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Article *in* Journal of Pharmacy and Pharmacology · August 2019

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GC-MS-based metabolomics, antibacterial and anti-inflammatory investigations to characterize the quality of essential oil obtained from dried *Xylopia aethiopica* fruits from Ghana and Nigeria

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Keywords
antibacterial effect; anti-inflammatory effect; essential oil; GC-MS-based metabolomics; *Xylopia aethiopica* fruits

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Received May 25, 2019
Accepted July 6, 2019

doi: 10.1111/jphp.13150

Abstract

Objectives This study sought to determine the quality of essential oil from *Xylopia aethiopica* fruits of different geographical origins using GC-MS-based metabolomics, bacterial quorum sensing and anti-inflammation assessment.

Methods Essential oil was obtained from eight batches of *X. aethiopica* fruits from Ghana and Nigeria by hydrodistillation, characterized using gas chromatography–mass spectrometry and differences therein found using metabolomics. The respective antibacterial activity of the oils was tested against four bacterial strains: two Gram-positive strains, *Staphylococcus aureus* (ATCC 25923) and *Bacillus licheniformis* (ATCC12759), and two Gram-negative strains, *Escherichia coli* (ATCC25922) and *Klebsiella pneumoniae* (ATCC 13883). Anti-inflammation was tested using RAW 264.7 macrophage cells.

Key findings The outcome of the study revealed that the oil of the Ghana-sourced samples exhibited superior antibacterial, cytotoxic and anti-inflammatory effects than those from Nigeria. This could be attributed to the higher levels of the bioactive compounds present in those samples. This distinction between the samples from the two countries was clearly established using the metabolomics approach, and 14 differential metabolites were found to be potential chemical markers.

Conclusions The study lends credence to the traditional uses of the essential oil of *X. aethiopica* as an antimicrobial and anti-inflammatory agent.

Introduction

The dried fruit of *Xylopia aethiopica* (Dunal) A. Rich is well known in many African countries for its culinary and medicinal uses. Traditionally, a decoction of the fruit is used to treat or manage various medical conditions including syphilis, boils, malaria, fungal infections, rheumatism, haemorrhoids, dysentery, flatulence, bronchitis, uterine fibroid and female infertility.[1] It is smoked and inhaled for the treatment of respiratory conditions. The fruit is also included in enemas and topical preparations for the treatment of boils and skin eruptions.[1]

Numerous studies have found the following pharmacological activity to be associated with its use: antimicrobial,[2] anti-inflammatory,[3] antidiabetic,[4] analgesic,[5] antiplasmodial,[6] antioxidant,[7] neuropharmacological,[8] antiproliferative effects,[9] etc.

The antimicrobial potential has been largely ascribed to the essential oil within various plant parts. These essential oils have been proven to exhibit varying levels of activity against various strains of Gram-positive and Gram-negative bacteria.[10–12] The antimicrobial effect is one that needs further investigation, considering the fact that there is resurgence in the search for new antimicrobial agents particularly, ones of natural origin. This interest is born out of the need for new, safe and cheap antibiotics due to the continual emergence of resistant bacterial strains.[13] There is also need to scientifically justify the traditional use of the essential oil as an anti-inflammatory agent since no scientific data exist to our knowledge in this regard.

Metabolomics, the total qualitative and quantitative analysis of the metabolome of any biological system under specific conditions, has emerged as a useful tool in systems biology.[14] It has been used successfully to distinguish

between closely related plant species as well as discriminate between same species of plant from different geographical locations.[15] Since metabolomics is a snapshot of a biological system under specific conditions, its application in plants can unveil the presence and levels of metabolites, that is primary and secondary metabolites. The primary metabolites are usually preserved structurally and abundance. The secondary metabolites however are needed for the plant to survive under abiotic stress conditions and differ vastly across the plant kingdom.[16] The levels of secondary metabolites captured through metabolomics can therefore provide vital information about the growth conditions of the plant and invariably the geographical location.

Available studies on the antimicrobial effect of *X. aethiopica* oil used one or a combination of methods such as agar disc diffusion, cup-plate agar diffusion and dilution assays but none used quorum sensing (QS) as an additional screening step. There has been no comparative metabolomic study until now on the essential oil of *X. aethiopica* sourced from Ghana and Nigeria. Herein, we sought to comparatively assess the quality of essential oil of *X. aethiopica* from the two countries using an untargeted gas chromatography–mass spectrometry (GC-MS)-based metabolomics approach. This study also sought to investigate whether or not differences of quality therein would translate into significant differential biological activity, in this instance, their antibacterial and anti-inflammatory effects.

Material and Methods

Plant material

Eight batches (i.e. four from each country) of *X. aethiopica* fruits were obtained from local producers at Techiman (Ghana) and Gombe (Nigeria) in August 2017. The samples were duly certified, voucher numbers assigned and deposited at the State Key Laboratory of Natural Medicines, Department of Pharmacognosy, China Pharmaceutical University.

Extraction of essential oil

The dried fruits were pulverized using AISITE electric blender. A 65 g quantity of the powdered *X. aethiopica* from each batch was weighed into the BP apparatus (i.e. Clavenger type). A 1040 ml volume of water was added and the mixture hydrodistilled for 4 h. The essential oils obtained were collected into amber-coloured airtight glass vials and kept at 4 °C in a refrigerator until analysis. Prior to that, the extracted oils were rid of water using anhydrous sodium sulphate. The yield of the oil was determined based on the weight of the dried powdered samples.

GC-MS analysis

The extracted oils were analysed using an Agilent 7890B series gas chromatograph (Ionization mode 70 eV) equipped with a fused capillary column (HP-5MS, 0.25 mm × 30 m, film thickness 0.25 μm) and coupled with a 5977A mass spectrometer (Agilent Technologies, Palo Alto, CA). The initial temperature of 50 °C was increased at a rate of 5 °C/min to 230 °C. The temperature was then increased at a rate of 10 °C/min to 280 °C. The input temperature was 190 °C. Helium at 49.9 KPa was used as carrier gas at a flow rate of 1.0 ml/min. Ethyl caprate 99 % was used as standard (molecular formula $C_{24}H_{12}O_2$, boiling point 245 °C, density 0.867). The method to analyse the samples consisted of 40 μl of the essential oil, 10 μl of standard and 50 μl of *n*-hexane.

Antibacterial effect assessment

Bacterial strains

Antibacterial effects of the essential oils were tested against four bacterial strains: two Gram-positive strains, *Staphylococcus aureus* (ATCC 25923) and *Bacillus licheniformis* (ATCC12759), and two Gram-negative strains, *Escherichia coli* (ATCC25922) and *Klebsiella pneumoniae* (ATCC 13883).

Determination of minimum inhibitory concentration

The micro-plate dilution method using the 96-well microplate[17] was used to determine the MICs of the oils. Overnight cultures of the test strains were diluted 1 : 100 into sterile nutrient broth and incubated at 37 °C with shaking at 250 rpm to OD600 value of 0.2 (low cell density, LCD) and OD600 value of 1.0 (high cell density, HCD) to determine the quorum-sensing effects of the oils in the test strains. A twofold serial dilution of each extract (100 μl) was prepared in 100 μl of sterile nutrient broth. Ampicillin (1000 μg/ml) was twofold serially diluted in 100 μl sterile nutrient broth to yield a final concentration of 0.25 μg/ml (positive control). Sterile nutrient broth (100 μl), with and without bacterial inoculation, was used as negative control and blank, respectively, to determine bacterial growth. The test strains (100 μl each) at each indicated density (LCD and HCD) was added to each well and incubated at 37 °C for 12–16 h. A 40-μl aliquot of 0.2 mg/ml *p*-iodonitrotetrazolium chloride (INT) was then added to each well and further incubated at 37 °C for 30 min. The growth of the bacteria in each micro-plate well was indicated by the formation of a red colour of INT reduced to formazan, while clear wells represented the absence of active bacterial growth. The lowest concentration at which a decreased red

colour is apparent compared to the next dilution was taken as the minimum inhibitory concentration (MIC) value. The assay was repeated three times.

Cell viability assay

Using the cell counting kit (CCK8 assay), the viabilities of RAW 264.7 cells were determined. The CCK8 assay consists of three steps. First, the RAW 264.7 cells (1.0×10^4/well) were seeded in a 96-well plate and incubated at 37 °C in 5% CO_2 for 12 h. In the second step, the *X. aethiopica* essential oils were dissolved in DMSO and diluted with PBS. Five concentrations (i.e. 10, 5, 1, 0.1, 0.05 and 0.01 μg/ml) of the oil samples were initially prepared and evaluated for their cytotoxic effects. Thereafter, three concentrations were selected and compared: 5 μg/ml + 1 μg/ml LPS (high), 1 μg/ml + 1 μg/ml LPS (medium) and 0.01 μg/ml + 1 μg/ml LPS (low). To the RAW 264.7 cells was added 100 μl of each concentration for 24 h. Finally, to the cells were added 10-μl aliquots of CCK8 (Dojindo, Kumamoto, Japan) and their viabilities measured at 450 nm using an ELISA reader (BioTek, Winooski, VT). The untreated cells which were incubated in DMEM (+10% FBS) were used as control.

Anti-inflammatory effect

The RAW 264.7 cells (1.0×10^6/well) were seeded in 96-well plates and incubated at 37 °C in 5% CO_2 for 12 h. The cells were then treated with the three main concentrations (i.e. 5 μg/ml + 1 μg/ml LPS, 1 μg/ml + 1 μg/ml LPS and 0.01 μg/ml + 1 μg/ml LPS) of *X. aethiopica* essential oil for 24 h. This procedure was repeated using same concentrations of dexamethasone-water soluble (CAS # 50-02-2). Until confluence, 50-μl aliquots of culture supernatants were mixed with the Griess reagent system (Beyotime, www.beyotime.com). After 10 min. of incubation, the absorbance was determined at 540 nm using an ELISA reader (BioTek). The standard 1 M $NaNO_2$ (Beyotime, www.beyotime.com) and its dilutions were used to plot the standard curve. The untreated cells that were incubated in DMEM (+10% FBS) were used as control.

Data processing and metabolite identification

Raw GC-MS files obtained were changed to mzData format using DA reprocessor (Agilent) at a peak height threshold of 5000 counts. Subsequently, peak finding, filtering and alignment were performed using R-Package XCMS. The

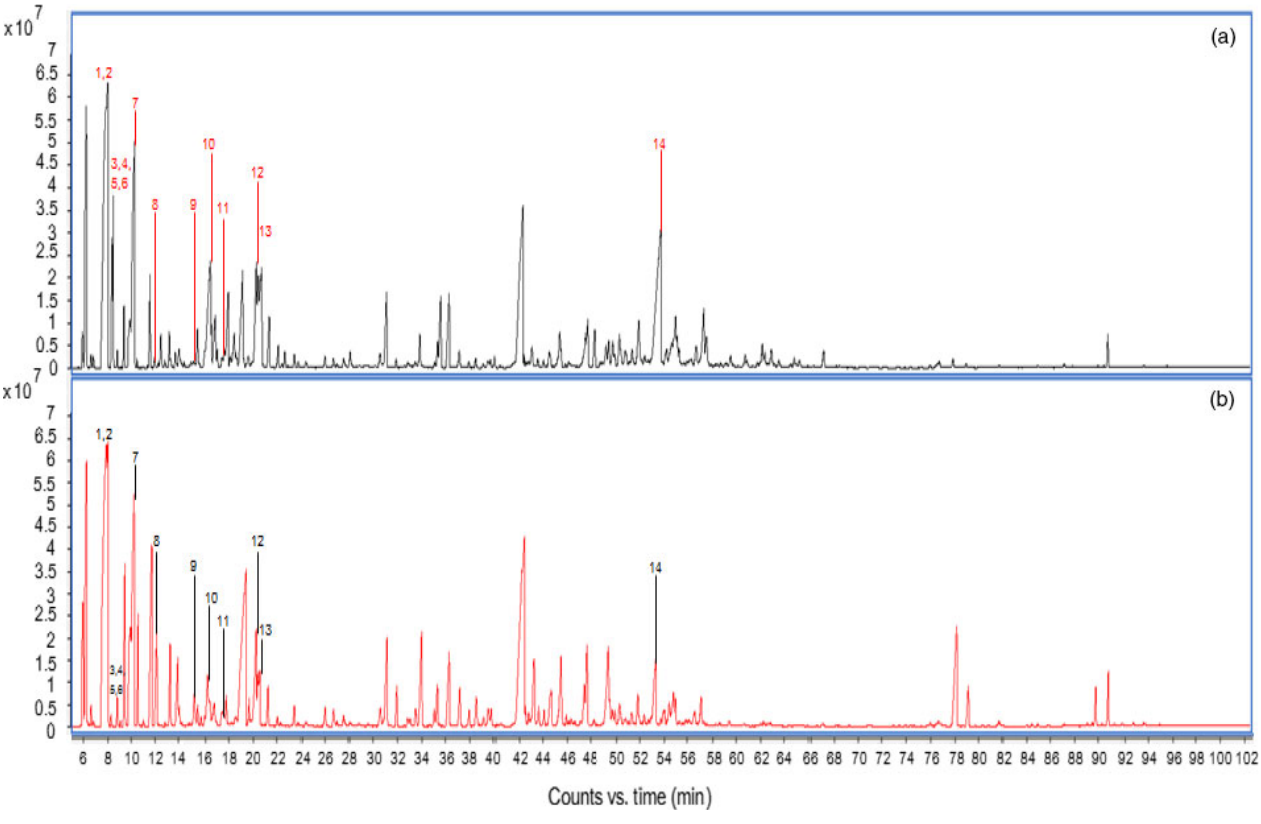

Figure 1 Representative total ion chromatograms (TIC) of essential oils extracted from the fruits of *Xylopia aethiopica* sourced from Ghana (a) and Nigeria (b). [Colour figure can be viewed at wileyonlinelibrary.com]

resultant data then were sequentially analysed using MetaboAnalyst 3.0 (http://www.metaboanalyst.ca). The main tasks accomplished with MetaboAnalyst 3.0 were same as reported earlier.[15] Using with MetaboAnalyst 3.0, the following were obtained: fold change, hierarchical cluster analysis (dendrogram), heatmap and *t*-test.

The normalized data were further analysed using SIMCA 14.1 (Umetrics, Umeå, Sweden). Using the SIMCA 14.1 software, multivariate data such principal component analysis (PCA), partial least squares-discriminate analysis (PLS-DA) and variable importance in projection (VIP) were obtained. The differences between the samples in terms of metabolite levels from the two countries were captured both with unsupervised PCA and supervised PLS-DA. The corresponding VIP values of the PLS-DA model were generated. The VIP values, fold change and *P*-values obtained from the *t*-test, were very crucial in determining the differential metabolites contributing to the observed differences between the samples. The criterion of VIP >1, fold change >2 and *P*-value <0.05 was relied upon to screen out the differential metabolites from the NIST Mass Spectral Library using their mass spectral data. Their identities were ascertained by a comparison of their linear retention indices (LRI) relative to (C8–C20) *n*-alkanes.

Statistical analysis

Source-dependent comparative levels of the differential metabolites between the essential oils, and differential cytotoxic and anti-inflammatory effects were assessed by one-way analysis of variance (ANOVA) with the Dunnett post hoc test using GraphPad Prism 6.0 (GraphPad Prism Software Inc., San Jose, CA, USA). *P*-values <0.05 were considered statistically significant while *P*-values <0.01 were deemed as highly statistically significant.

Results and Discussion

Yield of essential oil of fruits from Ghana and Nigeria

The amounts of the essential oil obtained from both the Ghana-sourced and Nigeria-sourced samples were generally the same (Table S1). However, the scent of the oil from the

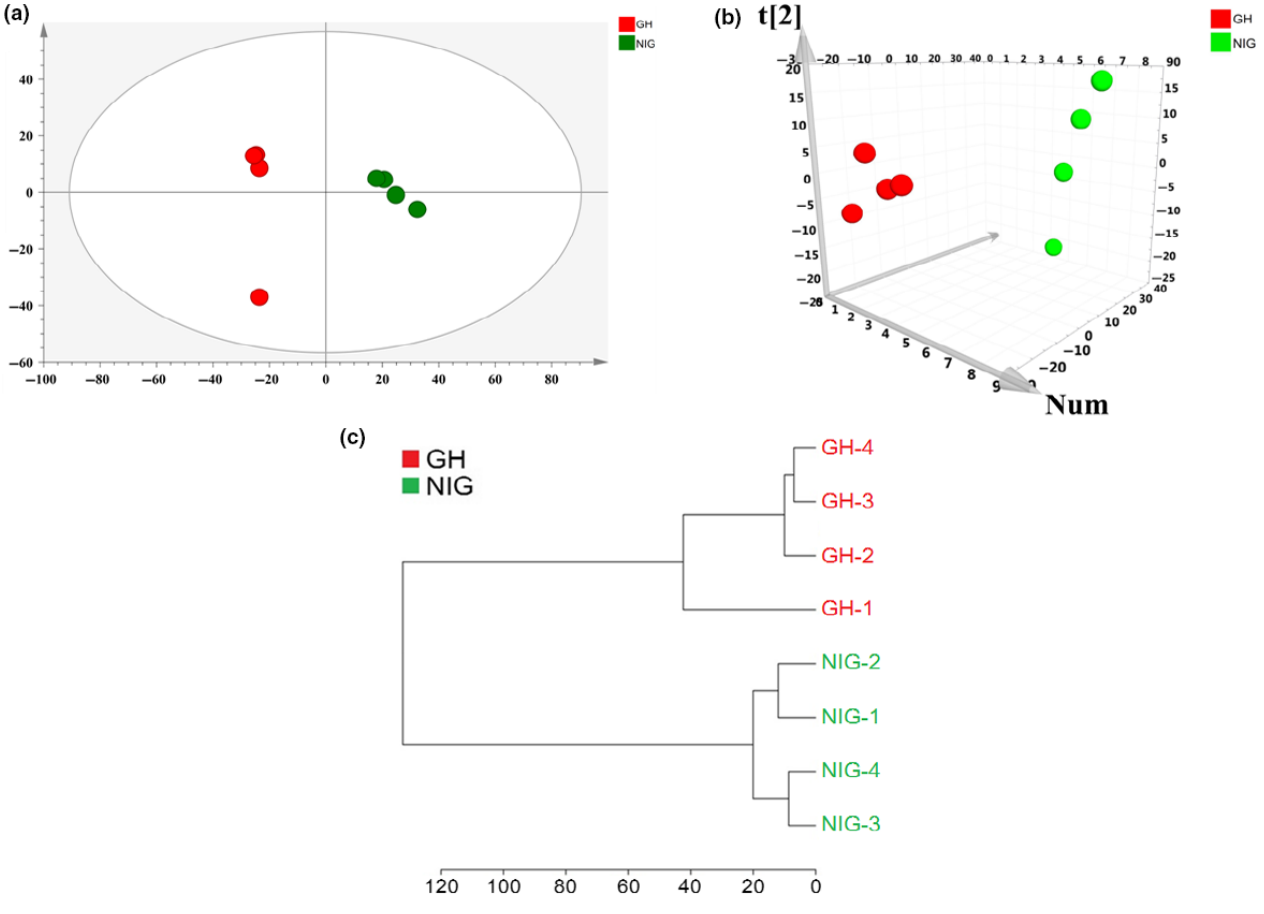

Figure 2 Various presentations of discrimination between essential oils of *Xylopia aethiopica* fruits from Ghana and Nigeria. (a) Unsupervised PCA score plot. (b) PLS-DA score plot [R^2Y (cum) = 0.998, Q^2 (cum) = 0.992]. (c) Dendrogram. [Colour figure can be viewed at wileyonlinelibrary.com]

two countries differed. The essential oils gotten from the Ghana-sourced *X. aethiopica* samples were strongly scented than those gotten from the Nigerian samples. The colour of the oil obtained was yellowish-green.

Metabolome differences between essential oil obtained from Ghanaian and Nigerian samples

Figure 1 presents representative total ion chromatograms of the essential oils gotten from the Ghana- and Nigeria-sourced samples of the *X. aethiopica* dried fruits. The difference found by visual examination relates to the differential levels of the metabolites present in the samples. Multivariate analysis better captured these differences and showed how statistically significant these differences are (Figure 2). The different representations of the differences between the samples, that is PCA (Figure 2a), PLS-DA (Figure 2b) and dendrogram (Figure 2c) clearly show the metabolomic differences. As can be noticed, a very obvious source-dependent grouping and clustering of the samples exist. Using chance permutation test ($n = 200$), the PLS-DA model [R^2Y (cum) = 0.998, Q^2 (cum) = 0.992] was found to be stable and reproducible.

Identification of differential metabolites

Relying on the established criterion, 14 metabolites were identified (Figure 1, Table 1). The *z*-score normalized quantities of these metabolites are presented as a heatmap in Figure 3 while their relative peak intensities are shown in Figure 4. The levels of all 14 differential metabolites were significantly higher in the essential oil obtained from the Ghana-sourced samples than those sourced from Nigeria (Figure 4). This difference could be attributed to the different geographical locations which invariably is influenced by different soil and climatic conditions. Our results are consistent with published literature, in that, the quality and quantity of essential oil obtained from plants depend to a large extent on the geographical location and variety of other factors.[18–20]

Differential antibacterial, cytotoxic and anti-inflammatory effects of essential oils of *X. aethiopica* fruits from Ghana and Nigeria

The results of the antibacterial tests (Table 2, Figure S1) show that the essential oil possessed antibacterial activity

Table 1 Details of differential metabolites identified

No.	Metabolite	Identity of metabolite	Formula	MW	CAS#	RT (min)	VIP	FC	*P*-value	LRI (iu)
1	M91T478	1R-α-Pinene	$C_{10}H_{16}$	136.1252	7785-70-8	7.13	1.21	4.87	4.45E-06	948
2	M135T479	β-Pinene	$C_{10}H_{16}$	136.1252	127-91-3	7.98	1.21	3.31	1.12E-06	970
3	M103T503	2-Carene	$C_{10}H_{16}$	136.1252	554-61-0	8.38	1.19	22.00	0.00267	996
4	M64T504	Cyclohexene,5-methyl-3-(1-methylethenyl)-,trans-(-)-	$C_{10}H_{16}$	136.1252	56816-08-1	8.40	1.20	18.08	0.001212	990
5	M52T506	Bicyclo[3.1.0]hexane,6-isopropylidene-1-methyl-	$C_{10}H_{16}$	136.1252	24524-57-0	8.43	1.20	19.20	0.001748	956
6	M111T611	Eucalyptol	$C_{10}H_{18}O$	154.1358	470-82-6	10.18	1.22	4.89	1.46E-05	1023
7	M70T731	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate	$C_{13}H_{22}O_4$	242.1518		12.18	1.22	2.33	0.000138	1090
8	M138T860	Isogeraniol	$C_{10}H_{18}O$	154.1357	5944-20-7	14.33	1.19	17.55	0.000414	1228
9	M95T926	α-Campholenal	$C_{10}H_{16}O$	152.1201	4501-58-0	15.43	1.23	3.58	1.11E-06	1102
10	M55T980	L-trans-Pinocarveol	$C_{10}H_{16}O$	152.1201	547-61-5	16.33	1.20	3.31	2.29E-05	1143
11	M50T1076	Pinocarvone	$C_{10}H_{14}O$	150.1045	30460-92-5	17.93	1.24	26.46	3.47E-06	1600
12	M107T1222	Myrtenal	$C_{10}H_{14}O$	150.1045	564-94-3	20.36	1.18	3.10	5.10E-05	1175
13	M152T1233	Myrtenol	$C_{10}H_{16}O$	152.1201	515-00-4	20.55	1.21	3.88	5.46E-06	1174
14	M205T3204	(-)-Spathulenol	$C_{15}H_{24}O$	220.1827	77171-55-2	53.40	1.08	3.99	0.000252	1619

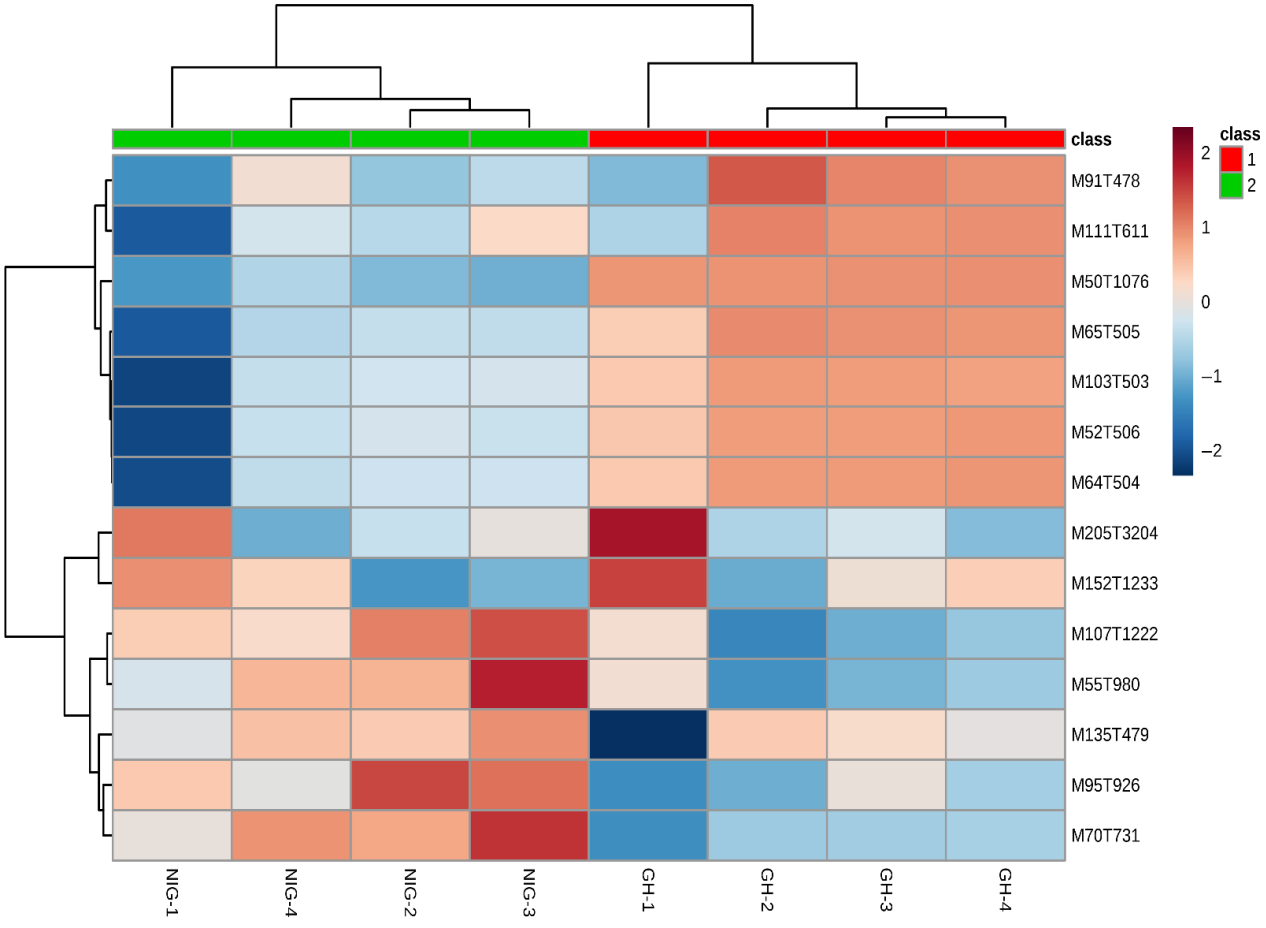

Figure 3 Heatmap pictorial presentation of 14 differential metabolites identified between the samples from Ghana and Nigeria. GH-1, GH-2, GH-3, GH-4 represent samples from Ghana while NIG-1, NIG-2, NIG-3, NIG-4 are samples from Nigeria. [Colour figure can be viewed at wileyonlinelibrary.com]

against all the four bacterial strains used (MIC values of 0.5–32 mg/ml at LCD and 8–64 mg/ml at HCD). Generally, the essential oils showed better antibacterial activity against the Gram-positive bacteria (MIC values of 0.5–8 mg/ml) than the Gram-negative bacteria (MIC value of 8–32 mg/ml). These results are in harmony with previous studies which reported the sensitivity of Gram-positive bacteria to essential oils compared with Gram-negative bacteria.[10,21,22] Also, it was observed that the test organisms were more sensitive to the samples from Ghana than those from Nigeria. This observation could be the result of the contrasting levels of bioactive compounds between the samples from the two countries. Previous studies have credited the antimicrobial activity of *X. aethiopica* oil to the presence of oxygenated monoterpenes as well as other constituents such as monoterpene hydrocarbons.[10,11,23] As evinced from the 14 differential metabolites (Figure 4, Table 1), the levels of the oxygenated monoterpenes including eucalyptol, α-campholenal, isogeraniol, L-trans-pinocarveol, pinocarvone, myrtenal and (-)-spathulenol were higher in the oil from the Ghana-sourced samples with respective fold changes of 4.89, 17.55, 3.58, 3.31, 26.46, 3.10, 3.88, and 3.99. The levels of monoterpene hydrocarbons such as 1R-α-pinene, β-pinene and 2-carene were also higher in the Ghana-sourced samples by 4.87-, 3.31- and 22.0-fold, respectively. Thus, the higher amount of these bioactive compounds (and many others) in the samples from Ghana account for their better antimicrobial effects. It is also worth mentioning that, the bacterial strains showed more sensitivity at LCD compared to HCD for all samples from both countries.

Bacteria use QS, a cell–cell signalling process to modulate communal behaviour and regulate gene expression by synthesizing, detecting and responding to changes in the population density through the use of signal molecules called autoinducers (AIs) in their surroundings.[24,25] QS controls bacterial behaviours, such as bioluminescence, type III and type VI secretion, biofilm formation, and motility[24,26] and contribute to the ability of the bacteria to survive changes in the environment. In *Pseudomonas*

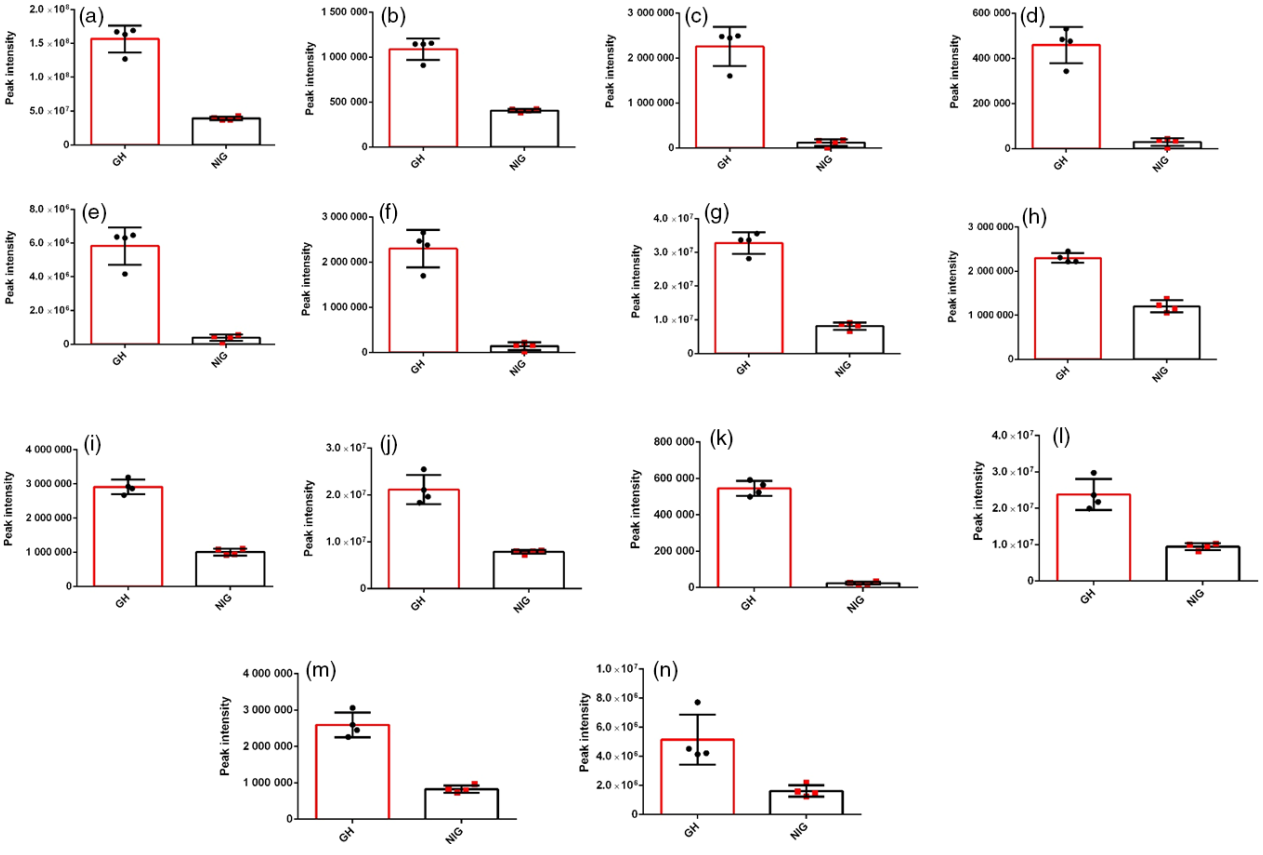

Figure 4 Relative peak intensities of 14 differential metabolites. (a). 1R-α-Pinene (b). β-Pinene (c). 2-Carene (d). Cyclohexene,5-methyl-3-(1-methylethenyl)-,trans-(-)- (e). Bicyclo[3.1.0]hexane,6-isopropylidene-1-methyl- (f). Eucalyptol (g). Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate (h). Isogeraniol (i). α-Campholenal (j). L-trans-Pinocarveol (k). Pinocarvone (l). Myrtenal (m). Myrtenol (n). (-)-Spathulenol. GH, Ghana; NIG, Nigeria. [Colour figure can be viewed at wileyonlinelibrary.com]

aeruginosa, QS is used to coordinate the expression of tissue-damaging factors and plays a major role in virulence.[27] Biofilm formation occurs at HCD resulting in antibiotic resistance compared with the planktonic cells at LCD which are more sensitive to antibiotics.[28] The formation of biofilms at HCD causes changes in membrane permeability of the bacterial cells, thereby resulting in antibiotic resistance.

Table 2 Antibacterial activity of essential oil obtained from *X. aethiopica* fruits from Ghana and Nigeria against selected Gram-positive and Gram-negative bacterial strains[a] (μg/ml)

	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus		*B. licheniformis*		*E. coli*		*K. pneumoniae*	
Sample	LCD	HCD	LCD	HCD	LCD	HCD	LCD	HCD
GH-1	0.0	4	1	8	1	16	8	8
GH-2	0.5	8	2	8	2	16	8	8
GH-3	0.5	4	1	8	1	16	8	8
GH-4	0.5	8	1	16	1	16	16	16
NIG-1	0.5	8	2	32	2	32	16	16
NIG-2	1	16	4	32	4	32	16	16
NIG-3	2	16	8	18	8	16	16	16
NIG-4	2	16	8	16	8	16	32	32
AMP (μg/ml)	4	256	8	256	4	128	4	64

AMP, ampicillin; GH, Ghana; HCD, high cell density; LCD, low cell density; NIG, Nigeria. [a]All determinations were done in triplicate.

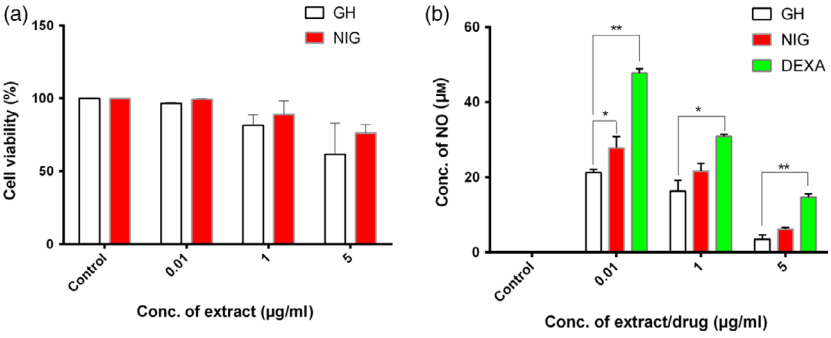

Figure 5 Effect of essential oils from Ghana- and Nigeria-sourced samples of *X. aethiopica* fruits on (a) Cell viability (b) NO production of LPS-induced RAW 264.7 cells. Cell viability was determined via CCK8 assay while the amount of NO produced was evaluated using Griess reagent. All determinations were done in triplicate and expressed as mean ± SD IC_{50} values from the cell viability assay for the Ghana- and Nigeria-sourced samples were respectively 3.825 µg/ml and 10.539 µg/ml. **P* value <0.05; ***P* value <0.01. DEXA: Dexamethasone-water soluble; GH, Ghana, NIG, Nigeria. [Colour figure can be viewed at wileyonlinelibrary.com]

The increased sensitivity at LCD may be due to the low number of planktonic cells present. However, at HCD, the bacterial quorum-sensing regulators activate genes that cause the bacteria cells to aggregate and form biofilms. Biofilm formation protects the bacterial cells from external environmental hazards including antimicrobials, leading to resistance.[29]

The results of the cytotoxicity studies against the murine macrophage cells, RAW 264.7 using CCK8 assay, showed that the oils from both countries exhibited their cytotoxic effects in a concentration-dependent manner as shown in Figure 5a. However, the oil from the Ghana-sourced samples generally exhibited higher activity than those from Nigeria as evinced from their respective IC_{50} values of 3.825 and 10.539 µg/ml. As a measure of anti-inflammation, the amount of NO produced was determined. The oils also inhibited NO production in LPS-stimulated RAW 264.7 cells concentration-dependently. The oils gotten from the Ghana-sourced samples exhibited better anti-inflammatory effects than those of Nigerian origin. At the lowest concentration (0.01 µg/ml), the difference in the anti-inflammatory effects between the oils from the two countries was statistically significant ($P < 0.05$). The difference was however not statistically significant at the medium (1 µg/ml) and high (5 µg/ml) concentrations. With reference to the standard drug (dexamethasone), the *X. aethiopica* oil from both countries exhibited better anti-inflammatory effects to statistically significant levels (Figure 5b). As multicomponent extracts, the synergistic roles of the various constituents of the oils could have accounted for the marked differences between them (i.e. the oils) and dexamethasone at all concentrations tested. The outcome herein lends credence to its traditional use in combating various inflammatory conditions such as skin eruptions.

Conclusion

Based on the findings of these studies, the following conclusions can be made: (1). the GC-MS-based metabolomics approach clearly established the difference between the essential oil obtained from the samples of *X. aethiopica* from the two countries, (2). The superior antibacterial and anti-inflammatory activity of the Ghana-sourced samples over those from Nigeria could be attributed to the higher levels of the bioactive compounds and (3). The 14 differential metabolites identified could serve as marker compounds to assess the quality of *X. aethiopica* essential oil. As recommendation for future studies, the sample size could be increased and other countries where *X. aethiopica* is grown could be included so as to determine the best place to obtain fruits with best antibacterial qualities. It would also be worthwhile investigating the effect of the oils against various strains of fungi as well as mechanistically determine their anti-inflammatory effects using *in vivo* models. Finally, fresh samples could be harvested and dried under same conditions so as to eliminate the effect of different drying conditions on the quality of the oil obtained.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Determination of minimum inhibitory concentration (MIC) of the essential oil of *X. aethiopica*.

Table S1. Yield of essential oil from *Xylopia aethiopica* dried fruits sourced from Ghana and Nigeria.