes of the broth were observed for turbidity (growth) the

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126 lowest concentration of the compound in the sterile broth which shows no turbidity was recorded
127 as the minimum inhibition concentration (MIC).

128 **Determination of minimum bactericidal/ fungicidal concentration (MBC/MFC)**

129 MBC/MFC was carried out to determine whether the test microbes were killed or only their
130 growth inhibition. Mueller agar and sabouraud dextrose agar were prepared, sterilized at 121°C
131 for 15 minutes, poured into sterile petri dishes and were allowed to cool and solidify. The
132 contents of the MIC in the serial dilutions were then sub-cultured onto the prepared media, the
133 bacteria were sub-cultured onto the Mueller agar while the fungi were sub-cultured onto
134 sabouraud dextrose agar, incubation was made at 37 °C for 24 hours for the bacteria and at 30 °C
135 for 1-7 days for the fungi, after which the plates of the media were observed for colony growth
136 MBC/MFC were the plates with lowest concentration of the compound without colony growth.
137 Results were recorded after 24 hours (Usman *et al.*, 2007).

138 **Results and Discussion**

139 **Characterization of PJZS 13 as Fagaramide**

140 Fraction PJZS- 13 was obtained as cream crystals with a melting point of 113-115 °C. It absorbed
141 UV when visualized after TLC in a UV-lamp. The ¹H-NMR spectrum showed a spin system for
142 two olefinic protons at δ_H 6.3 ppm (H-7) and δ_H 7.35ppm (H-8). The large coupling constant (J =
143 15.6Hz) exhibited between H-7 and H-8 was consistent with trans configuration of the olefinic
144 bond. Furthermore, the ¹H-NMR displayed signals at δ_H 3.32 ppm (-CH₂-m), 1.87 ppm (¹H,m),
145 0.93 (CH₃)₂, (d, J = 7.2Hz) indicating the presence of an isobutylamide chain in the compound.
146 The spectrum also showed the presence of three mutually coupled aromatic protons at δ_H 6.71
147 (H-2) and δ_H 6.93 (dd J = 8.2, 2.0 Hz, H-6) and δ_H 6.91ppm (d, J = 8.2 Hz, H-5) of a tri-
148 substituted benzene ring.

149 The ¹³C-NMR spectrum confirmed the presence of 14 carbon peaks. These peaks revealed the
150 presence of six aromatic carbons of which three (3) were CH appearing at δ_C 120.5 (C-2), δ_C
151 123.6 (C-5) and δ_C 129.5 (C-6) ppm respectively. Three (3) quaternary carbons at δ_C138.7 , δ_C
152 147.3 and δ_C 148.1 ppm assigned to C-1, C-3 and C-4 respectively. There was also a signal with
153 δ_C101.6 ppm corresponding to the methylene-dioxy group, a signal at δ_C166.14 ppm for the
154 olefinic carbons, lowfield CH₂ at δ_C 46.5 ppm near to the nitrogen atom. Signals for methyl
155 groups were observed at δ_C 20.3 and δ_C 21.3 ppm assigned to C-3' and C-4' respectively were
156 also observed.

157 Using the above ¹H and ¹³C-NMR spectral data and comparison with literature reports, fraction
158 PJZS 13 was characterized and identified as fagaramide. This compound has been isolated
159 previously from a number of plants including *Z. schinifolium* (Mbaze *et al*; 2009).

160 **Table 1: ¹HNMR and ¹³C NMR chemical shifts for PJZS-13**

161

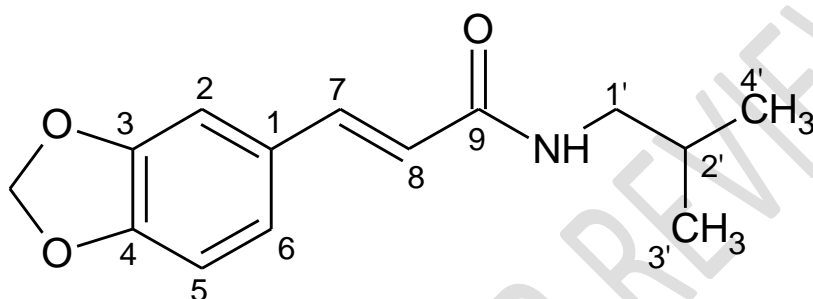
Spectroscopic technique	Data
Rf	0.61
Mp	113-115 ⁰ C
¹ HNMR(Acetone)	δ 6.71(H-2), 6.91(H-5), 6.93(H-6), 6.3(H-7), 7.35(H-8), 3.32(H-1 ¹), 1.87(H-2 ¹), 0.98(H-3 ¹), 0.85(H-4 ¹), 6.04(OCH ₂ O), 3.94(NH)

Comment [AP23]: Serial dilution

¹³C NMR(Acetone) δ 138.7(C-1), 120.5(C-2), 147.3(C-3), 148.1(C-4), 129.5(C-5), 123.6(C-6), 106.3(C-7), 108.8(C-8), 166.4(C-9), 46.5(C-1'), 28.4(C-2'), 20.3(C-3'), 21.3(C-4'), 101.6(OCH₂O)

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164

Figure 1: Chemical structure of compound PJZZ-13 (Fagaramide)

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167 Characterisation of PJZZ 80-85 as Pellitorine

168 The fraction coded PJZZ 80-85 was isolated as a white crystals and with molecular
 169 formula C₁₄H₂₅NO gave a molecular ion peak at M/z 223.0 in the E1-Ms spectrum. The infrared
 170 spectrum showed a strong absorption band at 3300cm⁻¹, which accounted for the presence of the
 171 NH group, a strong absorption band was observed at 1656cm⁻¹, belonging to the C=O of the
 172 amide group. Its melting point was obtained at 61-63°C. The ¹H-NMR spectrum of PJZZ 80-85
 173 showed three doublet signals at δ_H 5.78ppm for H-2, δ_H 0.89 for H-3', and δ_H 0.84ppm for H-4'.
 174 Two multiplet signals, which appeared at δ_H 6.05ppm and δ_H 6.01ppm, were due to the olefinic
 175 protons at C-4 and C-5. However, a multiplet occurring δ_H 1.79ppm, was due to the proton at C-
 176 2', coupling with the adjacent protons in the isobutyl moiety. A broad singlet at δ_H 5.74ppm was
 177 attributed to NH.

178 The ¹³C-NMR spectrum gave a total of 14 peaks and most of the carbon peaks appeared at
 179 the upfield region. The amide carbonyl carbon resonating at δ_C 166.60ppm was assigned C-1. A ³J
 180 correlation between δ_C 166.60 (C-1) and δ_H 3.13ppm (H-1') was observed and it validated the
 181 position of the isobutyl group. The location of the olefinic protons was confirmed by a ³J-
 182 correlation to the carbonyl carbon at δ_C 166.60ppm from a proton at δ_H 5.78 ppm. The chemical
 183 shifts of the proton and carbon NMR spectra were compared with literature values, and the values
 184 for both ¹H and ¹³C-NMR spectra were found to be in agreement. Thus, fraction PJZZ 80-85 was
 185 identified as deca-2E, 4E-dienoic acid isobutyl amide (Pellitorine).

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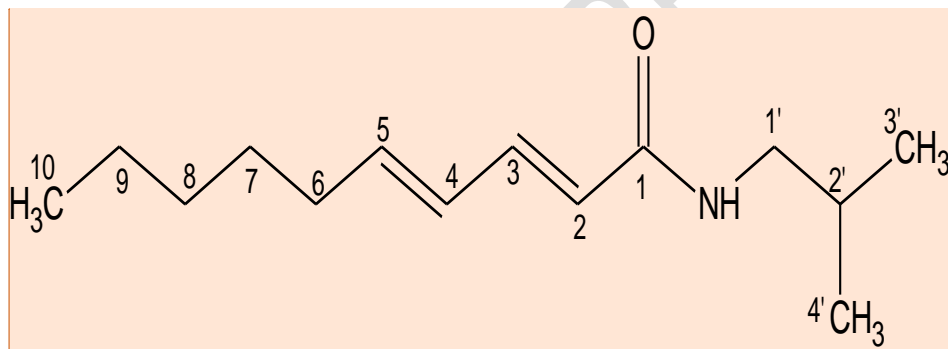
186 Table 2: ¹HNMR and ¹³C NMR chemical shifts for PJZZ-80-85

187

Spectroscopic technique	Data
IR(cm ⁻¹)	3300, 1656
Rf	0.58
Mp	60 – 62 ⁰ C
EI – Ms	223.0
¹ HNMR(Acetone)	δ 5.78(H-2), 7.16(H-3), 6.05(H-4), 6.10(H-5), 2.14(H-6), 1.39(H-7), 1.26(H-8), 0.89(H-10), 3.13(H-1 ¹), 1.79(H-2 ¹), 0.89(H-3 ¹), 0.84(H-4 ¹), 5.74(NH)
¹³ C NMR(Acetone)	δ 166.60 (C-1), 121.84(C-2), 141.40(C-3), 128.30(C-4), 128.30(C-5), 33.02(C-6), 28.71(C-7), 31.46(C-8), 22.58(C-9), 14.13(C-10), 47.02(C-1 ¹), 28.57(C-2 ¹), 20.24(C-3 ¹), 20.24(C-4 ¹)

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Comment [AP34]: Structure shall be draw using Chemdraw. Bonds have different length

190

191 **Figure 2:** Chemical structure of compound PJZZ: 80-85 (Pellitorine).

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198 **Table 3: Antifungal and sensitivity test, zone of inhibition (mm) of PJZZ80-85 and PJZS-13**
199 **versus vended drugs.**

Text organisms	PJZZ 80-85	PJZS 13	Fulcin	Kefeconazole

<i>Aspergillus flavus</i>	S (27)	R (0)	S (26)	R(0)
<i>Aspergillus fumigates</i>	S (29)	R (0)	S (28)	R (0)
<i>Aspergillus nigre</i>	R (0)	R (0)	S (30)	S (25)
<i>Coniophora puteana</i>	S (26)	S (30)	R (0)	S (23)
<i>Fibrophoria vaillantii</i>	S (28)	S (32)	S (28)	R (0)
<i>Fomitopsis pinicola</i>	R (0)	R (0)	S (30)	S (27)
<i>Fusarium oxysporum</i>	R (0)	S (31)	S (32)	R (0)
<i>Fusarium proliferatum</i>	S (29)	R (0)	S (27)	R (0)
<i>Rhizopus SP</i>	S (27)	R (0)	S (26)	S (28)
<i>Sclerotium rofsii</i>	R (0)	S (30)	R (0)	S (25)
<i>Serpula lacrymans</i>	R (0)	S (30)	S (30)	S (26)

200

201 **Table 4: Antibacterial and Sensitivity test, zone of inhibition (mm) of PJZZ 80-85 and PJZS**
 202 **13 Versus vende drugs.**

Text organism	PJZZ 80-85	PJZS 13	Sporfloxacin	Ciprofloxacin
<i>Methicillin resist staph aureus</i>	S (26)	R (0)	S (30)	R (0)
<i>Vancomycin resist enterococci</i>	S (28)	S (29)	S (29)	S (30)
<i>Staphylococcus aureus</i>	R (0)	S (30)	S (32)	S (26)
<i>Escherichia coli</i>	R (0)	R (0)	S (27)	S (37)
<i>Salmonella typhi</i>	S (28)	S (31)	R (0)	S (40)
<i>Pseudomona aeruginosa</i>	S (30)	S (30)	R (0)	S (25)

203 Key

204 R = Resistance

205 S = Sensitive

206 **Table 5: Minimum Inhibitory Concentration of PJZZ 80-85 and PJZS 13.**

Comment [AP35]: What does () means?

Serpula lacrymans - - 0* + ++

212 **Key:** - = No colony growth, 0* = MBC, + = scanty colonies growth, ++ = moderate colonies
213 growth, +++ = heavy colonies gro

UNDER PEER REVIEW

214 **Table 7: Minimum Inhibitory Concentration of PJZZ 80-85 and PJZS 13.**

Text organisms	PJZZ 80-85					PJZS 13				
	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
<i>Methicillin resist staph aureus</i>						-	-	-	0*	+
<i>Vancomycin resist enterococci</i>	-	-	-	0*	+					
<i>Staphylococcus aureus</i>	-	-	-	0*	+	-	-	-	0*	+
<i>Escherichia coli</i>						-	-	-	0*	+
<i>Salmonella typhic</i>	-	-	-	0*	+					
<i>Pseudomona aeruginosa</i>	-	-	-	0*	+	-	-	-	0*	+

215 Key: - = No turbidity (No growth)

216 0* = MIC

217 + = Turbid (light growth)

218 ++ = Moderate turbidity

219

220 **Table 8: Minimum Bactericidal Concentration (MBC) of PJZZ 80-85 and PJZS 13**221 **against test organism.**

Text organisms	PJZZ 80-85					PJZS 13				
	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
<i>Methicillin resist staph aureus</i>						-	-	0*	++	+
<i>Vancomycin resist enterococci</i>	-	-	0*	++						
<i>Staphylococcus aureus</i>	-	-	0*	++		-	-	0*	++	+
	+									
<i>Escherichia coli</i>						-	-	0*	++	+
<i>Salmonella typhic</i>	-	-	0*	++						
	+									
<i>Pseudomona aeruginosa</i>	-	-	0*	++		-	-	0*	++	+
	+									

222 **KEY:** - No colony growth 0* = MBC+ = scanty colonies growth, ++ = moderate colonies

223 growth

224

225 Form the biological studies, *Aspergillus Flavus*, *Aspergillus fumigates*, *Coniophora puteana*,
 226 *Fibrophoria vaillentis*, *Fusarium proliferation* and *Rhizopus SP* were sensitive to the
 227 compound PJZZ 80-85 (Table 3) while *Aspergillus nigre*, *Fomitopsis Pinicola*, *Furisarium*
 228 *oxysporum*, *Sclerotium rofsii* and *Serpula lacrymas* were resistant to compound PJZZ 80-85.
 229 The result showed that the organisms (fungi) exhibited and inhibition zone between 26-29
 230 mm which was consistent with the vended drugs ranging from 26-32mm for fulcin and 23-28
 231 mm for ketoconazole. *Coniophora puteana*, *Fibrophoria vailentii*, *Serpula lacrymans* was
 232 sensitive to PJZS-13 whereas the rest organisms under investigation were resistant to the
 233 compound. The zone of inhibition ranges from 30-32 mm (Table 3).

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234 The antibacterial result showed that *Methicillin resists staph aureus*, *Vancomycin resist*
 235 *enterococci*, *Salmonella typhi* and *Pseudomona aeruginosa* exhibited high sensitivity towards
 236 PJZZ 80-85 with inhibition zones ranging from 26-30 mm whereas *Staphylococcus aureus*
 237 and *Escherichia coli* were resistant. However, *Methicillin resist staph aureus* and *Escherichia*
 238 *coli* were resistant to PJZS13 while other microorganisms used were sensitive to PJZS-13.
 239 The results obtained were in consistent with that obtained when compared with the controlled
 240 drugs. The organisms exhibited an inhibitory zone ranging from 26-30 mm against PJZZ80-
 241 85 and 29-31 mm against PJZS13 (Table 4). *Howbeit*, *Salmonella typhi* exhibited the highest
 242 zone of inhibition against PJZS13 while the highest zone of inhibition against PJZZ 80-85
 243 was noticeable by *Pseudomona aeruginosa*. The sensitivity potencies of the microorganisms
 244 are evidences for numerous traditional medical uses of the plants.

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245 CONCLUSION

246 This present study investigated the isolation and characterization of Fagaramide and pellitorin
 247 from *Zanthoxylum zanthoxyloides* stem bark using physical and spectroscopic methods. The
 248 compounds were tested against some clinical and plant pathogens and the results showed an
 249 appreciable antibacterial and antifungal activities, thus, affirming the *trado-medical* uses of
 250 the plant. This is an evidence for the use of the isolated compounds as drug potential
 251 candidates for treatment of several fungi and bacteria diseases caused by the pathogens tested
 252 in the study. However, the study also revealed that the compounds could also serve as
 253 potential candidates for pesticides production.

Comment [AP41]: What?

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Comment [AP43]: Upper case for first letter

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