

PAPER • OPEN ACCESS

Antibacterial studies on fruit-skin and leaf extracts of *Annona muricata* in Ota, Nigeria

To cite this article: Wisdom O. Iyanda-Joel *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **331** 012029

View the [article online](#) for updates and enhancements.

You may also like

- [Bio-fabrication of Ni doped ZnO nanoparticles using *Annona Muricata* leaf extract and investigations of their antimicrobial, antioxidant and photocatalytic activities](#)
Vindhya P S, Kunjikannan R and Kavitha V T
- [Determination of the phytochemical screening, total polyphenols, flavonoids content, and antioxidant activity of sour sop leaves \(*Annona muricata* Linn.\)](#)
M T Nguyen, V T Nguyen, L V Minh et al.
- [Antihyperglycemic effectiveness comparison of ethanol extract of sour sop leaf \(*Annona muricata* L.\) against acarbose in streptozotocin-induced diabetic white rats](#)
R R Setiadi, A F M Z Zein and D Nauphar



ECS
The
Electrochemical
Society
Advancing solid state &
electrochemical science & technology

DISCOVER
how sustainability
intersects with
electrochemistry & solid
state science research

Antibacterial studies on fruit-skin and leaf extracts of *Annona muricata* in Ota, Nigeria

*Wisdom O. Iyanda-Joel, Emmanuel A. Omonigbehin, Emeka E. J. Iweala and Shalom N. Chinedu

[†]Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria

[‡]Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria

wisdom.iyanda-joel@covenantuniversity.edu.ng

Abstract. The current study examined the phytochemical constituents and antibacterial activity of leaf and fruit-skin extracts of *Annona muricata* Linn. The extracts were screened qualitatively for their phytochemical constituents following established protocols; disc diffusion method was employed for the antibacterial study on *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, after which their minimum inhibitory concentrations were determined via double dilution method. Furthermore, selected extracts were screened against 50 multidrug-resistant clinical isolates from immunocompromised patients who are already down with secondary acute respiratory infections. The phytochemical screening of the extracts collectively revealed the presence of tannins, saponins, terpenoids, steroids, phenols, flavonoids, coumarin, alkaloids, antocyanins and betacyanins. Only aqueous leaf (ALA) and fruit-skin ethanol (ESA) extracts showed remarkable activity with 15 – 17 mm inhibition diameter (DIH) against the test organisms. The minimum inhibitory concentrations (MIC) for the ALA and ESA were 25 and 6.25 mgmL⁻¹ respectively. The two extracts further showed activity (6-18 mm DIH) against 80% of the multidrug resistant isolates tested. It can therefore be inferred from this study that *Annona muricata* possibly contains potent bioactive antibacterials that can be developed into nature-friendly drugs to counter the effects of pathogenic bacteria, thereby preserving lives as well as sustaining a viable environment.

.Keywords: *Annona muricata*, antibacterials, minimum inhibitory concentration, multidrug-resistant isolates, phytochemicals.

1. Introduction

Provided the indispensable activity of a class of supramolecular complexes called viruses were not in existence, bacteria would have overrun the entire planet [1-3]! Bacteria are a set of highly prolific microscopic organisms that occupy any and everywhere called space. They fill up both habitable and non-habitable zones of the planet Earth. It is quite alarming to note that the total bacterial soil carbon in existence worldwide today, is estimated at the entire weight of the United Kingdom [4-7]. Be that as it may, bacteria can be as much beneficial as they are detrimental [8-10]. In the past few decades, Scientists have harnessed the use of bacteria through diverse technologies to better the lot of mankind, other living systems as well as the environment. In a bid to further ameliorate the impact of deleterious bacteria, natural herbs with ancient folklore claims are also being investigated worldwide to establish their ethnopharmacological effect [11-12]. Anyanwu and Okoye [13] more recently published a well



detailed review on the antimicrobial activity of Nigerian medicinal plants and effects of local herbs found in Nigeria against several pathogenic bacteria and viruses such as *Salmonella typhi*, *Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Aspergillus niger*, *Vibrio cholerae* to mention a few; with as low as 4.9 $\mu\text{g mL}^{-1}$ minimum inhibitory concentration (MIC). Some genus that were reviewed with remarkable activity are as follows: *Allium spp.*, *Amaranthus spp.*, *Cassia spp.*, *Eucalyptus spp.*, *Euphorbia spp.*, *Ocimum spp.*, *Pterocarpus spp.*, *Stachytarpheta spp.*, *Terminalia spp.*. Quite a number of specific species also studied include *Vernonia amygdalina*, *Psidium guajava*, *Jatropha curcas*, *Crinum jagus*, *Moringa oleifera*, *Anogeissus leiocarpus*, *Gongronema latifolium*, and *Fagara (Zanthoxylum) zanthoxyloides*, *Terminalia avicennioides*, *Stachytarpheta angustifolia* and *Struchium sparganophora*. Deploying state-of-the-art technologies into exploring these medicinal plants are most likely the way out of the currently emerging threats of antimicrobial resistance that is rendering modern medicine ineffective in so many ways. One of such herbs that has been designated as a crop for the future to combat bacterial infections amongst other disease conditions is *Annona muricata* Linn. *Annona muricata* also commonly called soursop is an evergreen low-branching, slender and bushy tree towering between 5 and 9 metres and 0.15-0.83 metres wide. The soursop tree is extravagantly distributed in the tropics largely because of the favourable climatic conditions such as annual rainfall above 1500 mm, altitudes below 1200 m above sea level, 60 to 80% relative humidity between and temperatures between 25 and 28 °C. A remarkable feature of the tree that makes it of utmost economic importance is its high and continuous productive profile [14-17]. For many decades now, the antibacterial activity of *A. muricata* have been explored on several gram-negative and gram-positive organism with interesting results [18]. A review report showed that *A. muricata* extract demonstrated antibacterial activity comparable to streptomycin which is a potent standard antibiotic [19]. One very key observation in the antibacterial activity studies of *A. muricata* is that extract activity is largely dependent on extraction solvent; denoting the fact that extracts prepared with organic solvents for instance, showed higher activity over aqueous extracts. Viera *et al.* [20] highlighted and confirmed this phenomenon, where ethanolic and methanolic *A. muricata* extracts were active against *Staphylococcus aureus*, whereas no such activity was observed in the aqueous extract of the fruit skin. As a result, the polarity of the organic solvent used in extraction further significantly influenced antibacterial activity. Amidst several studies, the current examination of the fruit-skin and leaf extracts of this unique crop is hereby presented.

2. Materials and Methods

Collection and identification of plant samples

Unripen mature fruits of *Annona muricata* were bought from local fruit markets in Lusada and Agbara, Ogun state, Nigeria; and the leaves were collected from trees on farmlands also in Ota, Ogun state, Nigeria. Fresh samples of the healthy fruits and leaves were deposited in the Forestry herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. The plant samples were identified and authenticated with Voucher referencing Number FHI. 110177.

Test Organisms

Clinical isolates of *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* coupled with those listed in Table 4 which were isolated from HIV/AIDS patients already down with secondary lower respiratory infections.

Culture media and plates

Mueller-Hinton agar (HiMedia Laboratories Ltd, Mumbai, India - ISO 13485-2003) and MacConkey agar (without salt: Rapid Labs), petri dishes and universal bottles were all purchased from standard scientific stores.

Methods

Preparation and extraction of plant samples

The plant samples of *A. muricata* were washed properly in clean running water. The fruits were peeled manually with knife, a portion of the peels (fruit skin) were blended and frozen while another portion was frozen directly for subsequent extractions. Also, a portion of the leaves were shade-dried and blended, while the remaining portion was frozen for respective extraction. Both fruit skin and leaves of *A. muricata* were extracted with five different categories of solvents following stipulated protocols itemized as follows. The extracts were concentrated using a rotary evaporator and the percentage (%) yield of the dry residue calculated as described by [21].

Aqueous extraction

Weighed amount of powdered leaves of *A. muricata* were soaked in distilled water (1:10) for 12 hours and sieved with muslin cloth to remove fiber particles; then filtered with a vacuum membrane filter device as well as filter paper into conical flasks. The filtrate was concentrated in a rotary evaporator and dried to powder in a water bath. The powder was weighed and percentage yield calculated. The aqueous leaf extract is labeled as subsequently referred to as ALA. The blended and frozen fruit skin were thawed and extracted with distilled water (1:5) for 12 hours, sieved and filtered. The filtrate was frozen and dried in a freeze dryer; it is labelled ASA [22]

Sodium chloride extraction

A specified volume of 1% sodium chloride was poured into weighed powdered leaves of *A. muricata* in a glass jar and left for 12 hours. The mixture was sieved, filtered and concentrated in a rotary evaporator and subsequently freeze-dried. The resulting powder was weighed and percentage yield calculated. Sodium chloride leaf extract is labelled as SLA. Frozen peels of *A. muricata* are weighed and blended into fine extractible particles; this was then soaked in a known volume of 1% sodium chloride for 12 hours. The mixture was also sieved, filtered and concentrated in a rotary evaporator and freeze-dried. Sodium chloride fruit skin extract is labelled as SSA [23-24].

Methyl-tert-butyl ether (MTBE) extraction

Fresh leaves and fruit skin of *A. muricata* were extracted with Methyl-tert-butyl ether (MTBE) amongst other solvents (MTBE/methanol/water: 10/3/2.5; volume by volume) for an accurate lipidome profiling. Methanol (970 mL) was added to 900g of blended fresh leaves of *A. muricata* in a cylindrical glass jar with lid; the content was shaken together to mix well. This was followed by adding 3.2 L of MTBE and incubating the mixture at room temperature for 55 minutes. After the incubation phase separation was induced with the addition of 810 mL of distilled water. Following 10 minutes of incubation at room temperature, the mixture was sieved with 8-fold muslin cloth and the resultant filtrate was poured into a separating funnel. The upper organic phase was collected, concentrated in a rotary evaporator and dried in a weighed beaker on a heat block set at 55 °C. Extracted lipids were kept in the refrigerator. Methyl-tert-butyl ether leaf and fruit skin extracts of *A. muricata* were labelled as MLA and MSA respectively [25].

Ethanol extraction

Powdered leaves of *A. muricata* were extracted with 95 % ethanol using soxhlet apparatus. Likewise the blended fresh peels of *A. muricata* were soaked in 95 % ethanol overnight (8-12 hours), sieved with an 8-fold muslin cloth to take out fiber particle and then filtered a vacuum membrane filter. Both the extract from the leaves and the filtrate from the fruit skin were concentrated in a rotary evaporator and dried in a weighed beaker on a heat block at 65 °C. The ethanolic leaf and fruit skin extracts of *A. muricata* were labelled as ELA and ESA respectively [26].

Isopropanol extraction

Frozen leaves and fruit skin of *A. muricata* were blended with a mixture of isopropanol/hexane/water in the ratio 55:20:25 (volume by volume) and incubated at 55.5 °C for 15 min with occasional shaking. After incubation, the mixture was sieved with an 8-fold muslin cloth and transferred to a separating funnel. The upper organic phase was collected with a micropipette into a weighed beaker and dried on a heat block set at 55 °C. isopropanol/hexane/water leaf and fruit skin extracts of *A. muricata* were labelled as ILA and ISA respectively [27].

Phytochemical screening

Selected qualitative phytochemical screening tests were carried out according to methods previously described on the extracts for the detection of the following compounds: alkaloids, anthocyanins & betacyanins, coumarins, flavonoids, glycosides, phenols, quinones, saponins, steroids, tannins, terpenoids and triterpenoids. Prior to the screening, the extracts were dissolved in their respective solvent [28-30].

Antibacterial studies

In vitro screening for antibacterial activity

The agar well diffusion method of sensitivity testing were used to determine the growth inhibition of bacteria by the plant extracts [31-33].

Determination of minimum inhibitory concentrations

Minimum inhibitory concentrations (MIC) of the extracts were determined by agar well dilution method as described by Russell and Furr (1972). Different dilutions of the extracts were prepared to give final concentration in the range of 100.0, 50.0, 25.0, 12.5, 6.25, 3.12 and 1.56 mg mL⁻¹. Two milliliter (2 mLs) of each dilution was mixed with 18 mL of Mueller Hinton agar (HiMedia Laboratories Ltd, Mumbai, India - ISO 13485-2003), poured into petri-dishes and allowed to set. The agar was streaked with an overnight broth culture of the bacterial isolates and incubated overnight. The plates were then examined for the presence or absence of growth. The minimum concentration that completely inhibited macroscopic growth was regarded as the minimum inhibitory concentration of the respective extracts [18].



Figure 1: *Annona muricata* fruit on the tree

3. Results and discussion

Qualitative phytochemical study

The qualitative phytochemical screening of the leaf and fruit-skin extract pairs of *A. muricata*, as shown in table 1 below indicated the presence of alkaloids, anthocyanins and betacyanins, coumarins, flavonoids, glycosides, phenols, saponins, steroids, quinones, tannins and terpenoids.

Table 1 Phytochemical screening results of extracts from the fruit-skin and leaves of *Annona muricata*

Test/Extract	ALA	ASA	SLA	SSA	MLA	MSA	ELA	ESA	ILA	ISA
<i>Alkaloids</i>	–	–	–	–	–	+	+	+	+	–
<i>Antocyanins- betacyanins</i>	–	–	+	–	–	–	+	–	–	+
<i>Coumarins</i>	–	–	+	+	–	–	–	–	–	–
<i>Flavonoids</i>	+	–	+	–	+	–	+	+	–	+
<i>Glycosides</i>	–	–	–	–	–	+	–	–	–	–
<i>Phenols</i>	+	–	–	–	–	+	–	+	+	–
<i>Quinones</i>	–	–	+	–	+	+	–	+	–	–
<i>Saponins</i>	+	+	+	+	+	+	–	+	–	+
<i>Steroids</i>	–	–	+	–	+	–	+	+	+	–
<i>Tannins</i>	+	–	–	+	–	–	–	+	+	–
<i>Terpenoids</i>	+	+	+	+	+	+	+	+	+	+
<i>Triterpenoids</i>	–	–	–	–	–	–	–	–	–	–

(+) denotes presence of phytochemical; (–) denotes absence or no trace of phytochemical in the extract tested. ALA: Aqueous leaf extract of *Annona muricata*, ASA: Aqueous skin extract of *Annona muricata*, SLA: 1 % Sodium chloride leaf extract of *Annona muricata*, SSA: 1 % Sodium chloride skin extract of *Annona muricata*, MLA: methyl tert-butyl ether leaf extract of *Annona muricata*, MSA: methyl tert-butyl ether skin extract of *Annona muricata*, ELA: ethanolic leaf extract of *Annona muricata*, ESA: ethanolic skin extract of *Annona muricata*, ILA: isopropanol leaf extract of *Annona muricata*, ISA: isopropanol skin extract of *Annona muricata*

Antibacterial sensitivity test for fruit-skin and leaf extracts of Annona muricata against Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa

Sensitivity testing on the five extract pairs of *A. muricata* against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* revealed antibacterial activity (Plates 4.14-4.16) for two of the extracts: ALA (16, 14 and 16 mm) and ESA (16, 16 and 17 mm) with zones of inhibition as shown in Table 2

Table 2: Activity of five extract pairs of *Annona muricata* against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*

SN	Organism	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
	Extract	Zone of inhibition (mm)		
1.	ALA ₁	16 ± 0.58 (+)	16 ± 1.15 (+)	14 ± 0.58 (+)
2.	ALA ₂	-	-	-
3.	SLA	-	-	-
4.	ELA	-	-	-
5.	MLA	-	-	-
6.	ILA	-	-	-
7.	ESA	17 ± 0.58 (+)	16 ± 0.00 (+)	16 ± 0.58 (+)
8.	MSA	-	-	-
9.	ISA	-	-	-
10.	ASA	-	-	-
11.	SSA	-	-	-

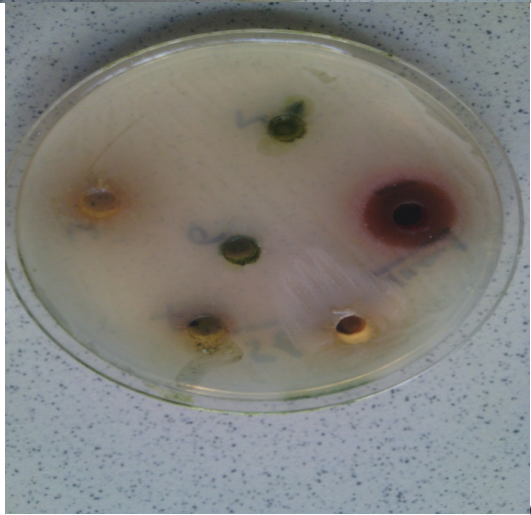
ALA: Aqueous leaf extract of *Annona muricata*; SLA: 1% sodium chloride leaf extract of *Annona muricata*; ELA: ethanol leaf extract of *Annona muricata*; MLA: methyl tert-butyl ether leaf extract of *Annona muricata*; ILA: isopropanol/hexane/water leaf extract of *Annona muricata*; ESA: ethanol fruit-skin extract of *Annona muricata*; MSA: methyl tert-butyl ether fruit-skin extract of *Annona muricata*; ISA: isopropanol/hexane/water fruit-skin extract of *Annona muricata*; ASA: Aqueous fruit-skin extract of *Annona muricata*; SSA: Aqueous fruit-skin extract of *Annona muricata*



A



B

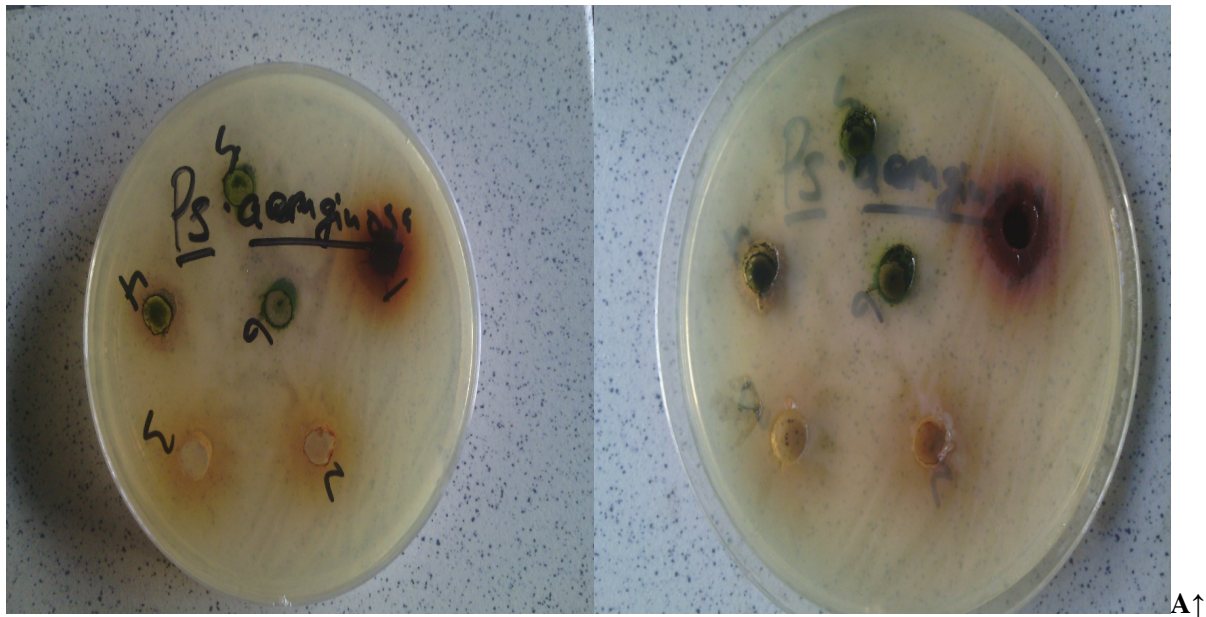


C



D

Plate 1 A, B, C and D: Agar diffusion plates showing the activities of *Annona muricata* extracts (100 mgmL^{-1}) against *Staphylococcus aureus*



A↑

and B↑



C

Plate 2 A, B and C: Antibacterial activity of *Annona muricata* extracts against *Pseudomonas aeruginosa*



Plate 3: Antibacterial activity of *Annona muricata* extracts against *Klebsiella pneumoniae*

Minimum inhibitory concentration (MIC) of aqueous leaf and ethanol skin extracts of Annona muricata against Staphylococcus aureus

The minimum inhibitory concentration (MIC) of aqueous leaf (ALA) and ethanol fruit-skin (ESA) extract of *Annona muricata* against *Staphylococcus aureus* are 25 mgmL⁻¹ and 6.25 mgmL⁻¹ respectively as shown in table 3.

Table 3: Minimum inhibitory concentration of aqueous leaf and ethanol skin extracts of *A. muricata* against *Staphylococcus aureus*

SN	Extract	Dilution in mg mL ⁻¹							Zone of Inhibition (mm)	
		100	50	25	12.5	6.25	3.12	1.56		
1.	ALA	16	14	12	-	-	-	-		
2.	ESA	17	13	11	9	7	-	-		

Antibacterial activity of aqueous and ethanol leaf extracts of Annona muricata against multidrug resistant (MDR) organisms isolated from immunocompromised (HIV/AIDS) patients with lower respiratory infections.

The two active extracts of *Annona muricata* (ALA and ESA) further screened against multidrug-resistant organisms isolated from immunocompromised patients co-infected with acute respiratory infections demonstrated antibacterial activity; and most of the organisms were sensitive to the extracts with zones of inhibition ranging from 7-18 mm (Table 4.6). Furthermore, a remarkable phenomenon was observed in the activity of ESA apart from showing a zone of inhibition such that the organisms appeared morphologically altered. This alteration occurs immediately after the zones of inhibition forming a purported zone of reversion which can be as wide as 28 mm in diameter.

Table 4 Antibacterial activity of aqueous leaf and ethanol skin extracts of *Annona muricata* against multidrug-resistant organisms isolated from immunocompromised (HIV/AIDS) patients with lower respiratory infections.

SN	Organism	MAR index	ALA Zone of Inhibition (mm)	ESA Zone of Inhibition (mm)
1.	<i>Klebsiella pneumoniae ozaenae</i>	0.2	14	15
2.	<i>Klebsiella pneumoniae pneumoniae</i>	0.5	10	7
3.	<i>Klebsiella pneumoniae pneumoniae</i>	0.7	14	9
4.	<i>Klebsiella pneumoniae ozaenae</i>	0.7	17	7
5.	<i>Klebsiella pneumoniae pneumoniae</i>	0.3	12	6
6.	<i>Klebsiella pneumoniae pneumoniae</i>	0.4	12	16
7.	<i>Klebsiella pneumoniae pneumoniae</i>	0.4	0	0
8.	<i>Klebsiella pneumoniae pneumoniae</i>	0.7	16	12
9.	<i>Klebsiella pneumoniae pneumoniae</i>	0.3	12	0
10.	<i>Klebsiella pneumoniae pneumoniae</i>	0.4	12	0
11.	<i>Klebsiella pneumoniae pneumoniae</i>	0.3	0	0
12.	<i>Klebsiella pneumoniae oxytoca</i>	0.1	0	0
13.	<i>Klebsiella pneumoniae ozaenae</i>	0.5	10	0
14.	<i>Klebsiella pneumoniae oxytoca</i>	0.7	0	0
15.	<i>Klebsiella pneumoniae pneumoniae</i>	0.3	11	0
16.	<i>Pseudomonas aeruginosa</i>	0.6	0	0
17.	<i>Klebsiella pneumoniae pneumoniae</i>	0.2	0	0
18.	<i>Klebsiella pneumoniae pneumoniae</i>	0.3	16	14
19.	<i>Klebsiella pneumoniae pneumoniae</i>	0.2	16	14
20.	<i>Klebsiella pneumoniae pneumoniae</i>	0.1	12	0

21.	<i>Pseudomonas putida</i>	0.6	15	13
22.	<i>Proteus mirabilis</i>	0.7	16	13
23.	<i>Burkholderia cepacia</i>	0.8	17	15
24.	<i>Burkholderia cepacia</i>	0.9	17	8
25.	<i>Shewanella putrefaciens</i>	0.8	16	12
26.	<i>Acinetobacter haemolyticus</i>	0.7	13	6
27.	<i>Enterobacter cloacae</i>	0.7	14	6
28.	<i>Burkholderia cepacia</i>	0.7	14	12
29.	<i>Burkholderia cepacia</i>	0.8	14	8
30.	<i>Acinetobacter baumannii</i>	0.7	13	15
31.	<i>Serratia marcescens</i>	0.9	12	0
32.	<i>Providentia stuartii</i>	0.8	12	15
33.	<i>Pseudomonas putida</i>	0.7	13	12
34.	<i>Acinetobacter baumannii</i>	0.7	15	11
35.	<i>Citrobacter diversus</i>	0.7	16	0
36.	<i>Escherichia coli</i>	0.7	16	10
37.	<i>Escherichia coli</i>	0.7	16	12
38.	<i>Burkholderia cepacia</i>	0.7	0	0
39.	<i>Acinetobacter iwoffi</i>	0.7	0	0
40.	<i>Pseudomonas aeruginosa</i>	0.9	14	16
41.	<i>Burkholderia cepacia</i>	0.7	14	8
42.	<i>Pseudomonas aeruginosa</i>	0.9	14	16
43.	<i>Burkholderia cepacia</i>	0.7	14	12

44.	<i>Pseudomonas aeruginosa</i>	0.9	14	16
45.	<i>Pseudomonas aeruginosa</i>	0.9	14	16
46.	<i>Pseudomonas aeruginosa</i>	0.9	14	16
47.	<i>Proteus mirabilis</i>	0.7	18	14
48.	<i>Proteus mirabilis</i>	0.7	14	13
49.	<i>Pseudomonas putida</i>	0.7	13	12
50.	<i>Staphylococcus aureus</i>	0.8	14	14

MAR: multiple antibiotic resistance; ALA: Aqueous leaf extract of *A. muricata*; ESA: Ethanol fruit-skin extract of *A. muricata*.

Antibacterial screening of extracts of *A. muricata* against test organisms responsible for specific acute respiratory infections, particularly pneumonia and tuberculosis; revealed bioactivity as earlier reported by Solomon-Wisdom *et al.* [34] and Radji *et al.* [36] respectively. Bussmann *et al.* [37] recorded huge antibacterial activities in screening aqueous and ethanol extracts of approximately a gross population of plant species including *Annona muricata* against selected gram-negative and gram-positive bacteria. They observed that 81% of the ethanol extracts inhibited *Staphylococcus aureus*, while 36% inhibited *Escherichia coli* with minimum inhibitory concentration range between 0.008 - 256 mgmL⁻¹. This result confirms the activity of extracts tested in this study, particularly the two extracts: ESA and ALA which showed the most remarkable antibacterial activity with MIC of 6.25 and 25 mgmL⁻¹ respectively. In the same vein, the diameter of inhibition for fruit-skin ethanol extract of *Annona muricata* against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*: 17 ± 0.58, 16 ± 0.00 and 16 ± 0.58 mm respectively, and for aqueous leaf extract: 16 ± 0.58, 16 ± 1.15 and 14 ± 0.58 mm; all agree with the findings of Solomon-Wisdom *et al.* [34] and Yakubu *et al.* [35] in inhibition zone and that of Martínez *et al.* [38] with Meléndez and Capriles [39]; who reported *Staphylococcus aureus* as one the most susceptible bacteria to plant extracts. The non-significant difference in antibacterial activities observed between the aqueous leaf and fruit-skin ethanol extracts of *A. muricata* implies that the antibacterial activities of both extracts against selected organisms are closely related; although several studies have proved that the extent of antibacterial activity for *Annona muricata* is solvent-dependent; affirming that ethanolic and methanolic extracts showed higher activity compared to aqueous extract which often times show low or no antibacterial activity [40]. The antibacterial activity demonstrated by ALA and ESA against 80% of the multidrug-resistant organisms isolated from immunocompromised patients co-infected with acute respiratory infections, with zones of inhibition ranging from 7-18 mm agree well with the findings of [40].

Conclusion

Extracts from the fruit-skin and leaves of *Annona muricata* are composed of diverse phytochemicals that possess both antibacterial and possible antimultidrug-resistant bioactive principles that may serve as potential antidote against deadly bacterial infections. There is need for more research on the fruit-skin extracts of *Annona muricata* Linn., as future findings from this may further enhance life quality via ameliorating targeted infections. Bioactive-directed fractionation of the fruit-skin extract to isolate pure bioactive compounds is also highly recommended.

Acknowledgements

We duly acknowledge the entire management of Covenant University for making requisite facilities available for scientific research and selected Technical staff in the Biochemistry for collaborative support and assistance in getting the project executed.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

We declare no conflict of interest whatsoever.

References

- [1]. Siddharth R. Krishnamurthy, Andrew B. Janowski, Zhao G, Barouch D, Wang D (2016). Hyperexpansion of RNA Bacteriophage Diversity. *PLOS Biology*, **14** (3): e1002409.
- [2]. Vitorino LC, Bessa LA (2017). Technological Microbiology: Development and Applications. *Frontiers in Microbiology*, **8**: 827.
- [3]. Forterre P (2010). Defining Life: The Virus Viewpoint. *Origins of Life and Evolution of Biospheres* **40**(2): 151–160.
- [4]. Brown JH (2014). Why are there so many species in the tropics? *Journal of Biogeography*, **41**(1): 8–22.
- [5]. University of Georgia (1998). First-Ever Scientific Estimate of Total Bacteria on Earth Shows Far Greater Numbers Than Ever Known Before. *Science Daily*. Retrieved 10 November 2014.
- [6]. Vaisberg HG, Eschweiler U, Reitz G, Wehner J, Willimek R, Strauch K (1995). Biological responses to space: results of the experiment "Exobiological Unit" of ERA on EURECA I. *Advances in Space Research*, **16**(8): 105–118.
- [7]. Zhang KD, Bieger-Dose A, Dillmann R, Gill M, Klein A, Meinert H, Nawroth T, Risi S, Stride C. (1995). ERA-experiment "space biochemistry". *Advances in Space Research*, **16**(8): 119–129.
- [8]. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (2005). Diversity of the human intestinal microbial flora. *Science*, **308**(5728):1635-1638.
- [9]. Bry L, Falk PG, Midtvedt T, Gordon JI (1996). A model of host-microbial interactions in an open mammalian ecosystem. *Science*. **273**(5280):1380-1383.
- [10]. Tlaskalova-Hogenova H, Stěpánková R, Kozáková H, Hudcovic T, Vannucci L, Tučková L, Rossmann P, Hrnčíř T, Kverka M, Zákostelská Z, Klimešová K, Příbylová J, Bártová J, Sanchez D, Fundová P, Borovská D, Srůtková D, Zídek Z, Schwarzer M, Drastich P, Funda DP. (2011). The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. *Cellular and Molecular Immunology*. **8**:110-120.

- [11]. Moreira D, López-García P. (2002). The molecular ecology of microbial eukaryotes unveils a hidden world (PDF). *Trends in Microbiology* 10 (1): 31–38.
- [12]. Kumamoto CA, Vences, M.D. (2005). Contributions of hyphae and hypha-co-regulated genes to *Candida albicans* virulence. *Cellular Microbiology* 7 (11): 1546–1554.
- [13]. Anyanwu MU, Okoye R.C. (2017). Antimicrobial activity of Nigerian medicinal plants. *Journal of Intercultural Ethnopharmacology*, **6**(2): 240–259.
- [14]. Iyanda-Joel WO, Adegbite OS, Ajetunmbi OB, Rotimi SO, Iweala EEJ, Chinedu SN. 2019. Phytochemical, antioxidant and mitochondrial permeability transition studies on fruit-skin ethanol extract of *Annona muricata*. Conference Proceedings of the 3rd International Conference on Science and Sustainable Development; May 6-8, 2019 at the Covenant University Center for Research, Innovation and Development, Covenant University, Ota, Nigeria.
- [15]. Badrie N, Schauss A. 2010. Soursop (*Annona muricata* L.): Composition, nutritional value, medicinal uses and toxicology. In: Watson R, Preedy V, editors. *Bioactive foods in promoting health*. Oxford: Academic Press; pp. 621–643.
- [16]. Pinto AC de Q, Silva EM. 1996. Graviola for Export. Technical Aspects of Production. Embrapa / SPI, Brasilia, Brazil.
- [17]. National Research Council. 1975. Underexploited tropical plants with promising economic value: report of an ad hoc panel of the Advisory Committee on Technology Innovation, Board on Science and Technology for International Development, Commission on International Relations. National Academy of Sciences. Washington DC., USA; Libraries Australia ID: 5664342. <https://trove.nla.gov.au/version/16206736>.
- [18]. Nwinyi OC, Chinedu N.S. and Ajani OO (2008). Evaluation of antibacterial activity of *Pisidium guajava* and *Gongronema latifolium*. *Journal of Medicinal Plants Research*, **2**(8): 189-192.
- [19]. Coria-Téllez AV, Montalvo-González E, Yahia EM, Obledo-Vazquez EN. *Annona muricata*: A comprehensive review on its traditional medicinal uses, Phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian J. Chem.*, 2018; 11(5):662–691.
- [20]. Viera GH, Mourao JA, Angelo AM, Costa RA, Vieira RH. Antibacterial effect (*in vitro*) of *Moringa oleifera* and *Annona muricata* against gram positive and gram negative bacteria. *Rev Inst Med Trop Sao Paulo*, 2010; 52:129–132.
- [21]. Lawal, I.O., Grierson, D.S. and Afolayan, A. J. (2015). Phytochemical and antioxidant investigations of a *Clausena anisata* Hook, a South African medicinal plant. *African Journal of Traditional, Complementary and Alternative Medicine*, **12**(1): 28-37.
- [22]. Larbie, C., Arthur, F.K.N., Terlabi, E.O. and Woode, E. (2011). Evaluation of acute and subchronic toxicity of *Annona muricata* (Linn.) aqueous extract in animals. *European Journal of Experimental Biology*, 1(4): 115–124.
- [23]. Igbinoba, V.I. (2014). Comparative analysis of the phytochemical and in vitro antioxidant properties of *Annona muricata* leaf extracts. Unpublished Dissertation. pp 31–38.

- [24]. Sarver, J.P., Blumer, L.S. and Denton, M.K. (2008). Methanol-free method of extracting secondary chemicals. In: Tested Studies for Laboratory Teaching, K.L. Clase (Editor). Proceedings of the 29th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 29: 376–388. Edeoga, H.O., Okwu D.E. and B.O. Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* **4**(7):685-688.
- [25]. Matyash, V., Liebisch, G., Kurzchalia, T.V., Shevchenko, A. and Schwudke, D. (2008). Lipid extraction by methyl-tert-butyl ether for high throughput lipidomics. *Journal of Lipid Research*, 49(5): 1137–1146. Kokate, C.K. (2000). In: Practical Pharmacognosy, Vallabh Prakashan, Delhi, India, p. 218
- [26]. Ronpirin, C., Charueksereesakul, T., Thongrakard, V. and Tencomnao T. (2014). Effects of ethanolic extract of *Annona squamosa* L. leaves on the expression of EGFR. *Journal of Chemical and Pharmaceutical Research*, 6(4): 791–797.
- [27]. Matyash, V., Liebisch, G., Kurzchalia, T.V., Shevchenko, A. and Schwudke, D. (2008). Lipid extraction by methyl-tert-butyl ether for high throughput lipidomics. *Journal of Lipid Research*, 49(5): 1137–1146.
- [28]. Harborne, J.B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis*. 3rd edition. Chapman and Hall, London: p 235. ISBN-13: 978-0-412-23050-9 e-ISBN-13: 978-94-009-5921-7 doi: 10.1007/978-94-009-5921-7
- [29]. Sofowora, A. (1993). *Phytochemical screening of medicinal plants and traditional medicine in Africa*. 2nd Edition Spectrum Books Limited, Nigeria: pp 150 – 156.
- [30]. Trease, G.E. and Evans, W.C. (1989). *Pharmacognosy*, 12th Edition, Balliere-Tindall, London: pp 241-260.
- [31]. Basu, S., Ghosh, A. and Hazra, B. (2005). Evaluation of the antibacterial activity of *Ventilago madraspatana* Gaertn., *Rubia cordifolia* Linn., and *Lantana camara* Linn.: isolation of emodin and physcion as active antibacterial agents. *Phytotherapy Research*, **19**: 888-894.
- [32]. Sateesh, V. and Naikpatil R.J.L (2011). Selective isolation and antimicrobial activity of rare actinomycetes from mangrove sediment of Karwar. *Journal of Ecobiotechnology*, 3(10): 48-53.
- [33]. Bauer, A.W., Kirby, W.M.M., Sherris J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45: 493–496.
- [34]. Solomon-Wisdom, G.O., Ugoh, S.C. and Mohammed, B. (2014). Phytochemical screening and antimicrobial activities of *Annona muricata* (L.) leaf extract. *American Journal of Biological, Chemical and Pharmaceutical Sciences* 2: 1–7.
- [35]. Yakubu, O.F., A.H. Adebayo, T.O. Famakinwa, O.S. Adegbite, T.A. Ishola, L.O. Imonikhe, O.A. Adeyemi, O.A. Awotoye, and E.J. Iweala (2018). Antimicrobial and toxicological

studies of *ricinodendron heudelotii* (Baill.). Asian Journal of Pharmaceutical and Clinical Research, 11(1): 299-305.

- [36]. Radji, M., Kurniati, M. and Kiranasari, A. (2015). Comparative antimycobacterial activity of some Indonesian medicinal plants against multi-drug resistant *Mycobacterium tuberculosis*. Journal of Applied Pharmaceutical Science, 5(1): 19–22.
- [37]. Bussmann, R.W., Malca, G., Glenn, A., Sharon, D., Nilsen, B., Parris, B. and Townesmith, A. (2010). Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. Journal of Ethnopharmacology, 132: 101–108.
- [38]. Martínez, M.J., Betancourt, J., Alonso-González, N. and Jauregui, A. (1996). Screening of some Cuban medicinal plants for antimicrobial activity. Journal of Ethnopharmacology, 52: 171–174.
- [39]. Meléndez, P.A. and Capriles, V.A. (2006). Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine, 13(4): 272–276.
- [40]. Viera, G.H., Mourao, J.A., Angelo, A.M., Costa, R.A. and Vieira, R.H. (2010). Antibacterial effect (*in vitro*) of *Moringa oleifera* and *Annona muricata* against gram positive and gram negative bacteria. Journal of the Institute of Tropical Medicine São Paulo, 52: 129–132.