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Original Article

Bioactive compounds and antimicrobial activity of extracts from fermented African locust bean (*Parkia biglobosa*) against pathogenic microorganisms

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Abstract

Background: The challenges of multiple antibiotic resistance by pathogenic microorganisms has necessitated the need for a continuous searching for new and effective antimicrobial bioactive compounds. **Objectives:** In this study, antimicrobial activity of extracts from fermented condiment from *Parkia biglobosa* was investigated against some pathogenic microorganisms. **Materials and Methods:** Gas chromatography and mass spectrometry (GC-MS) was used to identify bioactive compounds in *n*-hexane extract (oil). Aqueous and *n*-hexane extracts of locust beans were tested against clinical isolates; viz., *Klebsiella* spp., *Aeromonas hydrophilia*, *Citrobacter braakii*, *Enterobacter aerogenes*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Methicillin Resistant Staphylococcus aureus* (MRSA), *Aspergillus* spp. and *Candida albicans* using agar well diffusion method. **Results:** The study revealed the phytochemicals in oil as phenols (41.8 mg/100g), flavonoids of 19.37 mg/100g, saponins (16.7 mg/100g), alkaloids (22.9 mg/100g), steroids (6.9 mg/100g), terpenoids (10.0 mg/100g) and cardiac glycosides (3.3 mg/100g). The aqueous extract contains phenols (33.7 mg/100g), flavonoids (12.3 mg/100g), alkaloids (17.6 mg/100g), saponins (5.0 mg/100g) and cardiac glycosides (1.2 mg/100g). The bioactive compounds in the *n*-hexane extract were ricinoleic acid, p-cymene, octadecanoic acid, *n*-hexadecanoic acid and others. Oil from fermented locust bean exhibited zones of inhibition ranging from 5 mm to 14 mm against the tested isolates at 10 mg/mL, while the aqueous extract displayed inhibition zones of 4 mm to 10 mm at 10.0 mg/mL. **Conclusion:** The chemical constituents in locally fermented condiment (locust bean) are responsible for pronounced antimicrobial properties. Hence, the condiment can be exploited for medicinal purposes.

Keywords: Fermented food, condiment, *n*-hexane, phytochemicals and antimicrobials

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1. Introduction

Development of resistant genes in bacteria is one of mechanisms that support their natural adaptation to the presence of antimicrobial agents [1]. Resistance to drug by microorganisms are increasing despite the fact that pharmaceutical industries are producing a number of new antibiotics [2,3]. Infections as a result of recurrent multiple antimicrobial resistance have claimed at least 50,000 lives in many parts of the world [4]. It is estimated that if there is a continue rise in resistance levels by 2050 it would lead to 10 million deaths annually [4,5].

Plants play a pivotal role in the prevention or treatment of diseases and thus, reduce the adverse effects of conventional treatments [6]. An essential part in the investigation of plant is identification of biologically active compounds in plant, leading to further biological and pharmacological studies [7]. The plant kingdom represents a resource pool of species with potent medicinal potentials [8]. They constitute the richest source of pharmaceuticals, nutraceuticals and folk medicine products across the globe. The increasing side-effects of synthetic drugs on humans and their influence on the evolution of resistant microbial strains triggered research into plant resources and their derivatives as suitable alternative therapeutics [9]. The natural products in plant will be continued to be exploited towards meeting the urgent need to develop new and effective drugs, since plant plays a leading role in the treatment of human diseases [10].

P. biglobosa (Jacq.) R.Br. ex G.Don is a perennial dicotyledonous angiosperm that belongs to the family Fabaceae along with other tree legumes [11]. The roots, fruits and stem bark of *P. biglobosa* are used in the treatment of infertility and veterinary medicine respectively among the 'Igede' people of Benue State in Nigeria [12]. *P. biglobosa* leaves are traditionally used as antihypertensive agent in Benin, Nigeria [13], and a bark

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infusion of the plant is used as a tonic for diarrhea and anemia , as an analgesic drug, especially against dental pain with anti-inflammatory activities in Ivory Coast [14]. The bark was reported as viable remedy for toothache, leprosy, eye sores, fever, hypertension, wounds such as ulcer and snake bite [15]. Fermented locust bean, a pungent condiment called "Iru" in Yoruba land, "Dawadawa" in Hausa, "Ogiri okpe" in Igbo, as "Sumbala", "Netetou", and "Kainda" in some parts of Africa is commonly added to flavor most traditional stews, sauces and soups. It is produced from the fermentation of the dried, dehulled and boiled seeds of *P. biglobosa* [16]. The African locust bean has gained its popularity from consumption and economic value of its bean seeds [17]. Fermented foods are subjected to action of microbial enzymes, which cause significant modification to food as a result of desirable biochemical change. Natural fermentation can eliminate anti-nutrients and produce important nutrients [18]. Food condiments are prepared by traditional methods of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of protein and carbohydrates [19], yielding to more nutritional contents and higher therapeutic purposes [20]. The presence of nutrients and phytochemicals in African locust bean have been linked to its medicinal importance but with few reports on bioactivities of oil (*n*-hexane) and water extracts from the local condiment. In this study, the antimicrobial activity of extracts from fermented African locust beans was tested against some clinical microorganisms.

2. Materials and Methods

2.1 Source of indicator microorganisms

Clinical isolates namely; *Klebsiella pneumoniae, Klebsiella oxytoca, Aeromonas hydrophilia, Enterobacter aerogenes, Shigella dysenteriae, Pseudomonas aeruginosa, Salmonella typhi, Citrobacter braakii, Escherichia coli, Methicillin Resistant Staphylococcus aureus, Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus* and *Candida albicans* were gotten from Obafemi Awolowo University Teaching Hospital, Ile Ife and transported to Department of Microbiology, The Federal University of Technology, Akure, Nigeria.

2.2 Collection of samples

Fermented locust bean was purchased from King's market in Akure, the samples were taken to the Microbiology laboratory, The Federal University of Technology, Akure for further studies.

2.3 Preparation of extracts from Fermented Condiment

Fermented locust bean was sundried to reduce the moisture content. The dried sample was ground and 170 g was transferred into a Soxhlet extractor with *n*-hexane [21]. After extraction, *n*-hexane was removed using a rotary evaporator to generate fermented locust bean oil. The method of Azwanida [22] was utilized to obtain aqueous extract from fermented locust bean with some modifications. This was performed by soaking 50 g of pulverized sample in 200 ml of distilled water for one hour and was filtered with Whatman No. 1 filter paper. The extract was freeze dried (FD-10-MR, Xiangtan Xiangyi instrument Ltd, China) at -65 °C. The extracts were stored at - 4 °C before used.

2.4 Phytochemicals Assessment in Extracts from Fermented Condiment

Determination of alkaloid and tannins was carried out according to Trease and Evans [23], saponin [24] phenol [25], flavonoid [26], while steroids, cardiac glycosides and anthraquinones were determined according to the methods of Abioye *et al.* [27].

2.5 Identification of bioactive compounds in oil of fermented locust bean

Gas chromatography and mass spectrometry (GC-MS) was used to identify the various bioactive components present in oil from fermented locust beans [28]. The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions with a thickness of 1.00 µm, dimensions of 0.32 mm × 30 m and temperature limits of 60 °C to 325 °C. The column operates between 60 °C and 240 °C at a rate of 0.5 m/s with pressure of 100.2 Kpa. The injector and detector were at 250 °C and 200 °C, respectively. Helium gas was used as a carrier gas at flow rate of 0.46 m/s. The MS analysis was done based on comparative retention times, mass and peaks of chemical compounds using the computer-aided matching of unknown mass spectra of compounds with the known compounds stored in the software database library from the National Institute of Standards and Technology (NIST), Washington, USA, having more than 62,000 patterns as the reference database.

2.6 Determination of antimicrobial activity of oil and water extract obtained from fermented locust bean

Agar well diffusion method was used to evaluate antimicrobial activity of fermented locust bean extracts [29]. Briefly, tested microorganisms; inoculum turbidity was adjusted and compared to 0.5 McFarland standard. The absorbance of the solution was then checked using a spectrophotometer at 620 nm. Mueller Hinton agar was prepared according to manufacturer's specification and inoculated by spreading 0.1 ml of the test organisms (x 10^6) over the entire agar surface. Dimethyl sulfoxide (2% v/v) was used to reconstitute the oil extracts in order to make a stock solution and sterilization was carried out by filtration using 0.22 µm aqua membrane nylon filter disk (Benton Dickinson Company). Cork borer of 6 mm was used to bore holes on the plate. Reconstituted extracts (50 µl) of 10 mg/mL were dropped in each well. Antibiotics; chloramphenicol and nystatin were used as positive control against bacteria and fungi, respectively. Dimethyl sulfoxide (2% v/v) was used as negative control. The plates were incubated at 37 °C for 24 h and 25 °C for 3-4 days for bacteria and fungi, respectively. Zones of inhibition were measured in millimeter (mm). Minimum inhibitory concentration (MIC) was determined by varying the concentrations of extracts from 2.5-10.0 mg/mL. Minimum

bactericidal concentration (MBC) was recorded as lowest concentration of extract that showed no growth of tested microorganisms on the agar plates.

2.7 Experimental animal design

Wistar albino rats were obtained from the Department of Animal Production and Health, Federal University of Technology, Akure. The initial weights of the rats were 62-63 kg. They were allowed to acclimatize for seven days. The rats were fed basal diet, which comprises of animal feed with water. Some of the rats were inoculated with pathogenic microorganisms; they were carefully monitored due to effects of microorganisms on them, while some of them were not inoculated serving as the control. The experiment was carried out following the guideline stated institutional ethics and international standard of animal welfare described by National Research Council [30].

Rats (five) each was grouped as follows:

- CN 1: rats not infected but fed basal diet;
- CN 2: rats infected with *A. flavus* but not treated;
- CN 3: rats infected with *K. pneumoniae* but not treated;
- CN 4: rats infected with *Methicillin-resistant Staphylococcus aureus* but not treated;
- HEX 1: rats infected with *K. pneumoniae* and treated with oil of fermented locust bean;
- AQ 1: rats infected with *Methicillin-resistant Staphylococcus aureus* and treated with aqueous extract of fermented locust bean;
- HEX 2: rats infected with *A. flavus* and treated with oil of fermented locust bean;
- AQ 2: rats infected with *A. flavus* and treated with aqueous extract of fermented locust bean.

Infectivity was done according to the method of Komolafe *et al.*[31]. Stock cultures of *K. pneumoniae, methicillin resistant S. aureus* and *A. flavus* that were more susceptible to the aqueous and *n*-hexane extracts during in vitro assay were reactivated in nutrient broth and incubated at 37 °C for 18 hours. The broth culture was centrifuged at 2000 rpm for 10 min and the supernatant were discarded to obtain whitish pellet, which was serially diluted to106 CFU/mL in sterile distilled water. The test animals in each groups (five) were then orogastrically infected, while the control group were left uninfected.

2.8 Statistical Analysis

Experimental studies were carried out in replicates. Data obtained were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20, USA. Results were presented as mean ± standard deviation (SD).

3. Results

Quantity of phytochemicals in the oil and aqueous extract from fermented locust beans is shown in Table 1.

Table 1: Phytochemical contents (mg/100g) of extracts from fermented African locust beans

Phytochemicals	NE	AE
Phenol	41.8 ± 0.1	33.7 ± 0.3
Flavonoids	19.4 ± 0.5	12.3 ± 0.2
Saponin	16.7 ± 0.1	5.0 ± 0.0
Alkaloids	22.9 ± 0.0	17.6 ± 0.3
Steroids	6.9 ± 0.8	0.8 ± 0.0
Terpenoids	10.0 ± 0.0	8.0 ± 0.0
Cardiac glycosides	3.3 ± 0.0	1.2 ± 0.0

Values are mean of triplicates ± standard deviation. NE: *n*-hexane extract and AE: aqueous extract.

Phenols, cardiac glycosides, flavonoids, alkaloids steroids and saponin were present in the two extracts (oil and aqueous extract). Phenol had the highest value of 41.8 mg/100g and 33.7 mg/100g for oil and water extract, respectively. Alkaloid had the value of 22.9 mg/100g and 17.6 mg/100g for oil and aqueous extracts. Cardiac glycosides had the lowest value of 3.3 mg/100g and 1.2 mg/100g for oil and aqueous extract, respectively.

GC-MS spectrum confirmed the presence of various bioactive compounds with different retention times as illustrated in Figure 1.

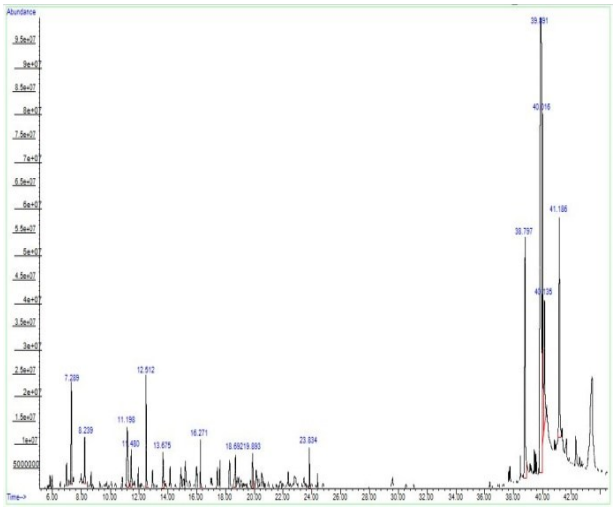

Figure 1: Peaks of bioactive compounds present in oil from fermented locust bean.

The bioactive compounds in oil; the most effective extract from fermented locust bean when subjected to GCMS were 1,3-dimethyl-p-xylene, o-xylene, 1-ethyl-3-methy-benzene, 1,2,3-trimethyl-mesitylene, 1,2,4-trimethyl-Benzene, 1,2,3-trimethyl-benzene, p-cymene, 1-methyl-2-(2-propenyl)-benzene, azulene, 2-methyl-naphthalene, *n*-hexadecanoic acid, 9,12-octadecadienoic acid, pentadecanoic acid, 7-methyl-9,12-octadiene and ricinoleic acid (Table 2).

Table 2: Bioactive compounds in oil from fermented African locust bean using Gas chromatography mass spectrometry

No	Retention time	Area (%)	Bioactive compound	Molecular Formula	Molecular Weight (g/mol)
1	7.28	3.93	1,3-dimethyl-p-Xylene	$C_6H_4(CH_3)_2$	106.17
2	8.23	1.55	0-Xylene	C_8H_{10}	106.17
3	11.19	2.84	1-ethyl-3-methy-Benzene	C_9H_{12}	120.19
4	11.48	1.35	1,2,3-trimethyl-Mesitylene	$C_9H_{11}NO_2$	120.19
5	12.51	4.04	1,2,4-trimethyl-Benzene	C_9H_{12}	120.19
6	13.67	1.26	1,2,3-trimethyl-Benzene	C_9H_{12}	120.19
7	16.27	1.75	p-Cymene	$C_{10}H_{14}$	134.21
8	18.69	1.83	1-methyl-2-(2-propenyl)-Benzene	$C_{11}H_{14}$	146.23
9	19.89	1.31	Azulene	$C_{10}H_8$	128.17
10	23.83	1.54	2-methyl-Naphthalene	$C_{11}H_{10}$	142.20
11	38.79	9.58	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.40
12	39.89	41.80	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.45
13	40.01	10.97	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.39
14	40.13	4.54	7-methyl-9,12-Octadiene	C_9H_{16}	316.26
15	41.18	11.71	Ricinoleic acid	$C_{18}H_{38}O_3$	298.46

Table 3 shows zones of inhibition of extracts from fermented locust beans on tested isolates. The oil (*n*-hexane extract) exerted the highest effect on *K. pneumoniae* at 10 mg/mL with diameter zone of inhibition of 14.00 mm, while the least effect (5.0 mm) was observed on *A. fumigatus* and *C. albicans*. The highest zone of inhibition of 10.0 mm was observed against *methicillin-resistant Staphylococcus aureus* when tested against aqueous extract at 10 mg/mL but *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *C. albicans* have no zone of inhibition.

Table 3: Inhibition zones (mm) displayed by extracts from fermented African locust beans against pathogenic bacteria at 10 mg/mL

Microorganisms	NE	AE	Chloramphenicol/ Nystatin
Escherichia coli	9.0 ± 0.0	7.0 ± 0.5	17.0 ± 0.2
Shigella dysenteriae	10.0 ± 0.1	5.0 ± 0.1	22.0 ± 0.1
Methicillin-resistant Staphylococcus aureus	8.0 ± 0.0	10.0 ± 0.5	11.0 ± 0.0
Pseudomonas aeruginosa	10.0 ± 0.1	0.0	17.0 ± 0.0
Salmonella typhi	9.0 ± 0.2	5.0 ± 0.2	9.0 ± 0.2
Citrobacter braakii	8.0 ± 0.0	7.0 ± 0.0	19.0 ± 0.0
Aeromonas hydrophilia	9.0 ± 0.2	6.0 ± 0.2	17.0 ± 0.3
Enterobacter aerogenes	10.0 ± 0.0	4.0 ± 0.0	21.0 ± 0.0
Klebsiella oxytoca	7.0 ± 0.0	0.0	22.0 ± 0.0
Klebsiella pneumoniae	14.0 ± 0.1	8.0 ± 0.1	18.0 ± 0.1
Aspergillus flavus	10.0 ± 0.5	8.3 ± 0.0	16.0 ± 0.0
Aspergillus niger	7.0 ± 0.0	6.0 ± 0.0	19.0 ± 0.0
Aspergillus fumigatus	5.0 ± 0.2	8.0 ± 0.0	18.0 ± 0.2
Candida albicans	5.0 ± 0.0	0.0 ± 0.0	22.0 ± 0.1

Values are means of triplicates ± standard deviation. NE: n-hexane extract and AE: aqueous extract

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of n-hexane and aqueous extracts of fermented locust bean against bacteria are shown in Table 4. The MIC of oil against tested microorganisms ranged from 2.5-10.0 mg/mL, while aqueous extract ranged from 5.0 -10 mg/mL. The MBC obtained in this study ranged from 5.0 - 20.0 mg/mL for oil extract and 20.0- 50.0 mg/mL for aqueous extract.

Table 4: Minimum inhibitory and bactericidal concentrations (mg/mL) for extracts of fermented African locust beans against microorganism

Bacterial isolates	NE	AE	NE	AE
	MIC		MBC	
Escherichia coli	5.0	10.0	10.0	20.0
Shigella dysenteriae	2.5	10.0	5.0	20.0
Methicillin-resistant Staphylococcus aureus	5.0	5.0	10.0	50.0
Pseudomonas aeruginosa	2.5	0.0	5.0	0.0
Salmonella typhi	5.0	10.0	10.0	20.0
Citrobacter braakii	5.0	10.0	10.0	20.0
Aeromonas hydrophilia	5.0	10.0	10.0	20.0
Enterobacter aerogenes	5.0	10.0	10.0	25.0
Klebsiella oxytoca	10.0	0.0	20.0	0.0
Klebsiella pneumoniae	5.0	10.0	10.0	20.0
Aspergillus flavus	5.0	10.0	10.0	25.0
Aspergillus niger	10.0	10.0	20.0	50.0
Aspergillus fumigatus	10.0	10.0	20.0	50.0
Candida albicans	10.0	10.0	20.0	50.0

NE: *n*-hexane extract and AE: aqueous extract

The hematological parameters of rats infected with pathogenic microorganisms and treated with aqueous and *n*-hexane extracts from fermented locust beans are shown in Table 5, while Table 6 shows Differential white blood cell count of rats infected and treated with extracts from fermented locust beans. The PCV of infected rats were lower (18-20%) compared to others, which ranged from 24.0 -38%. The WBC (109 g/L) of infected rats, which were not treated were higher as 11.8 to 14.8 when compared to treated groups with WBC ranging from 4.8-5.9. The lymphocytes ranged from 43.0 to 61.0%, neutrophils as 36.0-49.0%, eosinophil as 1.0-3.0% and monocytes as 1.0 to 2.0%.

4. Discussion

Parkia plants have been identified as source of tannins, saponins, protein and amino acid acids [32]. Phytochemical screening showed the presence higher proportion of phenols and flavonoids. They are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants [33]. Phenols and flavonoids have been shown to have a wide range of biological and pharmacological activities, which include anti-allergic, anti-inflammatory, antioxidant, anti-microbial, anti-cancer, and anti-diarrheal activities [34,35]. Cardiac glycoside was also one of the phytochemicals present in the oil. It served as the main medical treatment to congestive heart failure and cardiac arrhythmia, due to their effects of increasing the force of muscle contraction while reducing heart rate [36].

Table 5: Hematological parameters of rats infected with microorganisms and treated with extracts from fermented African locust beans

Sample	PCV (%)	HB (g/dl)	WBC (10^9g/L)	RBC (10^{12}g/L)	MCHC	MCV	MCH
CN 1	31.0 ± 0.0	10.3 ± 0.0	5.0 ± 0.0	3.4 ± 0.0	33.2 ± 0.0	90.1 ± 0.0	3.0 ± 0.0
CN 2	20.0 ± 0.0	6.8 ± 0.1	14.8 ± 0.0	2.4 ± 0.0	33.5 ± 0.0	82.3 ± 0.0	2.5 ± 0.0
CN 3	18.0 ± 0.0	9.4 ± 0.0	11.8 ± 0.1	2.1 ± 0.0	33.6 ± 0.0	81.5 ± 0.0	2.6 ± 0.0
CN 4	17.2 ± 0.0	7.5 ± 0.1	12.7 ± 0.0	2.1 ± 0.0	33.2 ± 0.1	81.7 ± 0.0	2.5 ± 0.0
AQ 1	30.0 ± 0.1	9.4 ± 0.0	5.4 ± 0.0	3.2 ± 0.0	33.6 ± 0.0	88.9 ± 4.0	3.0 ± 0.0
HEX 1	26.0 ± 0.5	5.4 ± 0.0	5.9 ± 0.0	3.0 ± 0.0	33.8 ± 0.0	89.2 ± 0.1	2.9 ± 0.0
AQ 2	38.0 ± 0.0	12.6 ± 0.0	5.4 ± 0.0	4.2 ± 0.0	33.2 ± 0.0	90.1 ± 2.0	3.0 ± 0.0
HEX 2	24.0 ± 0.0	8.0 ± 0.7	4.8 ± 0.1	5.4 ± 0.0	33.3 ± 0.0	90.3 ± 2.0	3.0 ± 0.0

Key: PCV-packed cell volume, Hb-Hemoglobin, WBC-white blood cell, RBC-red blood cell, MCHC- mean corpuscular hemoglobin concentration, MCV- mean corpuscular volume, MCH- mean corpuscular hemoglobin, CN 1: Rat not infected but fed basal diet and water, CN 2: rat infected with *Aspergillus flavus* but not treated, CN 3: rat infected with *Klebsiella pneumoniae* but not treated, CN 4: rats infected with *Methicillin-resistant Staphylococcus aureus* but not treated, HEX 1: Rat infected with *Klebsiella pneumoniae* and treated with *n*-hexane extract of fermented locust bean, AQ 1: Rats infected with *Methicillin-resistant Staphylococcus aureus* and treated with aqueous extract of fermented locust bean, HEX 2: rats infected with *Aspergillus flavus* and treated with *n*-hexane extract of fermented locust bean, AQ 2: rats infected with *Aspergillus flavus* and treated with aqueous extract of fermented locust bean.

Table 6: Differential white blood cell count (%) of rats infected and treated with extracts from African Locust beans

Sample	Lymphocytes	Neutrophils	Eosinophil	Monocytes	Basophile
CN 1	55.0 ± 7.3	41.0 ± 0.5	2.0 ± 0.0	1.0 ± 0.0	0.0
CN 2	61.0 ± 9.4	36.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.0
CN 3	43.0 ± 5.7	49.0 ± 5.0	1.0 ± 0.0	1.0 ± 0.0	0.0
CN 4	52.6 ± 3.1	45.3 ± 7.2	1.0 ± 0.0	1.0 ± 0.0	0.0
AQ 1	57.0 ± 4.7	37.0 ± 5.7	2.0 ± 0.0	1.0 ± 0.0	0.0
HEX 1	47.0 ± 7.1	49.0 ± 7.7	1.0 ± 0.0	1.0 ± 0.0	0.0
AQ 2	56.0 ± 8.2	38.0 ± 7.0	3.0 ± 0.0	2.0 ± 0.0	0.0
HEX 2	54.0 ± 5.7	40.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	0.0

Values are means of triplicate determinations ± standard error. Keys: CN 1: Rat not infected but fed with the normal diet and water, CN 2: rat infected with *Aspergillus flavus* but not treated, CN 3: rat infected with *Klebsiella pneumoniae* but not treated, CN 4: rats infected with *Methicillin-resistant Staphylococcus aureus* but not treated, HEX 1: Rat infected with *Klebsiella pneumoniae* and treated with *n*-hexane extracts of fermented locust bean, AQ 1: Rats infected with *Methicillin-resistant Staphylococcus aureus* and treated with aqueous extract of fermented locust bean, HEX 2: rats infected with *Aspergillus flavus* and treated with *n*-hexane extract of fermented locust bean, AQ 2: rats infected with *Aspergillus flavus* and treated with aqueous extract of fermented locust bean.

This is in accordance with previous studies where glycoside was found to be present in partially and completely fermented locust bean seed but lacking in the unfermented seeds [37]. Phytochemical screening showed the presence of saponins in *n*-hexane extract. Saponins in plant help humans to fight fungal infections, decrease blood glucose level, and lower cancer risks [38]. Phytochemicals serve as natural antibiotics, helping the body to fight infections and microbial invasions [39]. The presence of these phytochemicals in extracts of African locust bean and its fermented product has been linked to their antimicrobial activities [37,40]. The aqueous extract from fermented locust bean contains flavonoids with little amount of steroids, which is in accordance with Osemwegie and Dahunsi[41]. The researchers assessed the phytochemicals in the aqueous and ethanolic extracts of *Parkia biglobosa* root and stem. Daramola [42] reported that, the defatted samples of fermented locust bean contain high quantity of bioactive compounds like phenolic compounds, peptides, saponins, and amino acids. In the present study, p-cymene was one of the components of *n*-hexane extract of fermented locust bean. *p*-cymene as a medicinal bioactive compound is often found in more than 100 plant species[43]. *p*-cymene and its components are the most important constituents of essential oils produced through liquid extraction and steam distillation of edible and medicinal plants, it shows a range of biological activity including antioxidant, anti-inflammatory, antinociceptive, anxiolytic, anticancer and antimicrobial effects [44]. Rahman *et al.* [45] reported that p-cymene and *n*-hexadecanoic acid are part of the antimicrobial compounds present in methanol leaf extract of *Psidium guajava*.

Octadecanoic acid present in the *n*-hexane extract had the highest percentage as revealed by GC-MS. This is in accordance with Al-Jasass and Al-Jasser [46] who reported the presence of bioactive compounds (octadecanoic acid) in some spices and herbs; fenugreek, cress, mustard, black cumin, black pepper, and clove grown in Saudi Arabia. In this study, Pentadecanoic acid, a saturated fatty acid was identified as one of the constituents of fermented locust bean as revealed by GC-MS and this is in relation to previous studies where pentadecanoic acid was identified as one of the fatty acid constituents of *Peganum harmala* [47].

The aqueous extract of fermented locust bean inhibited the growth of methicillin-resistant *S. aureus* at concentrations 5, 10, 20, 50 and 100 mg/mL. This is in accordance with previous study that showed that the aqueous extract of the root of *P. biglobosa* has inhibitory effect on the growth of *Staphylococcus aureus* isolated from patients with urinary tract infection [40]. In another study, the fractionation of *P. biglobosa* seeds with different solvent

(*n*-hexane, chloroform and methanol) inhibited *Candida albican, Aspergillus fumigatus, Crytococcus neoformans, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, vancomycin resistance *Enterococcus* and Methicillin-resistance *Staphylococcus aureus* [48]. Jauro *et al.* [49] revealed inhibitory potential of *P. biglobosa* methanolic leaf extract on MRSA isolated from sheep and human at different concentrations of 100, 200 and 400 mg/mL. Abioye *et al.* [27] revealed that methanolic crude extract of *P. biglobosa* exhibited zones of inhibition of 14 mm and 28 mm against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. The researchers reported MIC of methanolic extract of *P. biglobosa* against tested isolates as 0.63 mg/mL to 5 mg/mL, while the MIC values of *n*-hexane and aqueous fractions were within 0.63 mg/mL and 10 mg/mL. In a similar study, the aqueous extract of the stem bark of *P. biglobosa* was reported to inhibit the growth of different microorganisms[50,51]. The oil of fermented locust bean exhibited better zone of inhibition than aqueous extract probably because not all the bioactive compounds have been extracted with water [52]. However, aqueous extract from fermented African locust still inhibited some pathogenic microorganisms. In a similar way, aqueous extract of the *P. biglobosa* displayed inhibitory zones of 6.5 to 19.5 mm against four spoilage microorganisms; *Bacillus subtilis, Cronobacter dublinensis, Pantoea agglomerans*, and *Bacillus* sp. isolated from mulberry fruit [53].

In this study, there was increase in the hemoglobin level of rats treated with oil and aqueous extract of fermented locust bean and this is a good one because decrease in hemoglobin level may result in anemia [54]. From the study, there was decreased in the packed cell volume of rats infected with each of the microorganism; *A. flavus, K. pneumoniae*, and Methicillin-resistant *S. aureus*. The treated group with aqueous extracts of fermented locust bean has increased packed cell volume than group treated with oil. This is similar to the findings of Sunday *et al.* [55] who revealed PCV values of rats treated with aqueous extract of *Brysocarpus coccineus* root bark were higher than that of untreated rats infected with bacterially induced diarrhea. The slight deviation of PCV observed in rats treated with oil could due to the presence of ricinoleic acid in the oil as revealed by GC-MS. This is in relation to the work of Momoh *et al.* [56] who studied the effect of oil from castor seed on the hematological parameters of albino rats and reported that the presence of ricinoleic acid in *Ricinus communis* caused a reduction in PCV. The white blood cell (WBC) increased in infected rats with pathogenic microorganisms. WBC is used as an immunological parameter to determine the case of infection [57], because the main type of phagocytic cells, which is required to participate in the phagocytosis in the ingestion of foreign bodies (like bacterial cells) are neutrophil and macrophage[58,59], so during infection with bacteria, the range of neutrophils increased compared to control. Basophil and eosinophil play a role in immunity, eosinophil increase in parasitic infections [60].

5. Conclusion

From this study, the growth inhibition exerted by aqueous and *n*-hexane extracts from fermented locust bean seed suggests that *P. biglobosa* have antimicrobial property, which may be as a result of the bioactive compounds present in the extracts. The oil and water extracts from fermented locust bean contained phytochemicals that exhibited noticeable antimicrobial activities. Hence, the bioactive compounds in the fermented condiment can be exploited for medicinal purposes.

Author contribution: OCO and BJA conceived and designed the research study. RNE, OCO and BJA performed the experiments. RNE and OCO interpreted the data and drafted the manuscript. OCO and BJA revised the manuscript. All authors read and approved the final manuscript.

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