Antimicrobial, antibiofilm, anti-quorum sensing and motility inhibition activities of essential oil from seeds of food spice *Xylopia aethiopica* (Dunal) A. Rich. on some pathogenic bacteria

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Abstract

Medicinal food excipients can be used to combat microbial infections especially essential oils. Xylopia aethiopica (Dunal) A. Rich is a highly consumed medicinal food spice in Africa and its seeds essential oil was extracted by hydro-distillation and characterized by GC-FID and GC-MS. Its antimicrobial, anti-biofilm, anti-motility and quorum sensing inhibition potentials were evaluated on some pathogenic microorganisms. The identified compounds were grouped as oxygenated monoterpenes (57.06%) and monoterpenes hydrocarbons (28.96%) as major components while sesquiterpene hydrocarbons (5.94%), oxygenated sesquiterpenes (2.29%) and diterpenes (1.79%) where minor constituents. The most abundant constituent in the essential oil is myrtenol (13.25%), an oxygenated monoterpene.

The most sensitive gram-positive and gram-negative bacteria were S. aureus and P. aeruginosa respectively with MIC values of 0.3125 µg/mL while the yeast C. albicans showed MIC of 0.625 µg/mL. Good antibiofilm results were found with highest inhibition percentage in S. aureus varying from 73.0±3.0 at MIC to 9.0±0.5 % at MIC/32. Biofilm inhibitions were higher in gram-positive bacteria than for gram-negative and yeast. Highest motility inhibitions were 45.32±0.10 and 63.84±3.50% in swimming and swarming models respectively at dose of 100 µg/mL. The essential oil of X. aethiopica showed good anti-quorum sensing activity with quorum-sensing inhibition zones of 22.0±0.5 mm at MIC. The good results show that consumption of X. aethiopica is potent biocontrol means to reduce severity and virulence of food pathogens and to reduce their resistance to antibiotics which is a global health problem

Keywords: *Xylopia aethiopica*, essential oil, GC-MS, antibiofilm, anti-quorum sensing.

Introduction

Foodborne disease (Any disease of an infectious or toxic nature caused by consumption of food) are sometimes life threatening and some people have a higher risk such as pregnant women, young children, older people and those with weak immune systems. Bacteria are mostly responsible for foodborne disease and their control are multi-disciplinary tasks requiring skills in the areas of clinical medicine, epidemiology, laboratory medicine, food microbiology and chemistry, food safety and food control and risk communication and management[34].

Despite advances in food safety, foodborne diseases still occur around the world caused by major foodborne pathogens which have developed multidrug resistance with time as a result of misuse of antibiotics[31]. New methods like biofilm and quorum sensing inhibition are currently employed as new strategies to fight the emergence of microbial resistant strains which are gradually constituting a global health problem.

Also, the search for more effective and safer alternative antibiotics from natural sources is growing. Based on this argument, researchers are increasingly investigating herbal products in the quest for new therapeutic and antipathogenic agents that might act as nontoxic inhibitors of quorum sensing (QS), thus controlling infections without encouraging the appearance of resistant bacterial strains[5,14]. Many pathogens attack plants and secret mycotoxins which can constitute negative effects on plants and animals and decrease crop production and the essential oils and their constituents can play the role of nontoxic biocides to control the pathogens and to improve the quality of crops and guarantee safety and consumer protection[4]. Plants use essential oils as chemical signals to interact with their surrounding environment such as to repel harmful insects and herbivores but attract beneficial insects. Due to their properties, man uses essential oils in aromatherapy, antimicrobial, antioxidant and food preservation.

Essential oils are a promising and suitable lead to this hypothesis since they are known to be good antimicrobial substances. A major food spice and essential oil rich plant

wildly used in Africa is *Xylopia aethiopica*. A very common example of an *Annonoceous* plant, *Xylopia aethiopica* (Dunal) A. Rich is an evergreen, slim, tall and aromatic fruit bearing tree that grows to 15–30 m high and 60–70 cm in diameter and is widely distributed in west and central Africa Savanna region which spreads across Ethiopia, Cameroon, Central African Republic, Chad, Egypt, Sudan, Ghana, Ivory Coast, Burkina Faso, Benin, Senegal and Nigeria where it is widely used as food condiments and as remedy to various ailments.[3,11,36]

Xylopia aethiopica is very rich in essential oils and studies have reported chemical characterisation of this essential oil and some of its medicinal importance. The essential oils from seeds of *Xylopia aethiopica* are known to possess antioxidant, antiradical, antifungal, antibacterial, anti-insecticidal, anti-cancer.[3,10,14,15,19,27,30]

Though few antimicrobial studies on essential oils from this plant exist, no study reporting the effect of *X. aethiopica* essential oil on microbial virulence factors exists. It should be noted that these virulence factors account for the development of microbial resistance and for the severity of microbial infection. This work examines the antimicrobial, antibiofilm and antiquorum sensing activity of essential oils from stored seeds of *Xylopia aethiopica* on various pathogenic bacteria.

Material and Methods

Plant material and hydro-distillation: The stored seeds of *Xylopia aethiopica* were purchased from the Bamenda food market, North-West Region of Cameroon. They were identified by Mr. T. Fulbert, a botanist working at the National Herbarium of Cameroon where a voucher specimen of this famous spicy plant is deposited under specimen number (39020/HNC).

The seeds were powdered and submitted to hydro-distillation to yield the essential oil[28]. Summarily, the essential oil was obtained by heating a mixture of 20 g of the seed powdered sample in 1000 mL of distilled water for 3 hours using a Clevenger-type apparatus.

The steam mixed with the essential oil was condensed forming two layers which were carefully separated on a separatory funnel to yield a light-yellow oil with a strong aroma. The oil was dried over anhydrous sodium sulfate and stored in a refrigerator at 4 ºC until it was used.

GC-MS analysis: The dried essential oil diluted with Et_2O, was subjected to GC-FID thermal gradient analysis, on an Agilent model 6280 gas chromatograph fitted with an HP-5® (30 m × 0.25 mm, 0.25 µm film thickness) capillary column. The initial temperature of the column was kept at 50°C for 5 min and was heated to 300°C at a rate of 4°C/min and then kept at final temperature for 20 min. The temperature of the detector and injector was kept at 250°C and 280°C respectively. Helium, as carrier gas, was used at flow rate of 1 mL/min. 1 µl of sample was injected with a split ratio of 1:20.

The GC-MS analysis was performed on similar conditions and parameters as described for GC-FID using Perkin-Elmer Clarus 500 gas chromatograph equipped with an HP-5MS® (30 m × 0.25 mm, 0.25 µm film thickness) capillary column. The MS was operated at standard conditions 70 eV and 250°C. The processed mass spectra of components from essential oil of propolis were identified by mass fragmentation patterns and by comparison with the electronic MS NIST library and the calculated retention indices (RI) with literature data.

Determination of minimum inhibitory concentrations: MICs were determined by a microtitre broth dilution method as recommended by the Clinical and Laboratory Standards Institute[7]. The MIC was defined as the lowest essential oil concentration that yielded no visible growth. The test medium was Mueller-Hinton Broth (MHB) and the density of bacteria was 5×10^5 colony-forming units (CFU)/mL. Cell suspensions (100 µL) were inoculated in to the wells of 96-well microtitre plates in the presence of essential oil with different final concentrations (10, 5, 2.5, 1,25, 0.625, 0.312 mg/mL). The inoculated microplates were incubated at 37ºC for 24 hours before being read.

Effect of essential oil on bacterial biofilm formation: The effect of essential oil at concentrations including 1, ½, ¼ and 1/8 MIC on biofilm-forming ability of test microorganisms was tested with a microplate biofilm assay[21,29]. Briefly, 1% of overnight cultures of isolates was added into 200 µL of fresh Tryptose-Soy Broth (TSB) supplemented with 0.25% glucose and cultivated in the presence and absence of essential oil without agitation for 48 hours at 37 ºC.

The wells containing TSB+cells served as control. After incubation, the wells were washed with water to remove planktonic bacteria. The remaining bacteria were subsequently stained with 0.1% crystal violet solution for 10 minutes at room temperature. Wells were washed once again to remove the crystal violet solution. A volume of 200 µL of 33% glacial acetic acid was poured in wells. After shaking and pipetting of wells, 125 µL of the solution from each well were transferred to a sterile tube and volume was adjusted to 1 mL with distilled water. Finally, optical density (OD) of each well was measured at 550 nm (Thermo Scientific Multiskan FC, Vantaa, Finland). Percentage of inhibition of the tested extracts was calculated using the formula:

$$\text{Biofilm inhibition (\%)} = \frac{OD\ 550control - OD550sample}{OD\ 550control} \times 100$$

Bioassay for quorum-sensing inhibition (QSI) activity using CV026: Quorum sensing inhibition was evaluated as described elsewhere [16, 29] with slight modifications. Five milliliters of warm molten Soft Top Agar (1.3 g agar, 2.0 g tryptone, 1.0 g sodium chloride, 200 mL deionized water) were seeded with 100 µL of an overnight CV026 culture and

20 µL of 100 µg/mL C_6HSL was added as exogenous AHL source. This was gently mixed and poured immediately over the surface of a solidified Luria Bertani Agar (LBA) plate as an overlay. Wells of 5 mm in diameter were made on each plate after the overlay had solidified. Each well was filled with 50 µL of sub-MIC concentrations filter sterilized essential oil.

A white or cream-colored halo around this well against a purple lawn of activated CV026 bacteria was an indication of QSI. The limit of detection of activity was also determined by applying serial dilutions of the essential oil (1:1 to 1:8, using LB broth as diluent). End points were estimated as the lowest dilution of essential oil giving discernible inhibition of violacein synthesis. Each experiment was done in triplicate and the assay plates were incubated at 30°C for 3 days after which the diameters of the quorum sensing inhibition zones were measured.

Swarming and swimming motility inhibition on Pseudomonas aeruginosa PA01: Inhibition of swarming motility assay was done as described previously[19, 29]**.** Briefly, overnight cultures of *P. aeruginosa* PAO1 strain were point inoculated at the center of swarming plates consisting of 1% peptone, 0.5% NaCl, 0.5% agar and 0.5% of filter-sterilized D-glucose with various concentrations of essential oil (50, 75 and 100 µg/mL) and the plate without the essential oil was maintained as control. Plates were incubated at an appropriate temperature in an upright position for 18 h. The swarming migration was recorded by following swarm fronts of the bacterial cells.

For swimming motility assay, the *P. aeruginosa* PAO1 strain was inoculated at the center of the swarming agar medium consisting of 1% peptone, 0.5% NaCl, 1.5% agar and 0.5% of filter-sterilized D-glucose with increasing concentrations of essential oil (50, 75 and 100 µg/ml). The plates were then wrapped with Saran Wrap to prevent dehydration and incubated at 37 °C in upright position for 16 h. The reduction in swimming migration was recorded by measuring the swim zones of the bacterial cells after 16 h.

Results and Discussion

Chemical composition of essential oil of *X. aethiopica*: Essential oil was obtained by hydro-distillation from seed powder of *X. aethiopica* in the yield 3.05 % (v/w). The seeds of this aromatic plant are richest part in essential oils, its leaves contain on average 30 times less essential oil, the bark 100 time less essential oil than the fruits. Variation of extraction yield of fruit essential oil against conservation conditions for this plant has been shown to have 4.39 % (v/w) content of essential oils[22]. This implies that conservation has an impact on the essential oil composition of this plant seed as the yield obtained here is slightly lower than that reported by Karioti and co-workers[15] who obtained 3.67% (v/w) and 3.33% (v/w) for fresh and dried fruits of *X. aethiopica* respectively.

It should be noted that this food spice is mostly consumed in conserved form which is relevant to this investigation. The chemical constituents of *X. aethiopica* seed essential oil characterized by GC-MS, are given in table 1.

Table 1
Chemical constituents of essential oil of *X. aethiopica* seeds

RT	Compound Name	RI HP-5	Amount %	Identification
5.838	α-Thujene	924	0.58	*RI, MS*
6.091	α-Pinene	951	1.45	*RI, MS, LIT*
7.502	Sabinene	972	1.46	*RI, MS*
7.674	β-pinene	978	4.03	*RI, MS, LIT*
9.509	α-Phellandrene	994	1.95	*RI, MS, LIT*
9.800	Eucalyptol	1022	3.84	*RI, MS, LIT*
11.341	3-Carene	1025	5.09	*RI, MS, LIT*
11.637	α-Limonene	1030	0.25	*RI, MS*
11.962	1,8-Cineole	1032	0.56	*RI, MS*
12.147	β-Phellandrene	1038	0.38	*RI, MS, LIT*
12.579	γ-terpinene	1055	4.72	*RI, MS, LIT*
12.712	Camphenilone	1060	0.42	*RI, MS, LIT*
12.793	trans-Linalool oxide	1070	0.93	*RI, MS*
13.251	α-Terpinolene	1081	1.34	*RI, MS, LIT*
13.528	allo-ocimene	1087	1.06	*RI, MS*
13.609	cis-Sabinene hydrate	1088	0.36	*RI, MS*
13.691	trans-Sabinene hydrate	1089	0.54	*RI, MS*
Monoterpene hydrocarbons			**28.96**	

14.020	linalool	1095	1.90	*RI, MS, LIT*
14.152	trans-Pinocarveol	1106	7.80	*RI, MS, LIT*
14.239	4-Isopropyl-1-methyl-2-cyclohexen-1-ol	1107	1.09	*RI, MS*
14.373	cis-Verbenol	1110	4.13	*RI, MS, LIT*
14.513	Camphor	1118	1.10	*RI, MS, LIT*
14.795	3-Pinanone	1143	1.74	*RI, MS*
14.919	Pinocarvone	1145	2.45	*RI, MS, LIT*
14.994	Myrtenal	1153	2.45	*RI, MS, LIT*
15.325	Cryptone	1155	0.56	*RI, MS*
15.403	Terpinen-4-ol	1159	4.93	*RI, MS, LIT*
15.531	Isopinocamphone	1176	0.39	*RI, MS*
15.762	Myrtenol	1177	13.25	*RI, MS, LIT*
16.005	Verbenone	1182	0.92	*RI, MS, LIT*
16.306	Cuminal	1216	3.72	*RI, MS, LIT*
16.366	Carvone	1223	6.92	*RI, MS, LIT*
16.749	Thymol	1277	3.71	*RI, MS, LIT*
Oxygenated monoterpenes			**57.06**	
16.874	δ-elemene	1325	0.69	*RI, MS, LIT*
17.256	α-cubenene	1350	0.44	*RI, MS*
18.095	α-Copaene	1363	0.91	*RI, MS, LIT*
18.171	β-elemene	1389	0.09	*RI, MS*
18.556	Cyperene	1401	0.34	*RI, MS*
18.634	Longifolene	1407	0.13	*RI, MS*
18.906	α-Gurjunene	1409	0.12	*RI, MS, LIT*
19.305	trans Caryophyllene	1418	0.33	*RI, MS, LIT*
19.416	β-Gurjunene	1439	0.20	*RI, MS, LIT*
19.961	α-humulene	1457	0.97	*RI, MS, LIT*
21.588	Germacrene D	1486	0.21	*RI, MS*
22.009	β-selinene	1488	0.15	*RI, MS, LIT*
22.736	Z-γ-bisabolene	1500	0.28	*RI, MS*
23.001	γ-cadinene	1513	0.53	*RI, MS, LIT*
26.350	δ-Cadinene	1523	0.18	*RI, MS, LIT*
27.016	α-Calacorene	1528	0.37	*RI, MS, LIT*
Sesquiterpene hydrocarbons			**5.94**	
27.652	Spathulenol	1563	0.71	*RI, MS, LIT*
28.902	Caryophyllene oxide	1568	0.20	*RI, MS, LIT*
29.566	Thujopsan-2-α-ol	1574	0.24	*RI, MS*
29.732	β-Eudesmol	1647	0.44	*RI, MS, LIT*
30.061	α-Eudesmol	1650	0.18	*RI, MS, LIT*
31.144	α-Cadinol	1676	0.52	*RI, MS, LIT*
Oxygenated sesquiterpenes			**2.29**	
41.766	Manoyl oxide	2002	1.54	*RI, MS, LIT*
42.526	Kaurene	2031	0.24	*RI, MS, LIT*
Diterpenoids			**1.79**	
Total percentage of identified constituents: 96.07				

RT=Retention Time, RI=Retention Indices, MS=Mass Fragmentation Patterns, LIT=Literature

The results show that most abundant of the constituents are oxygenated monoterpenes (57.06%) and monoterpenes hydrocarbons (28.96%) while sesquiterpene hydrocarbons (5.94%), oxygenated sesquiterpenes (2.29%) and diterpenes (1.79%) were less abundant. This is in conformity with reported constituents in fruits of *X. aethiopica*[22] but showed some difference to the constituents reported in *X. aethiopica* from other regions of Cameroon in which major compounds were monoterpene hydrocarbons.[3,23,27]

This difference may be due to the process of conservation which consists of packaging the dried seeds in polythene bags and storing under market conditions. There might possibly have been a loss in the hydrocarbon contents due to their volatility. Amongst the monoterpene hydrocarbons, the most abundant were 3-Carene (5.09%), β-pinene (4.03%), γ-terpinene (4.72%) and eucalyptol (3.84%). β-pinene and γ-terpinene have been reported as major monoterpene hydrocarbons in *X. xylopia* essential oil.[3,27]

The most abundant constituent in the essential oil is myrtenol (13.25%), an oxygenated monoterpene. Other major oxygenated monoterpenes were trans-Pinocarveol (7.80%), carvone (6.92%), terpinen-4-ol (4.93%), cis-Verbenol (4.13%), cuminal (3.72%) and thymol (3.71%) which have been reported in *X. aethiopica* essential oils as major constituents[22]. *Xylopia aethiopica* fruit finds uses in nutrition and pharmacology which may be accounted for by these chemicals which can confer reported functions in humans and other animals[6].

Mostly low molecular weight terpenoids constitute essential oils and they confer antimicrobial and antioxidant activities on the essential oils thereby making them suitable bio-preservatives in food and nutrition.

Antimicrobial activity of essential oils: Plant essential oils have been a subject of intense antimicrobial activity studies and for many centuries, they have been used as natural medicines to combat several pathogenic microorganisms such as fungi, bacteria and viruses[18,20]. The antimicrobial activity of the essential oil of *X. aethiopica* was assessed and the minimum inhibitory concentration (MIC) values defined as the minimum sample concentration with no observed bacterial growth are given in table 2.

Table 2
Minimal Inhibitory Concentrations (MIC) values of X. aethiopica essential oil on test microorganisms

Microorganism	MIC µg/mL
Staphylococcus aureus ATCC25923	0.3125
Enterococcus faecalis ATCC19433	0.625
Listeria monocytogenes ATCC7644	0.625
Pseudomonas aeruginosa PA01	0.3125
Escherichia coli ATCC25922	1.25
Candida albicans ATCC10239	0.625

For the gram-positive bacteria, the MIC values of the essential oils ranged from 0.3125 µg/mL for *Staphylococcus aureus* to 0.625 µg/mL for *Enterococcus faecalis* and *Listeria monocytogenes*. The most sensitive gram positive bacteria used in the study against essential oil was identified as *S.aureus*. For the gram-negative bacteria, the MIC values were 0.3125 µg/mL and 1.25 µg/mL for *Pseudomonas aeruginosa* and *Escherichia coli* respectively.

For the gram-negative bacteria, *P. aeruginosa* was the most susceptible. The yeast *Candida albicans* showed MIC of 0.625 µg/mL. The good antimicrobial activity of the fruit essential oil of *X. aethiopica* corroborates with previously reported data.[2,12,13,27,32,35] *S. aureus* and *P. aeruginosa* showed highest susceptibility to essential oils and in some study, these two bacteria were both having highest susceptibility to essential oil with highest antimicrobial inhibition zones compared to other microorganisms[33].

Since they are hydrophobic, essential oils can cross over the lipid membranes of bacterial cell wall, disrupting them and making them more permeable leading to leakage of cellular material and cell death.[8,9]

Antibiofilm activity of essential oil: Although many studies exist on antimicrobial activity of *X. aethiopica* essential oils, its antibiofilm and anti-quorum sensing activities have not been investigated. The antibiofilm activity of the essential oil on six microorganisms (S. *aureus, E. faecalis, L. monocytogenes, P. aeruginosa, E. coli and C. albicans*) was evaluated at MIC and sub-MIC concentrations and reported in table 3.

Table 3
Antibiofilm activity (Percentage inhibition) of essential oils on test microorganisms

Conc.	*S. aureus*	*E. faecalis*	*L. monocytogenes*	*P. aeroginosa*	*E. coli*	*C. albicans*
MIC	73.0±3.0	56.7±1.5	58.4±0.8	44.8±3.3	42.8±3.6	45.0±1.3
MIC/2	66.8±0.7	41.2±0.3	39.7±1.6	30.3±0.9	36.3±2.7	28.8±0.4
MIC/4	49.8±1.6	21.0±3.1	25.9±0.4	17.6±1.2	19.6±0.1	20.8±1.5
MIC/8	21.0±3.5	14.6±7.5	10.9±1.2	13.8±0.8	9.4±2.4	15.9±0.5
MIC/16	11.4±1.4	8.6±0.2	-	-	-	4.9±2.5
MIC/32	9.0±0.5	-	-	-	-	-
MIC/64	-	-	-	-	-	-

The percentage inhibitions of biofilm formations in the Gram-positive bacteria varied from 73.0±3.0 at MIC to 9.0±0.5 % at MIC/32 for *S. aureus*, 56.7±1.5 at MIC to 8.6±0.2 at MIC/16 for *E. faecalis* and from 58.4±0.8 at MIC to 10.9±1.2% at MIC/8 for *L. monocytogenes*. On the gram-negative bacteria, antibiofilm activity varied from 44.8±3.3% MIC to 13.8±0.8% at MIC/8 for *P. aeruginosa* and from 42.8±3.6% at MIC to 9.4±2.4% at MIC/8 for *E. coli*. For the yeast *C. albicans*, the biofilm inhibition percentage varied from 45.0±1.3 to 4.9±2.5% at MIC and MIC/16 respectively.

The biofilm inhibition varied in a dose-dependent manner and it is noted that the biofilm inhibitions were higher for the gram-positive bacteria than for gram-negative bacteria and *C. albicans*. Generally, the outer membrane consists of peptidoglycan in gram-positive bacteria, while in gram-negative bacteria, it consists of a double layer of phospholipids connected to an inner membrane by lipopolysaccharides. This makes gram-positive bacteria more susceptible to essential oils than gram-negative bacteria[4]. This may account for the higher biofilm formation inhibition in Gram-positive bacteria than in Gram-negative bacteria.

Bacterial biofilm consists of substances such as polysaccharides and certain proteins which help to protect the bacterial colonies from pressure, environmental stress, host immune system and antibiotics. Therefore, inhibiting or disrupting the constituted biofilm will expose the colonies to antimicrobial substances and help to invade the bacterial community, hence weakening its colonization and virulence in the host[26]. Therefore, the good biofilm inhibition of the essential oil of seeds of *X. aethiopica* as a substance can find applications in combating microbial resistance especially on food pathogens.

Swimming and swarming motility inhibition activity of essential oil: The swimming and swarming motility inhibitions of the essential oil were evaluated on *P. aeruginosa* PA01 and reported in table 4. At highest dose of 100 µg/mL, motility inhibition was 45.32±0.10 and 63.84±3.50% in swimming and swarming models respectively and at lowest dose (50 µg/mL), motility inhibition was 15.20±0.80 and 19.15±1.30% for the swimming and swarming models respectively.

Table 4
Swimming and swarming motility percentage inhibition of essential oil on *P. aeruginosa* PA01

Concentration µg/mL	Swimming	Swarming
100	45.32±0.10	63.84±3.50
75	31.01±4.50	42.75±2.00
50	15.20±0.80	19.15±1.30

The motility inhibitions varied in a dose-dependent manner as shown in fig. 1 and at same dose, it was higher in the swarming model than in the swimming model. The gram-negative bacterium, *P. aeruginosa* is suitable for this assay because it utilizes swimming motility predominantly mediated by hyperflagellation and surface-associated swarming and twitching motilities predominantly mediated by type-IV pili[1,17]. Swimming and swarming motility is important in microbial spread and possible surface colonization and they contribute to biofilm formation by bacteria which is a crucial environmental and clinical problem since it helps to increase resistance to antibiotics. The search of compounds which can disrupt microbial motility provides a suitable remedy to the limitation of bacterial surface spreading and colonization[24].

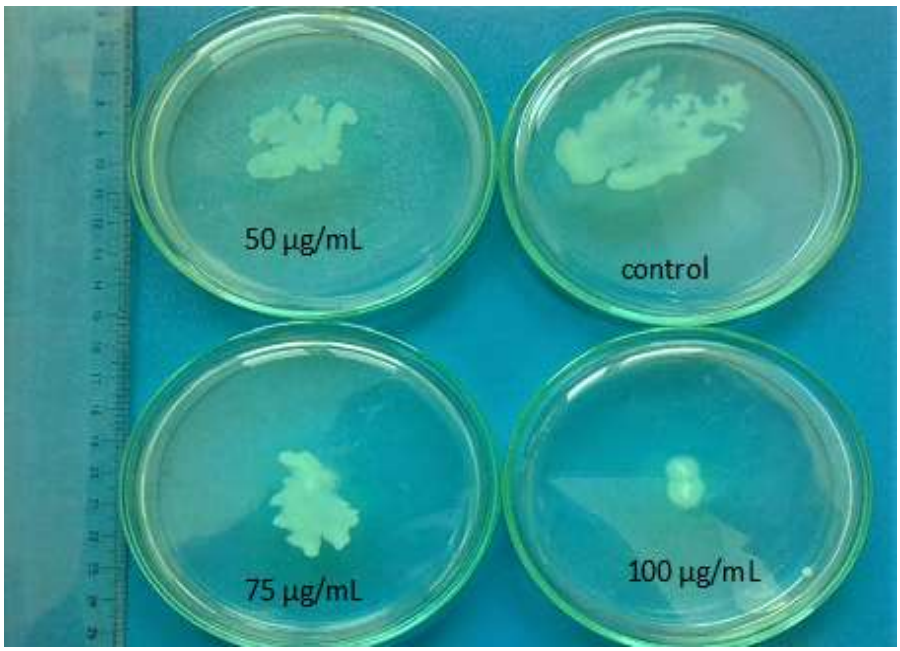

Fig. 1: *P. aeruginosa* PA01 motility inhibition plates

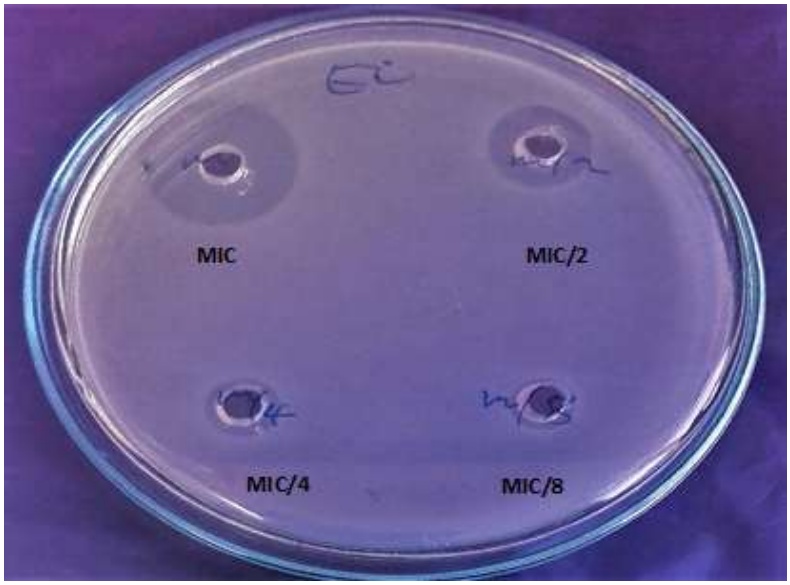

Fig. 2: *C. violaceum* CV026 quorum-sensing inhibition plate

Anti-quorum sensing activity of essential oil on *C. violaceum* CV026: Biofilm and swarming/swimming motility inhibitions are quorum-sensing mediated processes. Prior to anti-quorum sensing assay, the MIC value of the mutant strain *Chromobacterium violaceum* CV026 was measured and found to be 0.625 µg/mL. The QS inhibition zones corresponding to the cream or semitransparent zones in fig. 2 were determined (diameters measured in millimetres) at concentrations of MIC to MIC/8 and reported in table 5. The essential oil of *X. aethiopica* showed good anti-QS activity with QS inhibition zones of 22.0±0.5 mm at MIC, 15.7±3.0 mm at MIC/2 and 8.5±0.5 mm at MIC/4. No inhibition was observed at MIC/8.

Table 5
Anti-quorum sensing activity (Inhibition zones diameter in mm) of essential oil on CV026

Concentration	QS zone
MIC	22.0±0.5
MIC/2	15.7±3.0
MIC/4	8.5±0.5
MIC/8	-

-: No inhibition

This good anti-QS activity of the *X. aethiopica* essential oil is very important in preventing pathogenic microbial emergence since most bacteria regulate their virulence factors through quorum-sensing processes. Besides this, it displayed prominent antibacterial activity and is safer than conventional chemical antimicrobial agents since it is natural and is edible. The properties of essential oils which make them desirable in food include the fact that they confer non-toxic and desirable aroma and flavor to food products. Besides these aspects, they possess good antimicrobial and antioxidant properties. The good anti-quorum sensing activity of the essential oil from *X. aethiopica* is an indication that the severity of microbial contamination in food products can be reduced by the use of essential oils rich foods and spices since they have been shown to disrupt quorum sensing mediated processes in food pathogens.

Generally, antimicrobial activity of essential oils rich foods is very important and occurs through a number of ways including cell wall destruction, membrane protein damage, coagulation of cytoplasm, hydrolysis or decrease in synthesis of ATP, reduction of proton motive force, damaging cytoplasmic membrane and increasing permeability[4].

Conclusion

In conclusion, *Xylopia aethiopica* is an aromatic plant that belongs to the Annonaceae family and its fruits are highly commercialized in African markets and find uses as food spices and traditional medicine. It is commonly called African pepper, Guinea pepper or Ethiopian pepper and its nutritional and medicinal potentials have made this plant a subject of scientific investigations mostly for its chemical composition and bioactivities. This plant is highly rich in essential oils which also accounts for its bioactivities and hence its employment in nutrition and medicine. The essential oils obtained from seeds of this plant by hydrodistillation and characterized by GC-MS contained in monoterpene hydrocarbons (28.96%), oxygenated monoterpenes (57.06%), sesquiterpene hydrocarbons (5.94%), oxygenated sesquiterpenes (2.29%) and diterpenoids (1.79%). The essential oils showed good antimicrobial and antibiofilm activities on some pathogenic bacteria and antibiofilm activity was particularly higher on gram-positive bacteria than on gram-negative bacteria and yeast.

The essential oils disrupted bacteria cell-to-cell communication that was measurable from the quorum-sensing inhibition zones on *C. violaceum* CV026. Essential oils therefore have the potential to influence quorum-sensing

network of pathogens and reduce their virulence factors. This effect or phenomenon is important as it indicates that the essential oils from *X. aethiopica* can potentially reduce multidrug resistance by microorganisms which can be exploited as a biocontrol means to remedy infectious diseases, a global health problem.

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