



Gas chromatography - mass spectrometry characterization and antibacterial activity of stem-bark extracts of *Dacryodes edulis* (African pear)

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Abstract

This study investigates the antibacterial activities of the stem bark extract of *Dacryodes edulis*. The aim of the study was to deduce the potency of the crude extracts and their metabolites from stem-barks of *Dacryodes edulis* on drug-resistant microbial pathogen(s). The stem bark of the plant was extracted with five solvents of different polarities. While extraction with hexane, dichloromethane, ethyl acetate, and methanol were done using a soxhlet extractor, aqueous extract was obtained by soaking 250 g of powdered samples in 200 ml of distilled water for 18 hours.

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The five plant extracts were subjected to phytochemical screening that revealed the presence of alkaloids, flavonoids, carbohydrates, tannins, saponin, steroids, and triterpenes. The bioactivity of the extracts at varying concentrations of 250 mg/mL, 200 mg/mL, 150 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL were determined. Based on the result of the bioassay, the most active extracts were partitioned into acidic, basic, and neutral metabolites and the bioactivity tests were repeated on the metabolites. The extracts elicited antibacterial activity against all the strains tested zones of inhibition ranging between 1 mm to 36 mm. The bactericidal effects of the extracts on all the microorganisms tested were positive at 250 mg/mL and 200 mg/mL. Also, the acidic, basic, and neutral metabolites of the Methanol and the Ethyl acetate extracts exhibited bactericidal effects at 250 mg/mL. At 200 mg/mL, all the six samples except the neutral metabolite of the methanol extract (which was only effective against

Staphylococcus Aureus, *Salmonella typhi*, and *Salmonella Paratyphi*) exhibited antibacterial activity. The gas chromatography-mass spectrometry (gc-ms) spectra revealed 4-dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine and 1,2-bis(2-methyl propyl) benzene dicarboxylate as the most abundant compound in the basic metabolites of ethyl acetate and methanol extracts respectively. The most active metabolites were basic metabolites of ethyl acetate and methanol extracts. While the basic metabolites of ethyl acetate contain mostly alkaloids; the basic metabolites of methanol contain mostly terpenoids. These active metabolites possess antibacterial activity against *Staphylococcus aureus* and other pathogens tested.

Key Words: Phytochemicals; metabolites; *Dacryodes edulis*; Antibacterial activity

Introduction

Plants make an essential part of traditional ethnomedicinal preparations. The rural populace in some instances does not have access to basic health facilities owing to certain socioeconomic defects leading to non-availability or inability to afford such facilities; often rely on plants as remedies for their various health challenges (Johanna et al., 2005). They contain physiologically active substances which have been made use of in traditional medicine (Ajibesin, 2011). The search for plants with effective antimicrobial activities has attracted huge attention and thus its exploitation in relation to the resistance of microorganisms to drugs and the occurrence of awful side effects of some antibiotics (Soberon, 2007). Resistance of bacteria to antimicrobial drugs becomes a frightening challenge to microbial control. Biological resistance refers to a condition where pathogenic organism becomes less susceptible to a particular antimicrobial agent than has been previously observed. When

antimicrobial susceptibility has been lost to such an extent that the drug is no longer effective for clinical use, the organism is then said to have achieved clinical resistance (Forbes et al., 1998). Such failures are often due to the host, the antifungal/antibacterial agent, or the pathogen. The resistance of microorganisms to modern and frontline antimicrobial agents can however be addressed by exploiting the arsenal of antimicrobial natural products (Alves et al., 1997). These antimicrobial products can be derived from plants such as *Dacryodes edulis*. *Dacryodes edulis* (African Pear) belongs to the family of Burseraceae, which are basically shrubs and trees (Kengue, 2001). The African pear (*Dacryodes edulis*) fruit pulp is well known for its richness in protein, fat, fibre, minerals, and essential amino acids. They are majorly consumed during the months of April to September (Onuegbu et al., 2016). They are often softened by heating in hot water or ash and eaten as an accompaniment to roasted or boiled maize. However, the seeds are not eaten and are often discarded as waste or sometimes consumed by domestic animals if discovered before they are rotten (Onuegbu et al. 2016). Studies have shown that the bark of *Dacryodes edulis* has several health benefits ranging from anti-oxidant, anti-sickling, wound healing, antibacterial and anti-allergenic potency (Ajibesin, 2011; Amise et al., 2016). Few works have been reported regarding the effect of the stem bark extract using the polarity gradient extraction (PGE) method, where several solvents/reagents are involved in its extraction process in increasing order of polarity. The order used in this research is n-hexane, dichloromethane, ethyl acetate, methanol, and water. The effects of which are deduced experimentally against drug-resistant *Staphylococcus aureus* among others. The study aims to deduce the potency of the stem bark extracts of *Dacryodes edulis* on the drug-resistant microbial pathogen(s).

Materials and Methods

Sample preparation

Stem-bark of African pear (*Dacryodes edulis*) about 0.80 kg in weight was cut off from a tree at Etche Village, Rivers State in June 2016. The plant was identified and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria with Herbarium Number: UPH/V/1,245. The stem barks were cut into sizes, air-dried and ground for subsequent extraction.

Extraction

About 250g of the powdered sample was extracted with 1000mL each of n-hexane, dichloromethane, ethyl acetate, and methanol (for 6 h) in a soxhlet extractor equipped with a reflux condenser. The extract was concentrated using a rotary evaporator. The aqueous extract was prepared by soaking 250 g of powdered samples in 200 mL of distilled water for 18 h. The extract was then filtered and concentrated. Extracts were screened for the presence of phytochemicals. The bioactivity of the extracts was tested. The most active extract was used for the preparation of acidic, basic, and neutral metabolites. The metabolites were also tested for their bioactivities.

Phytochemical screening of the extracts

The methods employed for phytochemical screening were those previously described by Mohammed et al, (2014) and Gupta (2012).

Preparation of metabolites

Basic metabolites

The crude extract was treated with 30 ml of concentrated HCl and 30 ml of chloroform and vigorously shaken. With the aid of a separating funnel, the chloroform layer was separated and removed. To the HCl layer, dilute NaOH was added (at about pH 7). The mixture was evaporated to afford a gel that was soluble in ethanol and filtered. This filtrate was

evaporated and the gel obtained was used without further purification for antibacterial investigations (Akpan et al., 2017).

Acidic metabolites

The chloroform layer (removed from above) was placed in a separatory funnel and treated with 30ml of dilute NaOH solution. The mixture was homogenized with occasional releases of pressure and kept overnight at room temperature (20 - 25⁰C). Two layers were also formed. The chloroform layer was decanted and reserved. The NaOH layer was then treated with conc. HCl to a pH of 7. The precipitate formed was filtered and evaporated to dryness in an oven. This was dissolved in 95% ethanol, filtered and evaporated. The gel obtained was used without further purification for antimicrobial (Akpan et al., 2017).

Neutral metabolites

The chloroform layer obtained during the preparation of the basic metabolite above was evaporated completely to produce a gel using a rotary evaporator. The gel of neutral metabolite was used without further purification for antibacterial investigations.

Test microorganisms

Clinical isolates of the bacterial pathogens used were obtained as stock culture from the Microbiology Unit of Federal University of Technology Teaching Hospital, Owerri, Imo State, Nigeria. They were *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *Pseudomonas aeruginosa*, *Shigellasonnei*, *Shigella dysenteriae* and *Escherichia coli*.

Evaluation of antibacterial activity

Agar diffusion method was employed for the antibacterial activities of the compounds. From overnight broth cultures of the various bacterial isolates, a 1×10^8 cell/mL McFarland standard was prepared and 0.1 mL aseptically transferred to sterile Petri dishes before adding 20 mL molten Brain heart infusion agar cooled to 50

°C. The content was thoroughly mixed and then allowed to solidify. Five holes (5.0 mm) were made in each plate using a cork borer and 0.2 ml of the various extracts' concentrations (50 mg/mL, 100mg/mL, 150 mg/mL, 200 mg/mL and 250 mg/mL) of all the compounds transferred into each hole aseptically using a pipette. Plates were allowed to stand for pre-diffusion for 1h before incubation at 29 ± 2 °C for 24 h. Ethanol served as control. Zones of inhibition were measured and the average was calculated.

Gc-ms analysis

The analysis of the most active fraction was carried out using a gas chromatograph-mass spectrometer (gc-ms) on an Agilent 7890A gas chromatograph (gc) directly coupled to the mass spectrometer system (ms) of an Agilent 5975C inert MSD with triple-axis detector and BP20 (WAX) capillary column (30 m × 0.25 mm, 0.25 μm thickness, SGE). The injector temperature was 250°C. The ion source was set at 280°C and the ionization energy at 70 eV. The temperature was programmed from 100°C - 280°C at a rate of 5°C per min. The initial temperature was set at 40°C which increased to 150°C at the rate of 10°C/min. The temperature was again increased to 230°C at the rate of 5°C/min. The process continued till the temperature reached 280°C at the rate of 20°C/min which was held for 8 minutes. The sample extract was transferred into a 2ml vial for analysis. The injection volume was set at 0.5μL and the split ratio was 10:1. The MSD Chemstation was used to find all the peaks in the raw GC chromatogram. A library search was carried out for all the peaks using the NIST/EPA/NIH version 2.0, and the results were combined in a single peak table. The name, molecular weight, and structure of the components of the test materials were ascertained.

Results and Discussions

Phytochemical screening

The results of qualitative phytochemical screening of the components of the crude extracts of the stem bark of *Dacryodes edulis* were as presented in Table 1.

Not only the antimicrobial properties have been ascribed to the phytochemicals, but other biological activities including modulation of the immune system have been assigned to these compounds in plants. The phytochemical analysis of the stem bark of *Dacryodes edulis* revealed the presence of flavonoids in all the extracts; alkaloids in ethyl acetate, methanol, and aqueous extracts; carbohydrates in n-hexane, dichloromethane, methanol, and aqueous extracts; tannins in n-hexane dichloromethane and aqueous extracts; saponins in dichloromethane, methanol and aqueous extracts; and steroids/triterpenes in n-hexane, methanol and aqueous extracts. The results of phyto constituents of the aqueous extract are in support of the work of Ogboru et al., (2015) who remarked that the aqueous extract of *Dacryodes edulis* contains; alkaloids, flavonoids, tannins, saponins, and steroids, etc. The presence of alkaloids, saponins, flavonoids, tannins, and steroids in the extracts of *Dacryodes edulis* strongly indicates *Dacryodes edulis* as a potential source of antimicrobial agent (Nobori et al., 1994; Zablutowicz et al., 1996; Marjorie, 1999; Shimada, 2006 and Raquel, 2007).

Antibacterial activities of the crude extracts of *Dacryodes edulis*

The antimicrobial activity of the stem bark extract of *Dacryodes edulis* against pathogenic bacteria examined was assessed by the presence or absence of inhibition zones (figures 1 - 7). The result showed that all the extracts showed activities against all the pathogens. Methanol extract brought about the highest zones of inhibition on all the organisms tested except for *Salmonella paratyphi* where the highest inhibitory zone was observed with ethyl acetate extract. Meanwhile, the dichloromethane extract exhibited the lowest zones of inhibition,

as it was only active in the highest concentrations:

Table 1: Phytochemical Analysis of the Crude Extracts of The Stem Bark of *Dacryodes edulis*

Phytochemicals	N-Hexane extract	Dichloromethane extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Alkaloids					
Wagner's Test	-	-	+	+	+
Dragendorff's Test	-	-	-	-	+
Flavonoids					
Alkali Test	+++	±	+	+	±
Carbohydrate					
Molisch's Test	+++	-	-	+	±
Fehling's Solution Test	+++	+	-	-	-
Tannins					
	+++	+	-	-	+
Saponins					
	-	+	-	+	±
Steroids & Triterpenes					
Salkowski Test	+++	-	-	+	±

+++ = Strongly positive, + = Positive, ± = weakly positive and - = not detected

200 mg/mL and 250 mg/mL, followed by hexane, which remained active at 150 mg/mL (a lower concentration), then water the aqueous extract which was active at 100 mg/mL against only one of the seven microorganisms (*Escherichia coli*). However, methanol and ethyl acetate extracts of the plant demonstrated the best antimicrobial activity compared to the other extracts as they displayed inhibition against all the tested bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Salmonella*

paratyphi, *Pseudomonas aeruginosa*, *Shigellasonnei*, *Shigella dysenteriae* and *Escherichia coli*) over the concentration range of 100-250 mg/mL within 2.0 mm-36.0 mm. At 50 mg/mL, the antimicrobial activity of the ethyl acetate extract was sustained against *staphylococcus aureus* and *Salmonella paratyphi*. Higher antibacterial activity exhibited by the methanol and ethyl acetate extracts may be due to the presence of alkaloids and flavonoids in them, which are not found in other extracts.

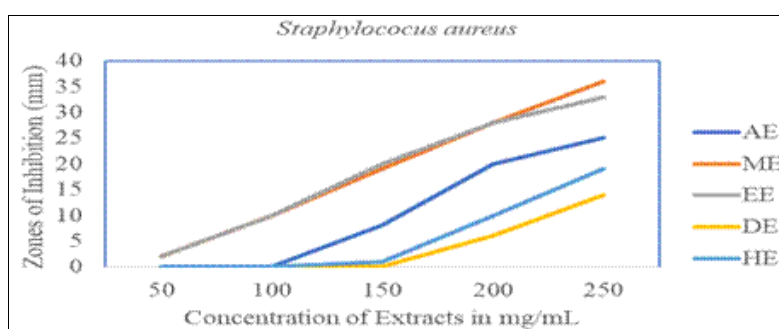


Fig. 1: Activity of plant extracts on *Staphylococcus aureus*. AE = aqueous extract of barks of *Dacryodes edulis*; ME = methanol extract of barks of *Dacryodes edulis*; EE = ethyl acetate extract of

barks of *Dacryodes edulis*; DE = dichloromethane extract of barks of *Dacryodes edulis*; HE = hexane extract of barks of *Dacryodes edulis*

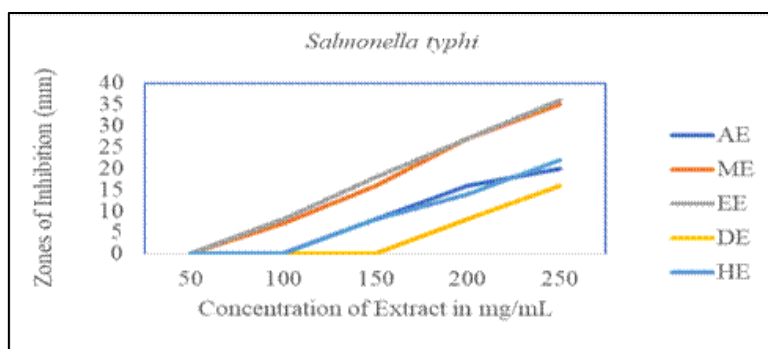


Fig. 2: Activity of plant extracts on *Salmonella typhi*

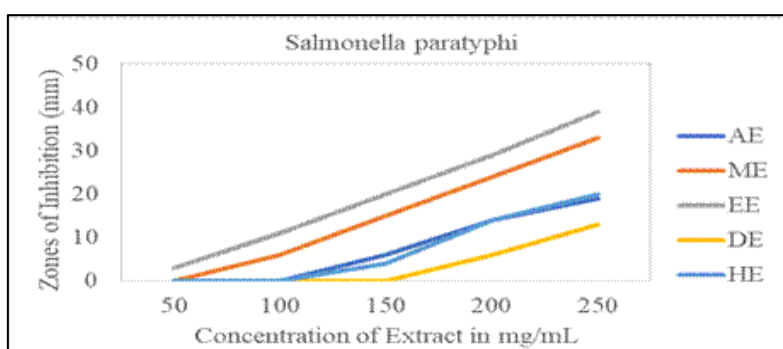


Fig. 3: Activity of plant extracts on *Salmonella paratyphi*

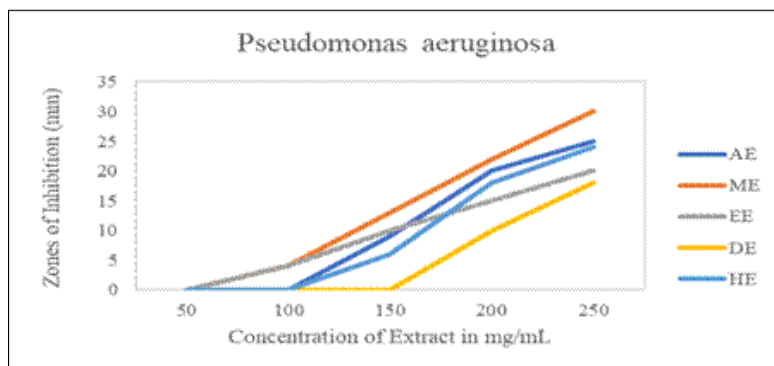


Fig. 4: Activity of plant extracts on *Pseudomonas aeruginosa*

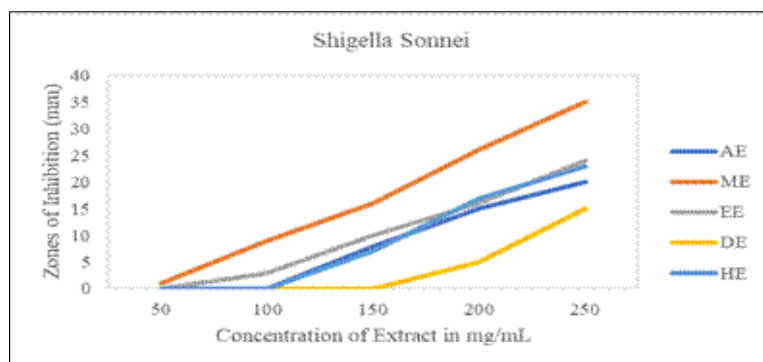


Fig. 5: Activity of plant extracts on *Shigella Sonnei*

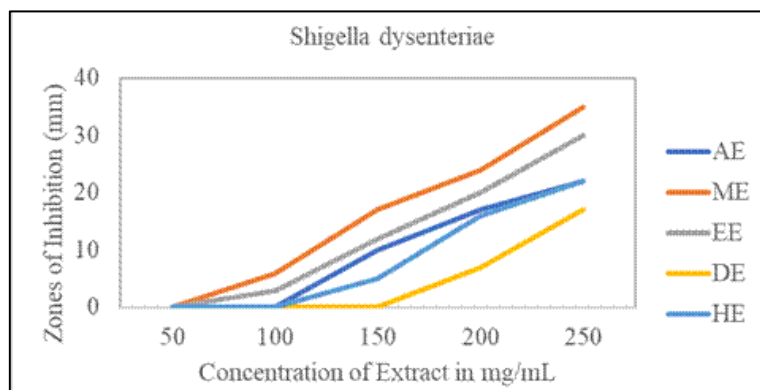


Fig. 6: Activity of plant extracts on *Shigella dysenteriae*

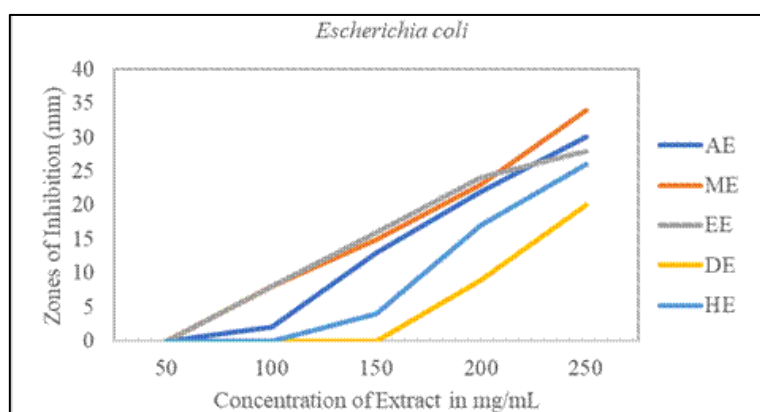


Fig. 7: Activity of plant extracts on *Escherichia coli*

Antibacterial activity of the acidic, basic, and neutral metabolites of the most active extracts (ethyl acetate and methanol) from the stem-bark of *Dacryodes edulis*. The acidic, basic, and neutral metabolites of the most active extracts; ethyl acetate and methanol, were tested against seven pathogenic bacteria. The results were shown as expressed in figures 8 - 14. The acidic and

basic metabolites of methanol and ethyl acetate showed antimicrobial activity against all the microorganisms at 250 mg/mL and 200 mg/mL. The acidic metabolites of the methanol extract (AME) were active against all the bacteria (at 150 mg/mL concentration) except *Shigella dysenteriae* and *Escherichia coli*

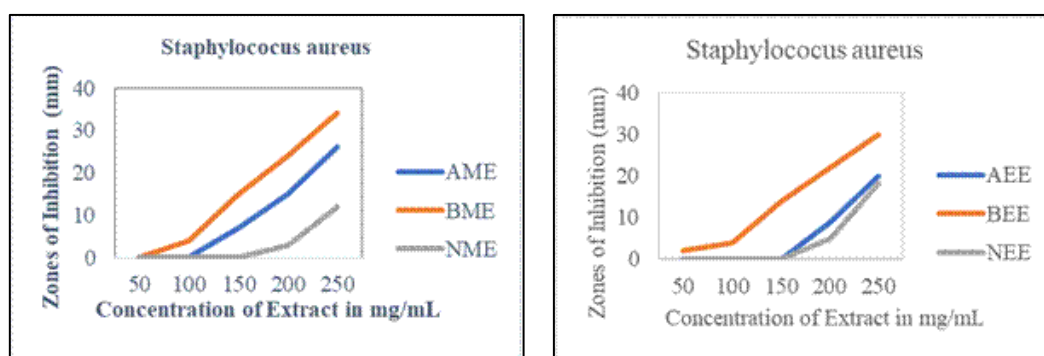


Fig. 8: Activity of metabolites of ME and EE on *Staphylococcus aureus*. AME – acidic metabolite of methanol extract of barks of *Dacryodes edulis*; BME – basic metabolite of methanol extract of barks

of *Dacryodes edulis*; NME – neutral metabolite of methanol extract of barks of *Dacryodes edulis*; AEE - acidic metabolite of ethyl acetate extract of barks of *Dacryodes edulis*; BEE - basic metabolite of ethyl acetate extract of barks of *Dacryodes edulis*; NEE - neutral metabolite of ethyl acetate extract of barks of *Dacryodes edulis*

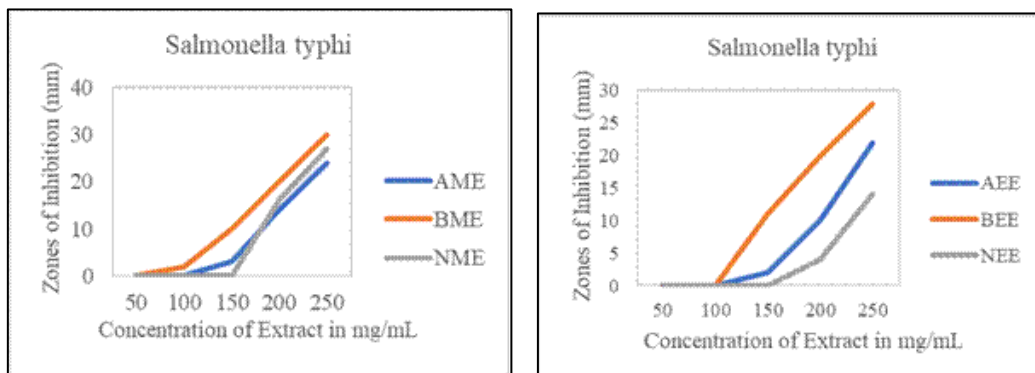


Fig. 9: Activity of metabolites of ME and EE on *Salmonella typhi*

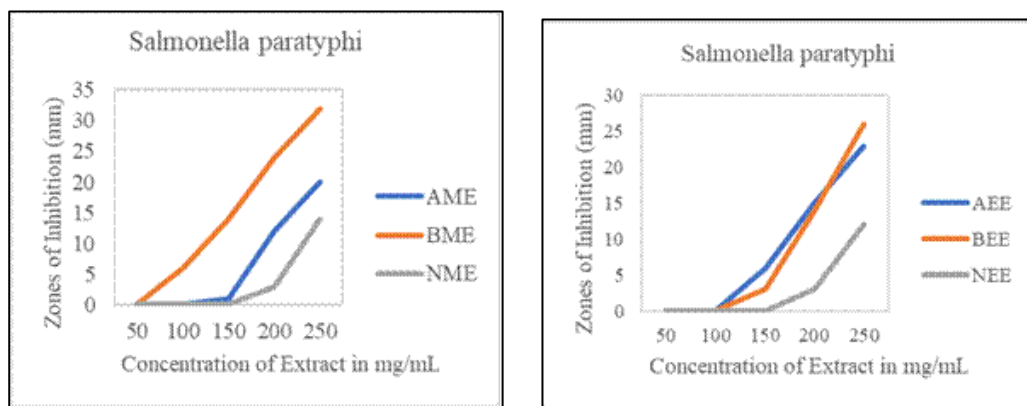


Fig. 10: Activity of metabolites of ME and EE on *Salmonella paratyphi*

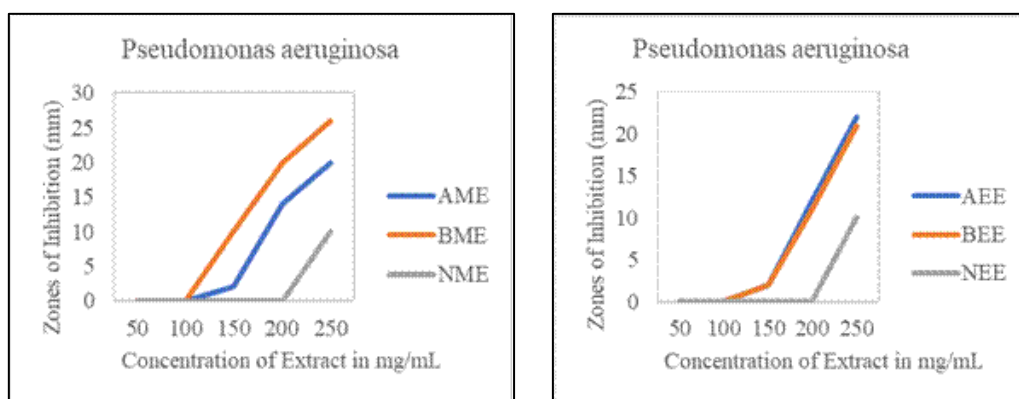


Fig. 11: Activity of metabolites of ME and EE on *Pseudomonas aeruginosa*

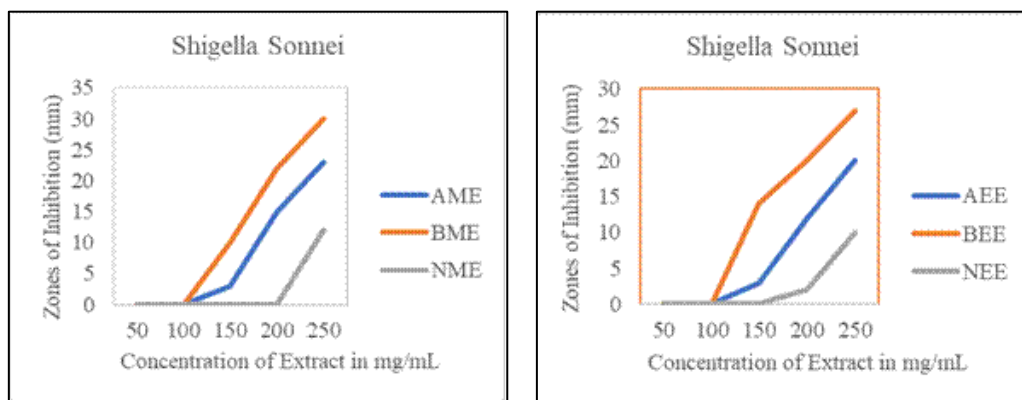


Fig. 12: Activity of metabolites of ME and EE on *Shigella Sonnei*

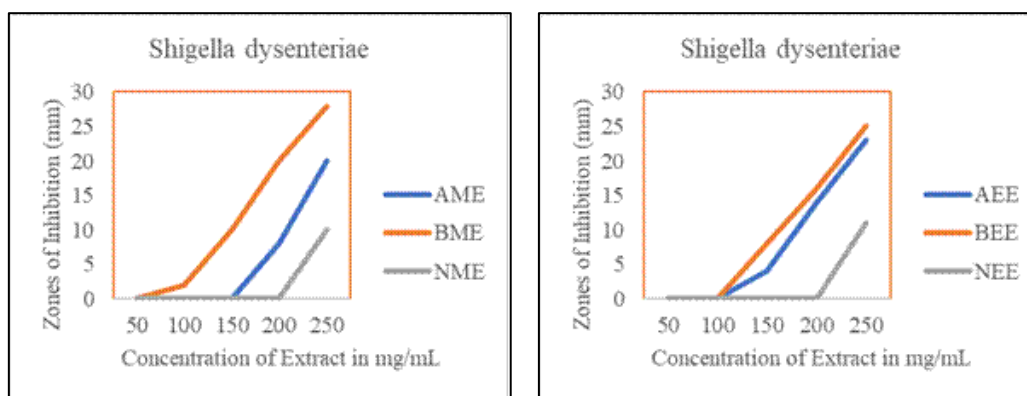


Fig. 13: Activity of metabolites of ME and EE on *Shigella dysenteriae*

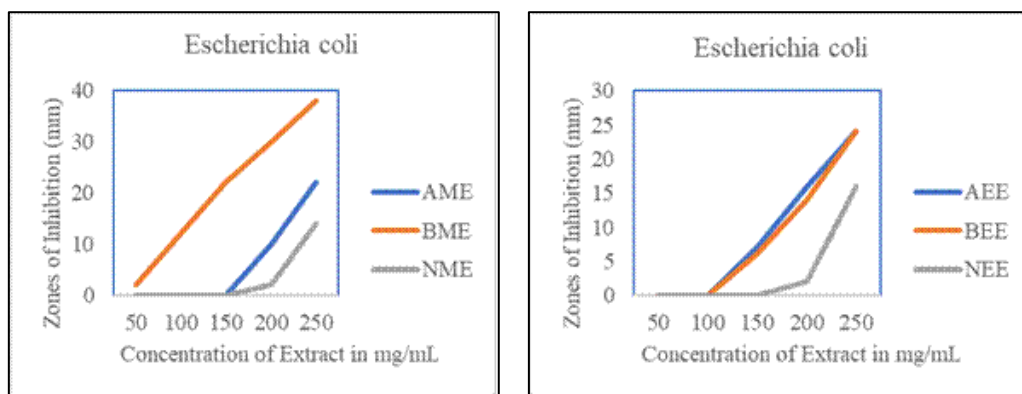


Fig. 14: Activity of metabolites of ME and EE on *Escherichia coli*

Gas chromatography-mass spectrometry (gc-ms) analyses

Based on the result of the antimicrobial activities of the acidic, basic, and neutral metabolites of methanol and ethyl acetate extracts, the most active of them; basic metabolites of Ethyl acetate and Methanol extracts, were subjected to gc-ms analyses. The result of gc-ms analysis revealed that basic metabolites of the ethyl acetate extract

contained four Phytochemical compounds. Figures 15 to 18 are the structures of the compounds of BEE. The four chemical compounds identified, the percentage of peak area of each compound, and their running times were as summarized in Table 2. The percentage of the peak area of 4-dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine [$C_{16}H_{14}N_2O_4$] with a molecular weight (MW) of 298 gmol^{-1}

and retention time (RT) of 13.82 min was 36.366 %; the highest among the four compounds identified and as such, the most abundant compound. Propanal,2-methyl-2,4-(dinitrophenyl) hydrazone [C₁₀H₁₂N₄O₄]

with a molecular weight of 252 g/mol and retention time of 10.75 min has the lowest percentage area of 14.187% and incidentally became the least abundant constituent compound.

Table 2: The chemical components identified in the gc-msanalysis of the basic metabolite of ethyl acetate extract

S/N	RT (min.)	Compound Name	Formula	MW (g/mol)	Area, %
1	10.75	Propanal,2-methyl-2,4-(dinitrophenyl)hydrazone	C ₁₀ H ₁₂ N ₄ O ₄	252	14.187
2	13.82	4-dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine	C ₁₆ H ₁₄ N ₂ O ₄	298	36.366
3	14.25	Cyclobarbital	C ₁₂ H ₁₆ N ₂ O ₃	236	28.881
4	19.92	2,4,6-cycloheptatrien-1-one-3,5-bis(trimethylsilyl)	C ₁₃ H ₂₂ OSi ₂	250	20.473

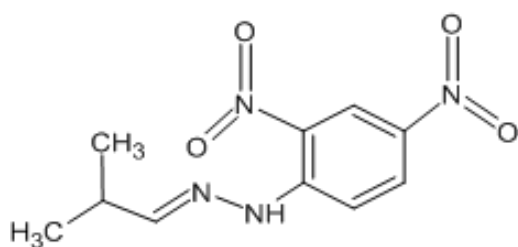


Fig. 15: Propanal,2-methyl-2,4-(dinitrophenyl)hydrazone

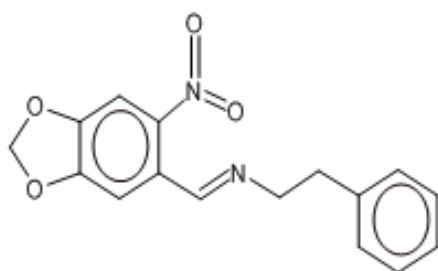


Fig. 16: 4-dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine

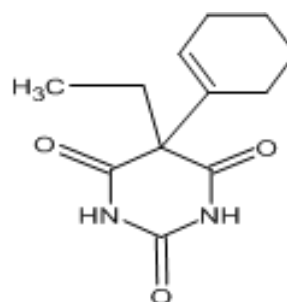


Fig. 17: Cyclobarbital

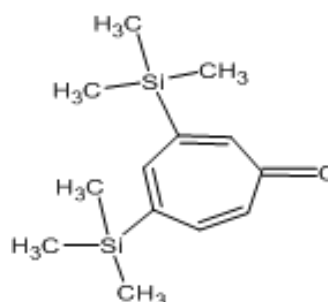


Fig. 18: 2,4,6-cycloheptatrien-1-one-3,5-bis(trimethylsilyl)

The gc-ms analysis of the basic metabolite of methanol extract, BME

The basic metabolite of the methanol extract contained six compounds. Chemical structures of the compounds of BME are as shown in figures 19 to 24. The six compounds identified in the BMF, their percentage of peak area, and their running times were as summarized in Table 3. 1,2

{Bis (2-methylpropyl)} Benzenedicarboxylate [$C_{16}H_{22}O_4$] with molecular weight; 278 gmol^{-1} and retention time of 7.26 min has the percentage area of 25.420 % is the most abundant compound. 2, 5-di(tertbutyl-1,4-benzoquinone) [$C_{14}H_{20}O_2$], with a molecular weight of 220 gmol^{-1} and retention time of 3.41 mins has a peak height (its percentage area) at 3.957 % is the compound with the lowest percentage area as such the least abundant compound.

Table 3: The chemical components identified in the gc-msanalysis of the basic metabolite of methanol extract

S/N	RT(min)	Compound Name	Formular	MW(g/mol)	Area, %
1	3.41	2,5-di(tertbutyl-1,4-benzoquinone)	$C_{14}H_{20}O_2$	220	3.957
2	5.94	2- Hexadecanol	$C_{16}H_{34}O$	242	14.260
3	6.25	Cholestan-3-ol-2-methylene-(3 β , 5 α)	$C_{28}H_{48}O$	400	22.397
4	6.80	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	15.496
5	7.26	1,2{Bis(2-methylpropyl)} Benzenedicarboxylate	$C_{16}H_{22}O_4$	278	25.420
6	8.24	2-(2,5-undecadienyloxy) tetrahydro- 2H-pyran	$C_{16}H_{24}O_2$	248	18.470

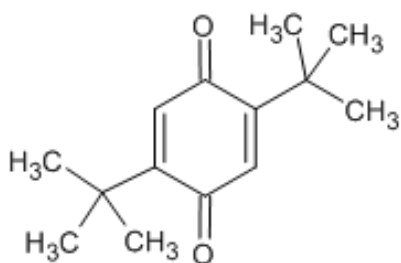


Fig. 19: 2,5-di (tertbutyl-1,4-benzoquinone)

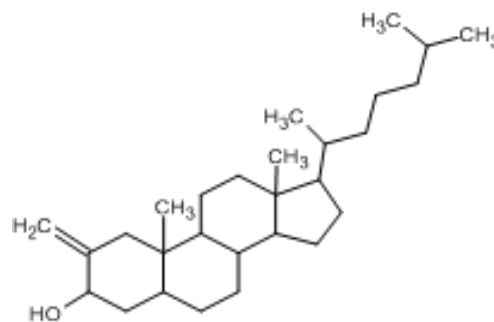


Fig. 21: Cholestan-3-ol-2-methylene-(3 β , 5 α)

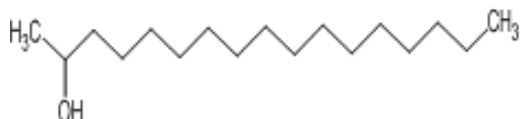


Fig. 20: 2- Hexadecanol

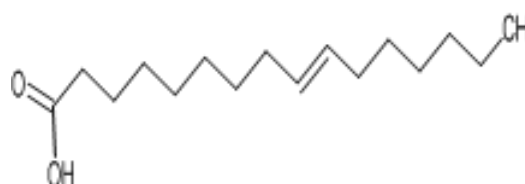


Fig. 22: 9-Hexadecenoic acid

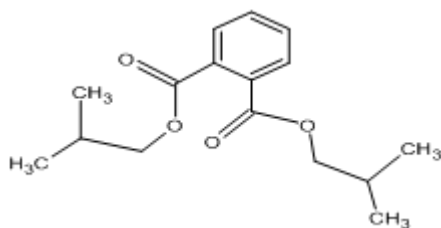


Fig. 23: 1,2 (Bis(2-methylpropyl)) Benzenedicarboxylate

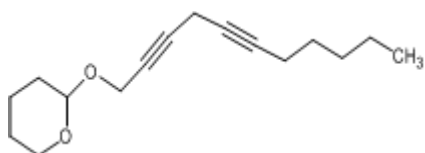


Fig. 24: 2-(2,5-undecadienyloxy) tetrahydro-2H-pyran

Gc-ms analysis of basic metabolite of ethyl acetate extract of *Dacryodes edulis* barks showed 4 different compounds. Each peak has a different retention time and abundance. The retention time showed the character of the compound. Compounds with higher polarity could be detected earlier than less polar compounds, because it has a different interaction with the immobile phase of the gas-chromatography column used. Moreover, the compound with a lower boiling point was detected earlier on gc-ms than the compound with a higher boiling point (Schwender, 2009). The retention time of the basic metabolite of ethyl acetate extract ranged from 10 to 20 minutes. The first peak was detected at minute 10.75 and read as Propanal,2-methyl-2,4-(dinitrophenyl) hydrazone, a member of the alkaloid group, while the last peak was on minute 19.92 and read as 2,4,6-cycloheptatrien-1-one-3,5-bis(trimethylsilyl), which is a member of terpenoid group (Table 2). Results of the basic metabolite of methanol extract of *Dacryodes edulis* barks showed five terpenoid compounds and one steroid compound [Cholestan-3-ol-2-methylene-(3 β , 5 α)] (Table 3). 2,5-di(tertbutyl)-1,4-benzoquinone was eluted first after 3.41 minutes while 2H-pyran, tetrahydro-2,2,5-undecadienyloxy was eluted

last after 8.24 minutes. These compounds are believed to be responsible for the higher antibacterial activities observed in the basic metabolite of ethyl acetate extract and basic metabolite of methanol extract of *Dacryodes edulis* barks. The biological activity of the compounds identified in the gc-ms characterization of the two basic metabolites was reported by many researchers. 9-hexadecenoic acid, 1,2{Bis(2-methylpropyl)} benzenedicarboxylate and propanal,2-methyl-2,4-

(dinitrophenyl)hydrazone were reported to possess antimicrobial properties by Chandrasekaran *et al.* (2011); Govindappa *et al.* (2014) and Rollas and Küçükgül (2007) respectively. n-Hexadecanoic acid is used as an antimicrobial and antioxidant (Abdullah *et al.*, 2020). 1,2{Bis(2-methylpropyl)} benzenedicarboxylate was also reported to exhibit α -Glucosidase inhibition and in vivo hypoglycemic effect (Govindappa *et al.*, 2014) while 2,4,6-cycloheptatrien-1-one-3,5-bis(trimethylsilyl) was said to possess antioxidant activity (Devi *et al.*, 2018). Hosttetman and coworkers isolated two novel benzoquinones heliotrophinones from the aerial parts of *Heliotropium ovalifolium* (Abraham *et al.*, 2011). These two quinones demonstrated antifungal activities against *Cladosporium cucumerinum* and *Candida albicans* as well as antibacterial activity against *Bacillus subtilis*. Judging from here, it is very clear that 2,5-di(tertbutyl)-1,4-benzoquinone might have contributed to the antibacterial activities of basic metabolites of methanol extract. Thymoquinone (2-propyl, 5-methyl-1,4-benzoquinone), which has a structure similar to 2,5-di(tertbutyl)-1,4-benzoquinone was reported by Hadi *et al.*, (2016) to possess anti-cancer activity. Hadi *et al.*, (2016) and Shareef *et al.*, (2016) also documented that Cholestan-3-ol-2-methylene-(3 β , 5 α); exhibited anti-

inflammatory and cytotoxic activities. Cholestan-3-ol, 2-methylene-, (3a,5a)-, a steroid, has been reported to exhibit antimicrobial activity (Archana *et al.*, 2014); antimicrobial, anticancer, diuretic, anti-asthma, anti-arthritic (Jegadeeswarriet al., 2012). No activity has been reported for 4-dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine and 2-(2,5-undecadienyloxy) tetrahydro-2H-pyran from the available literatures. Saeedi and Morteza-Semnani (2009) reported n-hexadecanol among the constituents of essential oil of *Heliotropium europaeum* was and found effective as antimicrobial agent against *Bacillus subtilis* PTCC 1023, *Staphylococcus aureus* PTCC 1112, *Escherichia coli* PTCC 1330, *Salmonella typhi* PTCC 1639, *Aspergillus niger* PTCC 5011, and *Candida albicans* PTCC 5027. 2-methyl-1-Hexadecanol was also reported by Sarada *et al.*, (2011) to possess antimicrobial activity. These compounds were believed to function in synergy either: to interfere with cell wall or nucleic acid synthesis or to inhibit a metabolic pathway or protein synthesis of the pathogens (Tenover, 2006).

Conclusion

The following conclusions are drawn from the study. That the crude extracts contain alkaloids, saponins, flavonoids, tannins, carbohydrates, and steroids. That the most active metabolites were basic metabolites of ethyl acetate and methanol extracts. While the basic metabolites of ethyl acetate contain mostly alkaloids, the basic metabolites of methanol contain mostly terpenoids. These active metabolites possess antibacterial activity against *Staphylococcus aureus* and other pathogens tested.

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