

RESEARCH

Open Access



Phytochemical screening, gas chromatograph/mass spectrometer (GCMS) analysis and molecular toxicological potential of *Hunteria umbellata* aqueous fruit extract against *Staphylococcus aureus* in accessory gene regulators (AGRs)

Titilola Fausat Salisu^{1*} , Muinah Adenike Fowora², Tajudeen Olanrewaju Yahaya³ , Sulaimon Adebisi Aina¹, Benjamin Thoha Thomas⁴, Latifat Aderonke Ademola¹ and Precious Oluwabukola Jimoh¹

Abstract

Background Fruits of *Hunteria umbellata* (HU) have been utilized in folk medicine as potent against *Staphylococcus aureus* (SA) infections, particularly skin and nasal related conditions. However, there is scarcity of literature concerning toxicological evaluation of graded doses of HU fruit, especially at molecular level, specifically targeting the accessory gene regulator (AGR) system to prevent abuse of doses in the treatment of bacterial infections. This research evaluated molecular toxicological property of SA exposed to varied concentrations of aqueous HU fruit extract ranging from 500 to 3.90625 µg/µL using broth microdilution method and quantification of AGR I and II genes' expression employing two-step reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). We first identified quality and quantity of chemical compounds in aqueous fruit of HU using phytochemistry and hybrid gas chromatograph–mass spectrometer (GC–MS) technique. Additionally, preliminary bactericidal potential of HU was assessed before molecular toxicology.

Results Results revealed six phytochemicals and twenty analytical grade compounds from a standard library were identified from chromatograms of HU fruit extract. Some main compounds detected are *n*-Hexadecanoic acid (25.24%), 2-Pentadecanone, 6,10,14-trimethyl (16.08%), Cuparene (16.63%), Tetradecanoic acid (6.21%) and 9-Octadecenoic acid, (E)- (5.70%). Bactericidal activity shows significant ($p < 0.05$) toxicity in the tested (8) concentrations of HU fruits in a dose-response relationship compared to the controls (positive and negative). The quantified expression of AGR I and II genes in SA was most significantly increased ($p < 0.05$) at both 250 and 500 µg/µL of HU fruit extract while least significant increase ($p < 0.05$) was recorded at 125 µg/µL compared to control.

Conclusions Notably, the study highlighted a potential risk of augmented bacterial infection especially with higher doses of HU extracts during boils' treatment and other epidermal infections instigated by Staph. Expression of both AGR genes at higher doses (250 and 500 µg/µL) is indicative of further expression of several other genes

*Correspondence:
Titilola Fausat Salisu
titilola.salisu@oouagoiwoye.edu.ng
Full list of author information is available at the end of the article

responsible for virulence factors in a variety of human bacterial infections. Thus, consumption of HU fruit to treat boils and blisters should be with great caution especially at high doses.

Keywords *Hunteria umbellata*, Molecular-toxicology, AGR genes, *Staphylococcus aureus*, Bioactive compounds

Background

The emerging integration of modern molecular technology in toxicological evaluation of medicinal plants has become an integral part of decoding progression of infectious diseases and possible development of promising drugs and vaccines. This is because quantitative molecular technique such as RT-qPCR used for gene expression profiling from targeted bacterial will create an intrinsic understanding of the roles of virulence genes in disease pathogenicity [31]. *Staphylococcus aureus* (SA) emerges as one out of five leading gram-negative antibiotic-resistant bacteria causing death globally with the highest prevalence in sub-Saharan Africa super-region as recently documented by antimicrobial resistance collaborators [25]. Antibiotic-resistance of *S. aureus* may be associated with acquisition of novel virulence via one lateral gene transfer event or more, and subsequently modifies an earlier nonpathogenic strain to a hypervirulent and/or multidrug resistant [23]. Currently, there is a focus on development of preventive alternate strategies (vaccination and phage therapy) through AGR regulatory genes to forestall occurrence of staphylococcal infections [8]. The regulation of AGR has been described to be imperative in the development of staphylococcal infections involving formation of biofilm [29]. Recurrent Staph. infection has become an important source of health loss through colonization of the skin and nostrils of human host [27]. The frequency of infections and persistent evasion of most antimicrobials of SA colonizing strain has led to alternative treatment in traditional medicine by means of various parts of medicinal plants' parts, especially in Africa. Most communities in Africa including Nigeria still rely on indigenous herbs as most sourced health treatments since these plants are easily accessible and affordable [38]. *Hunteria umbellata* (HU) fruit has been popularly employed in folk medicines without satisfactory doses for the management and treatment of many disease conditions caused by pathogenic bacterial in humans.

Although, several therapeutic potentials of many parts have been highlighted in the literature [37, 39]. *Hunteria umbellata* have been extensively harnessed in traditional herbal medicine as anti-diabetic potent agents with hyperlipidaemic activities [6], natural erectogenic and antioxidant extracts [35]. However, under exploitation of the in vitro molecular toxicity of varying doses from low to high of HU fruit in SA still persists. Hence, this study design was necessitated to precisely offer evidence based

scientific findings on in vitro molecular toxicology in virulent genes' expression of SA as the causative agent of boils when treated with HU fruit extracts. Additionally, to establish basis for possible mechanisms of response underlying medicinal plant-resistance as implicated in noxious exhibition in human staphylococcal communicable diseases.

Methods

Preparation of fruit extract

The HU fruit samples which were purchased in the second month (February) of the year 2023 from a local resident marketplace in Dopemu, Lagos State, Nigeria (6° 07' N to 7° 00' N latitude and 3° 43' E to 4° 00' E longitude) had been identified, authenticated and assigned voucher LUH 8996 as earlier illustrated by Salisu et al. [43]. Extraction employed was modified using the method of maceration [2]. The process involved preparing the fresh fruits by washing, slicing, oven-drying at 40 °C and grinding into a powder using electronic milling machine grinder, Lab. Mill, Serial No. 4745, Christy and Norris Ltd. The ground fruit (254 g) was macerated in 1.4 L of absolute sterile water within 72 h. A thin cloth and cotton wool were used to filter homogenate to get rid of every possible residue. The filtrate was placed in a fan assisted oven at 20 °C to dry. The aqueous extract (paste like form) yielded 3.95 g was stored in glassware and used for further analysis.

Qualitative phytochemical examination

Crude aqueous extract (20 mg) from HU fruit was mixed gently with 6 ml of 1% HCl, warmed, filtered, then tested for availability of alkaloids, anthraquinone and cardiac glycosides, saponins, phenol, flavonoids, tannins, phylloba-tannins, terpenoids as well as steroids. The presence (+) and absence (–) of tested phytochemicals were observed and recorded.

Test for alkaloids

Wagner's assay Dilute HCl and Wagner's reagent (1 ml) were added to fruit filtrate (3 ml) and shook well. Afterward, colored precipitate (reddish–brown) was formed as the presence of alkaloids [40].

Tests for glycosides

Borntrager's test of anthraquinone glycoside Addition of 5% H₂SO₄ (1 ml) to fruit extract solution (1 ml) made

a mixture that was boiled, filtered, shaken with the same volume of chloroform and kept to still for 5 min. Then the lower layer of chloroform was shaken with half of its volume with dilute ammonia. Nonappearance of bright red coloration of ammoniacal layer indicates absence of anthraquinone glycosides in the fruit [40].

Keller–Killiani test of cardiac glycoside A small amount of fruit extract (5 mg) were treated with anhydrous acetic acid (1 ml) and few drops of ferric chloride solution in a test tube. Then, concentrated sulphuric acid (2 ml) was added cautiously to prepared mixture. An observed reddish–brown ring at the junction of two layers and development of aqua at the lower layer indicated cardiac glycosides existence [45].

Test for saponins

A mixture of fruit sample (1 g) and distilled water (10 ml) was prepared, boiled for 5 min and shaken vigorously and observed in a test tube. Absence of froths indicates lack of saponins [15].

Test for phenol

A few fruits sample (1 g), ethanol (10 ml) and three drops of phenol solution were mixed in a labeled test tube. Nonappearance of a deep green coloration specifies absence of phenol [16].

Test for flavonoids

A mixture of HU fruit extract (1 g), 10 ml of ethanol and 2 drops of FeCl_3 was prepared. Observed dark green color specifies flavonoid [15].

Test for tannins

Decoction of fruit extract (1 g) with 10 ml of distilled water was prepared by boiling for 10 min, filtered, cooled and mixed with 0.1% ferric chloride reagent. An observed blue–black precipitate implies tannins availability as delineated in a modified method of Banso and Adeyemo [10].

Test for phyloba-tannins

Aqueous hydrochloric acid (1 ml) was stirred with fruit extract (2 ml), boiled and allowed to cool down. Availability of phyloba-tannins was implicated in precipitate (red) observed. [10].

Test for terpenoids

Some concentrated sulphuric acids (5 ml) were gently added to fruit extract sample (0.5 g) in a test tube. Observation revealed reddish brown coloration indicating terpenoids' presence [9].

Salkowski test for steroids

The crude fruit extracts (2 mg each) were dissolved with chloroform (2 ml) and mixed gently by shaking to form a mixture. Oil of vitriol (2 ml) was poured in prepared mixture to observe red topmost layer in the test tube. This indicates existence of terpenoid [9].

Gas chromatograph/mass spectrometer (GCMS) analysis of bioactive components of *Hunteria umbellata* fruits

Gas Chromatograph/Mass Spectrometer (GCMS) analysis as reported earlier [42] was carried out in Central Research Laboratory, University of Lagos Akoka, using 7890A Gas chromatography system attached to VL/MSD 5975C mass spectrometer (GC–MS Agilent Technologies, Santa Clara, USA) instrument employing the following settings: Column HP5MS fused silica capillary column [30 m (length) \times 0.32 mm (diameter) \times 0.25 μm (film thickness)] composed of 100% dimethyl polysiloxane. 1 g of ground fruit samples was dissolved in 10 ml chromatographic grade methanol, filtered by using syringe filter and the clear sample was later injected in GCMS column. Helium gas (99.9999%) was used as the carrier gas at constant flow rate of 1 ml/min and an injection volume of 1 μl was employed with injector temperature at 250 $^{\circ}\text{C}$ and pressure at 8.802 psi. The oven temperature was programmed originally from 80 $^{\circ}\text{C}$ (held for 2 min) with an increase of 5–120 $^{\circ}\text{C}/\text{min.}$, then 10–240 $^{\circ}\text{C}/\text{min.}$, to hold for 6 min. The total GC running time for the fruit sample was 24 min. The area under a peak accurately represents the quantity of the component present in the sample. Software adopted to handle mass spectra and chromatogram was a ChemStation. The interpretation on mass spectrum of GCMS was done by means of the database of National Institute of Standard and Technology (NIST) version 2, year 2015 library. The mass spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library to establish the name, molecular weight and structure of the components of the fruit extract.

Collection of bacterial isolates and antibacterial assay

A gram-positive organism, *S. aureus* strain MBSA2208, was used for this study. The bacteria isolate was obtained from the Department of microbiology, Nigerian Institute of Medical Research (NIMR), plated on salt agar (Mannitol) and then brooded (at 40 $^{\circ}\text{C}$ for 24 h) to obtain pure culture. Antibacterial activity of HU fruit sample was tested by method of broth micro-dilution. A sterilized well (96) microplate was prepared for the experiment. Each well was filled with 90 μL of nutrient broth. In the standard wells, 10 μL of 1 mg/ml vancomycin (a

susceptible antibiotic) was added, while the control well, received 10 µL of sterile broth as a sterility control [44]. Minimal inhibitory concentration (MIC) was evaluated by preparation of 0.4 µL of a stock concentration HU extract (250 mg/ml) to arrive at a working solution of 500 µg/µL. This working solution was then serially diluted to obtain two-fold dilutions. The resulting concentrations in column 3 to 11 were 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, and 3.90625 µg/µL. Each well, except for the blank well in column 12, was filled up to 200 µL with nutrient broth that had contained bacteria already inoculated at a standard density of 0.5 McFarland, equivalent to 1.5×10^8 (CFU/mL). This setup allowed for the estimation of the bacterial activity of fruit extract by observing a noticeable growth or inhibition of the bacteria in each well of the microplate. After setting up the microplate with the appropriate concentrations of the extract, standard antibiotic and control. The incubation of the plate was done at 37 °C on a shaker in not more than 24 h. After incubation period, measurements of absorption values in each well with microplate reader at 600 nm absorbance were recorded. This measurement allowed for the determination of the growth difference between the wells before and after incubation, indicating the effectiveness of the extract in inhibiting bacterial growth.

To determine the point of inhibition for each extract, preparation of resazurin dye (0.8 mg/ml) was done. After the 24-h incubation period, A small volume of prepared resazurin solution (30 µL) was dropped in individual well of microplate. Resazurin is a dye that changes color from blue to pink in the presence of oxygen. The microplate was then incubated for an additional 3 h to allow the color change to occur. This color change visually indicated microbes' growth inhibition due to smallest amount of HU fruit extract in each well.

RNA extraction and purification

For the extraction of single-stranded RNA from the aqueous extracts of *H. umbellata*, three concentrations were chosen: the highest (500 µg/µL), a moderate (250 µg/µL) and the lowest (125 µg/µL). The extraction was performed via the NIMR Biotech extraction kit according to manufacturer's description.

The extraction process involved several steps. First, the cells in the extracts were lysed to disrupt cell membranes and make RNA available freely. Lysed samples were then incubated to facilitate the extraction process. After incubation, RNA precipitation was carried out to isolate the RNA from other cellular components. The cell debris and impurities were removed through a washing step to obtain a purified RNA sample. The elution step utilized a spin column-based method, where the purified

RNA was eluted from the column, leaving behind any contaminants.

Finally, the extracted single-stranded RNA was stored at –20 °C until further use. This freezing temperature helps maintain the stability and integrity of the RNA for future analysis or experiments.

Dual-step reverse transcriptase quantitative polymerase chain reaction (RT-qPCR)

A separate reverse transcription coupled with qPCR was carried out. First, extracted RNA was converted to complementary DNA (cDNA). Synthesis mix of FIREScript RT cDNA (Solis Biodyne, Estonia) was used, alongside random primers and Oligo (DT). Prepared reaction mixture consisted of 6.8 µl of double-distilled, molecular-grade water, 2 µl of mastermix, 0.1 µl each of forward and reverse primers in Table 1 as manufactured by the NIMR-MTN Oligo Synthesis Laboratory and 1 µl of the RNA template. These components were combined to create a 10 µl reaction mixture as followed by manufacturer's manual. Transcription (reverse) process took place for a duration of 30 min at a temperature of 50 °C. After 5 min, transcriptase enzyme was deactivated at a lesser temperature of 85 °C using a BioRad thermal cycler.

The second step was the quantitative Polymerase chain reaction (PCR). This reaction was executed to quantify amplifications of expressed genes. The number of amplifications of virulence genes (AGR I and II genes) and the house keeping gene (SA442) of *S. aureus* used in this study was determined using a real-time system (BioRad CFX96 Deepwell), in accordance with the manufacturer's description. A 10 µl reaction mixture was prepared containing 5.8 µl double distilled, nuclease free water, 2 µl Solis Biodyne Eva green mastermix, 0.1 µl each of both forward and reverse primers and 2 µl template coding deoxyribonucleic acid (cDNA) for the two primers separately. The processes involved are; initial and final denaturation, annealing, initial and final extension. First denaturation (DNA unwinding) occurred at 95 °C for 2 min, final unwinding took place for just 30secs at the same temperature as the first melting of DNA, annealing was set for 30secs at 54 °C and repeated for 40cycles

Table 1 List of Nucleotide Primers designed for Virulence Genes in Real-Time PCR Technique

Gene name	Sequences (forward and reverse)	Reference
AGR I	Forward: 5'-CAC TTA TCA TCA AAG AGC C-3' Reverse: 5'-CCA CTA ATT ATA GCT GG-3'	[3]
AGR II	Forward: 5'-GTA GAG CCG TAT TGA TTC-3' Reverse: 5'-GTA TTT CAT CTC TTT AAG G-3'	

followed by extension step with temperature range of 55–80 °C with 0.5 °C increment for 5secs.

Ct values (cycle threshold) were generated at the completion of the reaction and used to calculate relative quantification $2^{-(\Delta\Delta Ct)}$.

Data analysis

The cycle threshold (ct value) generated from the melt curves plotted by the thermal cyclor was used to calculate relative expression using Microsoft Excel (2016 version). Relative expression values were further subjected to IBM SPSS statistics version 22, to determine the increment or decrease in expression of the virulence genes (AGR I and II). All data entries were given as descriptive average alongside standard error means (mean \pm SEM), mean difference was compared using a one-way ANOVA analysis and a post-hoc test of LSD (least significance difference). A confidence interval of 95% was used as the criterion for significance to show variations between compared sets.

Results

Phytochemical analysis of chemical components of *Hunteria umbellata* fruits

The screening of phytochemicals in HU fruit, aqueous extracts revealed six (6) phytochemicals which include specific secondary metabolites like alkaloids, cardiac glycosides, flavonoids, tannins, phyloba-tannins and terpenoids. Four compounds (anthraquinone glycosides, saponins and steroids) were absent in the fruit (see Table 2.)

Bioactive components of *H. umbellata* fruit from chromatograms of GC–MS

Twenty (20) non-polar volatile compounds appeared in HU fruit (methanol extract) as analyzed from GCMS

chromatograms in Fig. 1 below. Individual peak denotes a compound with dissimilar quality and quantity based on percentage by ChemStation's calibration mode (Software). All compounds identified with their molecular formula, molecular weight (MW), retention time (RT), area percentage composition (quantity), quality (matching factor > 80) and chemical structure of fruit extract are presented in Table 3. The presence of compounds that combine both the matching factor (> 80) and percentage composition (> 5%) were classified as main compounds while others below both quality (matching factor > 80) and quantity (> 5%) were regarded as minor compounds. The five major compounds (Table 4) detected in HU fruit are *n*-Hexadecanoic acid (99; 25.2%), 2-Pentadecanone, 6,10,14-trimethyl (96;16.1%), Cuparene (95; 6.6%), Tetradecanoic acid (95; 6.2%) and 9-Octadecenoic acid, (E)- (83; 5.7%). The 15 minor compounds comprise 1-Methylenespiro [2.4] heptan-4-one (43; 1.1%), 2-Butynedioic acid, di-2-propenyl ester (49; 2.1%), Phthalic acid, butyl oct-3-yl ester (86; 2.2%), 7-Hexadecene, (Z)- (97; 2.6%), camphor (42; 1.6%), Methyl 14-methylpentadecanoate (97; 3.3%), 1-Octadecene (97; 4.7%), Methyl linoleate (95; 1.1%), Methyl elaidate (99; 1.4%), Bromoacetic acid, octadecyl ester (49; 1.76%), Cyclohexanethanol, 4-methyl-.beta.-methylene-, trans-(45; 5.8%), Octadecanoic acid (70; 2.31%) and 1-Octadecene (97; 2.5%).

Bactericidal property of aqueous *Hunteria umbellata* fruit extract on *Staphylococcus aureus*

Result obtained from the antibacterial assay showed that Vancomycin, a standard drug for *Staphylococcus* infection greatly displayed repressive properties on proliferation of SA. None of eight tested concentrations of *H. umbellata* with 500 μ g/ μ L being the highest stalled the growth of *S. aureus* in comparison to control as shown below (Fig. 2). This was confirmed in all the wells containing resazurin that changed from blue to pink. The change of resazurin from blue indicates the presence of viable organisms (producing oxygen).

Amplification of AGR I and II genes' expression in SA predisposed to three selected doses of HU fruit extracts

The amplification and melt curves of the two AGR genes in SA exposed to aqueous HU fruit extracts were provided in Figs. 3 and 4 respectively. The amplification curves are indicative of the binding of DNA to SYBR green fluorescent dye present in the master mix which started after the 20th cycles in AGR I and 30th cycle in AGR II of real time PCR. The melt curves began after 60 °C for AGR I and 75 °C for AGR II.

Table 2 Presence (+) & absence (–) of Phytochemicals in *Hunteria umbellata* Fruit

Phytochemicals	Presence/absence
Alkaloids	+
Anthraquinone glycosides	–
Cardiac glycosides	+
Saponins	–
Phenol	–
Flavonoids	+
Tannins	+
Phyloba-tannins	+
Terpenoids	–
Steroids	–

+ Presence, – Absence

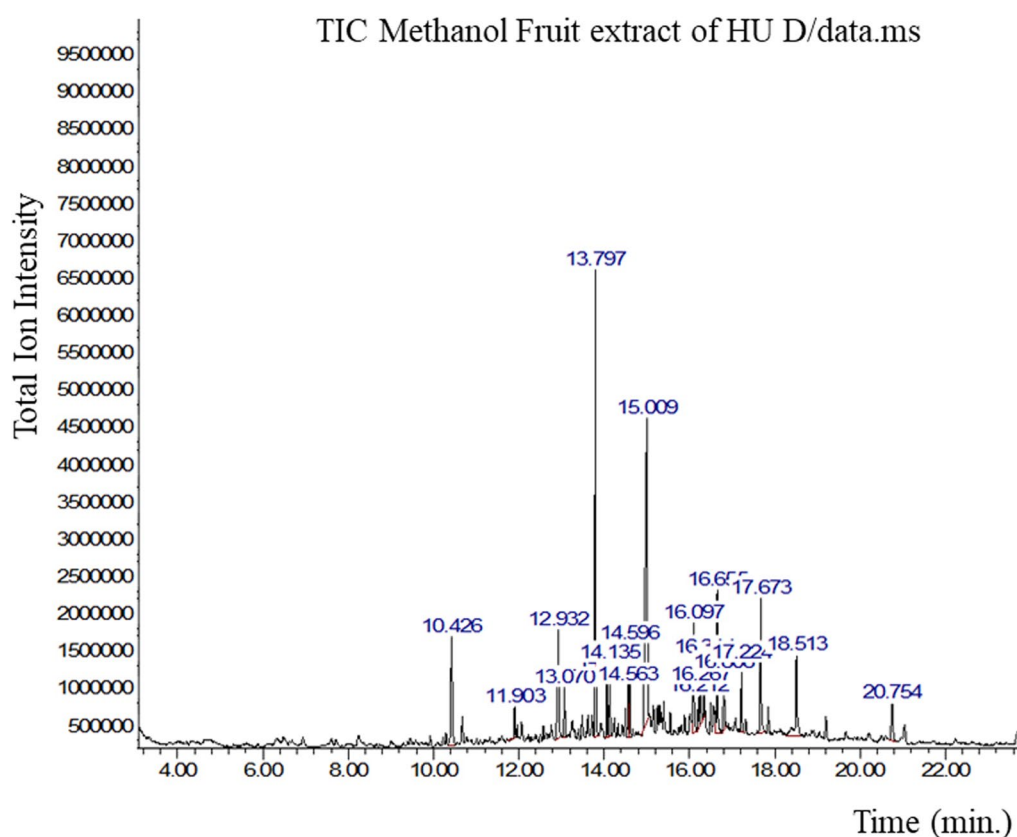


Fig. 1 Total Ion GCMS Chromatogram of methanol fruit extract of *Hunteria umbellata*

Quantification of AGR I and II gene in *Staphylococcus aureus* after exposure to HU aqueous fruit extract

The result obtained after the quantitative real time PCR showed increased expression of AGR I and II genes at 500 and 125 $\mu\text{g}/\mu\text{L}$ compared to control. However, AGR I and II genes were more significantly ($p < 0.05$) expressed at 250 $\mu\text{g}/\mu\text{L}$ of *H. umbellata* aqueous fruit extract relative to the other two doses and control. (see Fig. 5).





















Discussion

The safety profile of certain plants, their potential therapeutic benefits and considerations of toxicity effect have been criticized as a result of ambiguity in treatment prescriptions by indigenous traditional healers. This is due to ancestral believe in guaranteed safety with no side effect in any consumption of most parts of medicinal plants without adequate doses [36]. Regrettably, this supposition has translated to numerous cases of health complications and in some cases demise of the users [21]. This often results to patients overdosing of herbs and herbal products, as regulatory bodies are totally not in control over the usage of herbal medicines. Despite the goodness of herbal medication, certain plant species have been documented to exhibit cytotoxic effects at elevated doses.

The implication is that a whole plant or its parts considered highly safe can become toxic at elevated doses. Serious concerns related to potential toxicity-related to phytomedicine or phytotherapy including issues like cancer-inducing effect, liver toxicity, mutation inducement and genetic damage have been brought to the forefront [30]. Therefore, prevailing perception among users of herbal medicines that these remedies are inherently safe without adverse effects due to their natural origins in the plant kingdom calls for serious concern [32].

Secondary metabolites of different parts of HU have consistently been documented in therapeutic intervention or management of numerous ailments. The results of phytochemical screening of HU fruit revealed certain specialized metabolites including alkaloids, cardiac glycosides, flavonoids, tannins, phyloba-tannins and terpenoids. This result is in accordance with the compounds found in HU methanol seed as well as their known bioactivities as reported by Salami and Ladokun [41], Aderale et al. [5] and Akinrotaye et al. [7]. Another study reported 21 different compounds from chromatograms of both seed and leave methanol extracts [28]. The GCMS analysis from this study resolved 20 compounds from HU fruit, comprising five

Table 3 Compounds recognized in methanol fruit extract of *H. umbellatta*

Peak No	Phyto-compounds	Molecular Formula	Retention Time (min.)	Molecular Weights (gmol ⁻¹)	Area Composition (%)	Quality (%)	Chemical Structure
1	Cuparene	C ₁₅ H ₂₂	10.42	202.33	6.63 ^c	95	
2	1-Methylenespiro[2.4] heptan-4-one	C ₈ H ₁₀ O	11.90	122.16	1.13	43	
3	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	12.93	228.37	6.21 ^d	96	
4	2-Butynedioic acid, di-2-propenyl ester	C ₁₀ H ₁₂ O ₄	13.07	196.20	2.08	49	
5	2-Pentadecanone, 6,10,14-trimethyl	C ₁₈ H ₃₆ O	13.80	268.48	16.08 ^b	96	
6	Butyl octyl phthalate	C ₂₀ H ₃₀ O ₄	14.07	334.45	2.19	86	
7	7-Hexadecene, (Z)-	C ₁₆ H ₃₂	14.13	224.42	2.59	97	
8	Camphor	C ₁₀ H ₁₆ O	14.56	152.23	1.62	42	
9	Methyl 14-methylpentadecanoate	C ₁₇ H ₃₄ O ₂	14.60	270.45	3.26	97	
10	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	15.01	256.42	25.24 ^a	99	
11	1-Octadecene	C ₁₈ H ₃₆	16.10	252.50	4.73	97	
12	Methyl linoleate	C ₁₉ H ₃₄ O ₂	16.21	294.47	1.10	95	
13	Trans-13-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	16.27	296.49	1.41	99	
14	Bromoacetic acid, octadecyl ester	C ₂₀ H ₃₉ BrO ₂	16.34	391.40	1.76	49	
15	Cyclohexaneethanol, 4-methyl-.beta.-methylene-, trans-	C ₁₂ H ₂₄ O	16.66	184.32	5.80	45	
16	Octadecanoic acid	C ₁₈ H ₃₆ O	16.81	284.48	2.31	70	
17	1-Octadecene	C ₁₈ H ₃₆	17.23	252.50	2.45	97	
18	9-Octadecenoic acid, (E)-	C ₁₈ H ₃₄ O ₂	17.67	282.50	5.70 ^e	83	
19	1-Hexadecanol, 2-methyl-	C ₁₇ H ₃₆ O	18.51	256.47	5.27	66	
20	2 (Isobutoxycarbonyl) benzoic acid	C ₁₂ H ₁₄ O ₄	20.75	222.24	2.42	46	

abcde: The top 5 abundant phyto-compounds in descending order (highest to smallest)

Table 4 Nature and reported bioactivity of the major phyto-compounds in methanol fruit extract of *H. umbellatta*

Phyto-compounds	Nature	Quality	Quantity (%)	Reported bioactivity	References
n-Hexadecanoic acid	Palmitic acid	99	25.24	Anti-bacterial	[22]
Octadecanal	Aldehyde	96	16.08	Anti-oxidant	[51]
Cuparene	Sesquiterpene	95	6.63	Anti-cancer activity	[11]
Tetradecanoic acid	Myristic acid	96	6.21	Virulence regulation	[26]
9-Octadecenoic acid, (E)-	Stearic acid	83	5.70	Anti-inflammatory	[50]

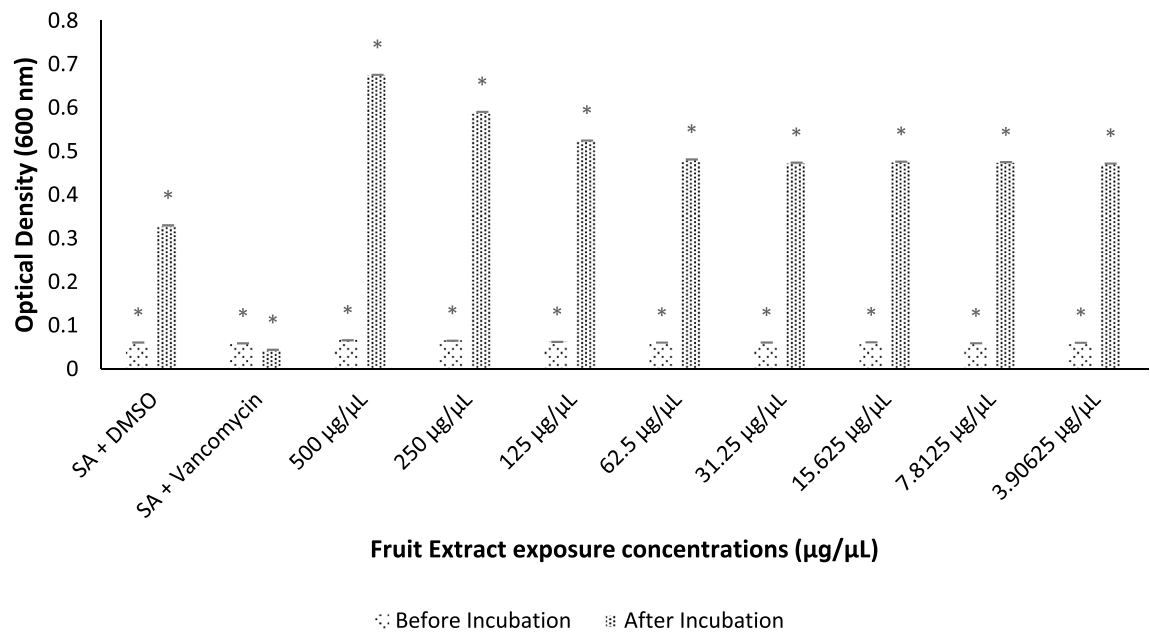


Fig. 2 Graphical representation of Microbial growth in *Hunteria umbellata* fruit extract before and after 24 h of incubation

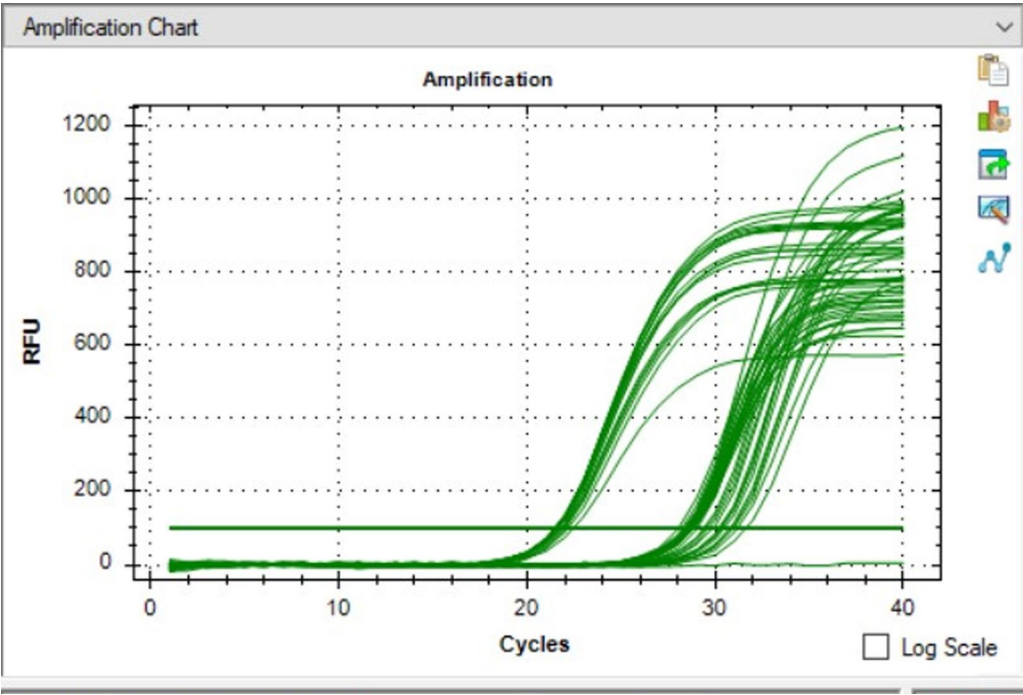


Fig. 3 Amplification curves of AGR (I and II) genes of SA treated with HU fruit extracts. RFU represent relative fluorescence units

major compounds and 15 minor compounds based on quality and quantity. The major compounds are palmitic acid, aldehyde, sesquiterpene (terpene), myristic acid and stearic acid. Palmitic acid (*n*-hexadecanoic acid) constituted the most abundant with almost 100%

matching factor (Table 4). Notably, maximum concentration of 50 µg/ml, *n*-hexadecanoic acid has established only modest antagonistic bacterial activity against *S. aureus*, as specified by Ganesan et al. [22]. This means overdose of plant rich in palmitic acid in

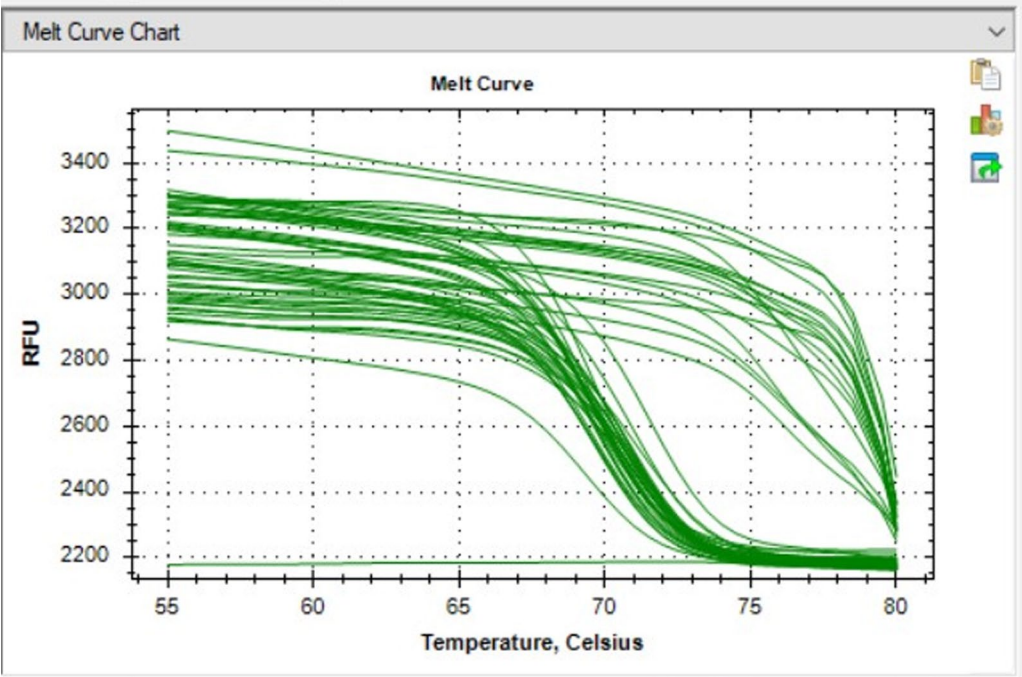


Fig. 4 Melt curves of AGR (I and II) genes of SA treated with HU fruit extracts. RFU represent relative fluorescence units

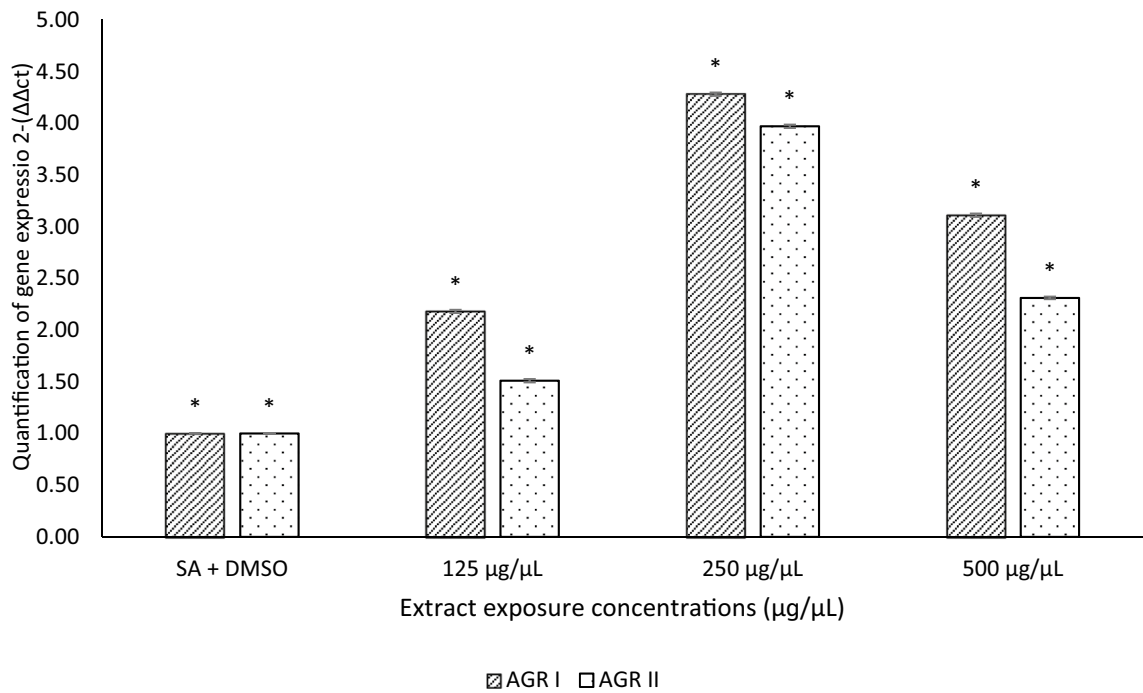


Fig. 5 Graphical representation of AGR I gene expression of *Staphylococcus aureus* exposed to *Hunteria umbellata* aqueous fruit extract

the treatment of bacterial infection could be lethal. Void activity of fatty aldehydes against clinical pathogenic bacteria has been documented by Xie et al. [49]

while literature has described terpenes as promising antibacterial especially in multiple drug resistance (MDR) bacterial strains [20, 33]. Though the precise

molecular machinery responsible for the antibacterial effects of terpenes are yet to be fully understood. Most plant parts possess fatty acids (FA) as good sources of carbon with structural functions and bactericidal properties [14]. However, sublethal concentrations of some of these fatty acids serve as signaling molecules in modulating bacterial virulence as explained by Cortes-López et al. [17]. Despite the antibacterial activity of most fatty acids, palmitic, myristic and stearic acids have been associated with increase in pathogenicity of bacteria [26]. Although their probable impact on the effectiveness of anti-virulence treatments coupled with participation in intricate network of pathogenicity regulation are increasingly recognized, representing a promising strategy to manage antibiotic-resistant bacterial diseases.

Several reports have shown that *Hunteria umbellata*, possesses antagonistic bactericidal efficacy against the two major representative of bacteria (gram positive and negative), *S. aureus* and *E. coli* bacteria respectively, at doses less than 150 mg/ml [5, 7]. Contrarily to these reports, our findings revealed negative antibacterial activity of *H. umbellata* aqueous fruit extract against *S. aureus* tested. Increase in growth was recorded in *S. aureus*, the causative agent of these staph infections, when it was exposed to *H. umbellata* aqueous fruit extract at doses ranging from 3.9 to 500 µg/µL. The increment could be indicative that the extract supports the growth of Staph infections in the body. This difference could also be attributed to the growing evolution and resistance of pathogenic bacteria [18, 34]. Nevertheless, our study corroborates minimal and inconsequential inhibitory effects of the fruit pulp (water extract) noted against SA in old fact-findings of Igbe et al. [24] as reviewed by Fadahunsi et al. [19].

The expression levels of gene have been generally regarded as an excellent match for protein expression and thus be harnessed to deduce expression of either protein and/or virulence susceptibility. Equivalent data will offer more enlightened strategy for the choice of medicinal plants for the development of drugs to manage persistent evaded pathogenic processes. Our previous study has established increase in the amount of Laf A and ExsE genes' expressions in, as a main causative agent of gastroenteritis, when exposed to high dosages of both *H. umbellata* seed and stem extracts [43]. This present study further evaluated the AGR system of *S. aureus* which is a vital component underlying numerous genes' expression that code for virulence tendencies, formation of biofilms during the pathogenicity of bacterium and inducement of both type 2 and interleukin-17-dependent epidermal inflammation in host cell [47]. Significant increase in expression of both AGR

genes of SA exposed to the three tested doses (500, 250 and 125 µg/µL) is in accordance with expression of AGR I and II which has been ascribed to invasive infection like bacteraemia [12].

Every pathogen has virulence genes associated with them, these genes are critical for triggering diseases when the host has been colonized successfully to cause bacterial infections [48]. Our studies revealed persistent increase of AGR I gene followed by AGR II gene in the tested doses as compared to control, in agreement with other study that both genes are the most common allelic groups of AGR system [1]. The AGR I system is often connected with strains of *S. aureus* which produces the Pantone-Valentine leukocidin (PVL-SA) toxin, commonly implicated in necrotic skin infection and persistent cellulitis [13]. AGR II is frequently found in antibiotic (methicillin) resilient *S. aureus* strains, linked with hospital-acquired infections [46]. Variation in amplification peaks during quantification of the AGR genes revealed difference in expression of AGR genes in the three tested concentrations of the extract (500, 250 and 125 µg/µL) compared to normal control. This further indicates the presence of the genes in the *S. aureus*. AGR I and II genes were expressed significantly ($p < 0.05$) at 250 µg/µL and 500 µg/µL doses while dose 125 µg/µL significantly decreased ($p < 0.05$) genes' expression. This suggests that AGR I and II genes would be expressed as doses of *H. umbellata* fruit extract increases. However, this molecular toxicity at genetic level is scarce in the literature, as most studies have been focused on the antibacterial activity of *H. umbellata* aqueous fruit extract. Until this present study, there isn't any recorded side effects of *H. umbellata* at molecular level. Adeneye et al. [4] indicated in his study that HU exhibits a notably low level of oral toxicity. That is, consumption of the HU fruit is not dangerous or toxic. Yet, this study confirms the expression of both types of AGR genes (I and II) as the colonization factor for *S. aureus* in the development of staphylococcal related infections. These findings affirmed the importance of molecular toxicity especially at gene and protein levels to validate conventional and modern therapeutics in either prevention or treatment of microbial infectious diseases globally. This will further elucidate the toxicity pathways underlying antimicrobial resistance associated with virulence.

Conclusion

Consumption of indiscriminate dosages of *H. umbellata* extracts may aggravate the staphylococcal diseases such as boils, abscess and staph food poisoning. Hence, *H. umbellata* fruit extract maybe be dangerous to health when consumed in high quantities.

Recommendation

Extensive in vitro studies should be conducted on different herbal plants and concoctions to verify their antibacterial activities. Most especially, molecular toxicity tests must be incorporated in the toxicology of medicinal plants to fully ascertain its safety and safe dosage. In addition, enhancing awareness about potential health risks linked to the utilization or excessive use of medicinal plant parts and quality control guidelines should be encouraged and enforced on traditional use of herbal plants in any disease management.

Abbreviations

HU	<i>Hunteria umbellata</i>
SA	<i>Staphylococcus aureus</i>
AGR	Accessory gene regulator
RT-qPCR	Reverse transcriptase quantitative polymerase chain reaction
GCMS	Gas chromatograph/mass spectrometer
NIST	Nigerian institute of standards and technology
MIC	Minimal inhibitory concentration
CFU	Colony forming unit
OD	Optical density
RNA	Ribonucleic acid
cDNA	Complementary deoxyribonucleic acid
CT	Cycle threshold
ANOVA	Analysis of variance
LSD	Least significant difference
PCR	Polymerase chain reaction

Acknowledgements

Not applicable

Author contributions

TFS conceptualized the research, contributed to supervision, participated in the laboratory work, performed data analysis and drafted the paper. MAF and LAA were involved in primers' selection, laboratory work and data collation. TOY, MAF and BTT were involved in selection of microorganisms, participated in laboratory work, data collection and contributed in writing the manuscript. SAA and POJ ensured quality assurance, contributed to validation and revised the manuscript. TFS, TOY and LAA revised and edited the final manuscript. All authors have read and approved the final manuscript.

Funding

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The HU fruit samples used in this study were identified, authenticated by a taxonomist and assigned voucher numbers, LUH 8995 and LUH 8996 respectively in the Department of Botany, Faculty of Science, University of Lagos. The fruit samples were further deposited in the herbarium of the same University. This is in alignment with national guideline and legislation required for the study of indigenous plant.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. ²Department of Molecular Biology and Biotechnology, Nigerian Institute of Medical Research, 6, Edmund Crescent, P.M.B.2013, Lagos, Nigeria. ³Department of Biological Sciences, Federal University Birnin Kebbi, PMB 1157, Birnin Kebbi, Kebbi State, Nigeria. ⁴Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Received: 5 January 2024 Accepted: 19 March 2024

Published online: 28 March 2024

References

1. Abdulgader SM, van Rijswijk A, Whitelaw A, Newton-Foot M (2020) The association between pathogen factors and clinical outcomes in patients with *Staphylococcus aureus* bacteraemia in a tertiary hospital, Cape Town. *Int J Infect Dis* 91:111–118. <https://doi.org/10.1016/j.ijid.2019.11.032>
2. Abdullahi RA, Mainul H (2020) Preparation of medicinal plants. Basic extraction and fractionation procedures for experimental purposes. *J Pharm Bioallied Sci* 12(1):1–10. https://doi.org/10.4103/jpbs.JPBS_175_19
3. Achek R, El-Adawy H, Hotzel H, Hendam A, Tomaso H, Ehrich R, Neubauer H, Nabi I, Hamdi TM, Monecke S (2021) Molecular characterization of *Staphylococcus aureus* Isolated from human and food samples in Northern Algeria. *Pathogens* (Basel, Switzerland) 10(10):1276. <https://doi.org/10.3390/pathogens10101276>
4. Adeneye AA, Adeyemi OO, Agbaje EO (2010) Anti-obesity and antihyperlipidaemic effect of *Hunteria umbellata* seed extract in experimental hyperlipidaemia. *J Ethnopharmacol* 130(2):307–314. <https://doi.org/10.1016/j.jep.2010.05.009>
5. Aderole OR, Rasaq AK, Momoh JO (2020) Phytochemical screening, mathematical analysis and antimicrobial activity of methanolic seed extract of *Hunteria umbellata*. *Eur J Med Plants* 31(16):1–17. <https://doi.org/10.9734/ejmp/2020/v31i1630325>
6. Ahajumobi EN, Anderson PB (2022) *Hunteria umbellata* extract is a potent agent for effective diabetes control. *Asian J Med Health* 20(8):26–36. <https://doi.org/10.9734/ajmah/2022/v20i830479>
7. Akinrotaye KP, Akinduti P, Lanlokun O, Adetogun C (2020) Synergistic Evaluation of *Moringa oleifera*, *Hunteria umbellata* and *Azadirachta indica* with antibiotics against environmental MRSA isolates: an in-vitro study. *Am J BioSci* 8(4):91–98. <https://doi.org/10.11648/jajbio.20200804.11>
8. Andreas FH, Lassi L, Paolo D, Clarissa P, Tarcisio B, Matthew JB, Fabio B, Isabel D (2023) In vivo gene expression profiling of *Staphylococcus aureus* during infection informs design of stemless leukocidins Luke and -D as detoxified vaccine candidates. *Microbiol Spectr* 11(1):e02574–e2622. <https://doi.org/10.1128/spectrum.02574-22>
9. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008) Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Trop J Pharm Res* 7(3):1019–1024. <https://doi.org/10.4314/tjpr.v7i3.14686>
10. Bansa A, Adeyemo S (2006) Phytochemical screening and antimicrobial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium*. *Biokemistri* 18(1):39–44. <https://doi.org/10.4314/biokem.v18i1.56390>
11. Beretta GL, Ribaudo G, Menegazzo I, Supino R, Capranico G, Zunino F, Zagotto G (2017) Synthesis and evaluation of new naphthalene and naphthoquinone derivatives as anticancer agents. *Arch Pharm (Weinheim)* 350(1):e1600286. <https://doi.org/10.1002/ardp.201600286>
12. Bibalan MH, Shakeri F, Javid N, Ghaemi A, Ghaemi EA (2014) Accessory gene regulator types of *Staphylococcus aureus* isolated in Gorgan, North of Iran. *J Clin Diagn Res* 8(4):DC07–DC09. <https://doi.org/10.7860/JCDR/2014/6971.4219>
13. Cai H, Li X, Zhang C, Zhong H, Xie Y, Huang L, Zhang B, Long Y, Zhou Z, Liang B (2023) Molecular characterization of *Staphylococcus aureus* in school-age children in Guangzhou: associations among agr types, virulence genes, sequence types, and antibiotic resistant phenotypes. *BMC Microbiol* 23(1):368. <https://doi.org/10.1186/s12866-023-03126-y>

14. Calder PC (2015) Functional roles of fatty acids and their effects on human health. *JPEN* 39(1 Suppl):185–325. <https://doi.org/10.1177/01486071155595980>
15. Chaudhary S, Negi A, Dahiya V (2010) The study of in vitro antimicrobial activity and phytochemical analysis of some medicinal plants in chamoli garhwal region. *Phcog J* 2(12):481–485. [https://doi.org/10.1016/S0975-3575\(10\)80035-5](https://doi.org/10.1016/S0975-3575(10)80035-5)
16. Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P (2012) Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereals breads. *LWT Food Sci Technol* 10(12):548–555. <https://doi.org/10.1016/j.lwt.2011.11.009>
17. Cortes-López H, Juárez-Rodríguez M, García-Contreras R, Soto-Hernández M, Castillo-Juárez I (2020) Old acquaintances in a new role: regulation of bacterial communication systems by fatty acids. In: Rai VR, Bai JA (Eds.), *Trends in quorum sensing and quorum quenching*. (Taylor & Francis Group, pp 47–58). <https://doi.org/10.1201/9780429274817>
18. Dufrene YF, Persat A (2020) Mechanomicrobiology: how bacteria sense and respond to forces. *Nat Rev Microbiol* 18(4):227–240. <https://doi.org/10.1038/s41579-019-0314-2>
19. Fadahunsi OS, Adegbola PI, Olorunnisola OS, Subair TI, Adepoju DO, Abijo AZ (2021) Ethno-medicinal, phytochemistry, and pharmacological importance of *Hunteria umbellata* (K. Schum) Hallier f. (Apocynaceae): a useful medicinal plant of sub-Saharan Africa. *Clin. Phytosci* 754:1–17. <https://doi.org/10.1186/s40816-021-00287-z>
20. Freitas PR, de Araújo AC, dos Santos Barbosa CR, Muniz DF, da Silva AC, Rocha JE, de Moraes Oliveira-Tintino CD, Ribeiro-Filho J, da Silva LE, Confortin C, do Amaral W (2020) GC-MS-FID and potentiation of the antibiotic activity of the essential oil of *Baccharis reticulata* (ruiz & pav.) pers. and α -pinene. *Ind Crops Prod* 145:112106. <https://doi.org/10.1016/j.indcrop.2020.112106>
21. Frenzel C, Teschke R (2016) Herbal hepatotoxicity: Clinical characteristics and listing compilation. *Int J Mol Sci* 17(5):588. <https://doi.org/10.3390/ijms17050588>
22. Ganesan T, Subban M, Christopher Leslee DB, Kuppannan SB, Seedeve P (2022) Structural characterization of n-hexadecanoic acid from the leaves of *Ipomoea eriocarpa* and its antioxidant and antibacterial activities. *Biomass Conv Bioref*. <https://doi.org/10.1007/s13399-022-03576-w>
23. Haag AF, Fitzgerald JR, Penadés JR (2019) *Staphylococcus aureus* in animals. *Microbiol Spectr*. <https://doi.org/10.1128/microbiolspec.gpp3-0060-2019>
24. Igbe I, Ozolua RI, Okpo SO, Obasuyi O (2009) Antipyretic and analgesic effects of the aqueous extract of fruit pulps of HUK. Schum (Apocynaceae). *Trop J Pharm Res* 8(4):331–336. <https://doi.org/10.4314/tjpr.v8i4.45226>
25. Ikuta KS, Swetschinski LR, Robles AG, Sharara F, Mestrovic T, Gray AP, Davis W, Wool EE, Han C, Gershberg HA, Aali A, Abate SM, Abbasi-Kangevari M, Abbasi-Kangevari Z, Abd-El salam S, Abebe G, Abedi A, Abhari AP, Abidi H, Aboagye RG (2022) Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet* 400(10369):2221–2248. [https://doi.org/10.1016/S0140-6736\(22\)02185-7](https://doi.org/10.1016/S0140-6736(22)02185-7)
26. Juárez-Rodríguez MM, Cortes-López H, García-Contreras R, González-Pedrajo B, Díaz-Guerrero M, Martínez-Vázquez M, Rivera-Chávez JA, Soto-Hernández RM, Castillo-Juárez I (2021) Tetradecanoic acids with anti-virulence properties increase the pathogenicity of *Pseudomonas aeruginosa* in a murine cutaneous infection model. *Front Cell Infect Microbiol* 10:597517. <https://doi.org/10.3389/fcimb.2020.597517>
27. Krismer B, Weidenmaier C, Zipperer A, Peschel A (2017) The commensal lifestyle of *Staphylococcus aureus* and its interactions with the nasal microbiota. *Nat Rev Microbiol* 15(11):675–687. <https://doi.org/10.1038/nrmicro.2017.104>
28. Ladokun OA, Abiola A, Okikiola D, Ayodeji F (2018) GC-MS and molecular docking studies of *H. umbellata* methanolic extract as a potent anti-diabetic. *Inf Med Unlocked* 13:1–8. <https://doi.org/10.1016/j.imu.2018.08.001>
29. Le KY, Otto M (2015) Quorum-sensing regulation in staphylococci—an overview. *Front Microbiol* 6:1174. <https://doi.org/10.3389/fmicb.2015.01174>
30. Leonti M, Stafford GI, Cero MD, Cabras S, Castellanos ME, Casu L, Weckerle CS (2017) Reverse ethnopharmacology and drug discovery. *J Ethnopharmacol* 198:417–431. <https://doi.org/10.1016/j.jep.2016.12.044>
31. Lin Q, Di YP (2020) Determination and quantification of bacterial virulent gene expression using quantitative real-time PCR. *Methods Mol Biol* (Clifton, N.J.) 2102:177–193. https://doi.org/10.1007/978-1-0716-0223-2_9
32. Mensah ML, Komlaga G, Forkuo AD, Firempong C, Anning AK, Dickson RA (2019) Toxicity and safety implications of herbal medicines used in Africa. *Herb Med IntechOpen* 63:1992–2849. <https://doi.org/10.5772/intechopen.72437>
33. Muniz DF, Dos Santos Barbosa CR, de Menezes IRA, de Sousa EO, Pereira RLS, Júnior JTC, Pereira PS, de Matos YMLS, da Costa RHS, de Moraes Oliveira-Tintino CD, Coutinho HDM, Filho JMB, Ribeiro de Sousa G, Filho JR, Siqueira-Junior JP, Tintino SR (2021) In vitro and in silico inhibitory effects of synthetic and natural eugenol derivatives against the NorA efflux pump in *Staphylococcus aureus*. *Food Chem* 337:127776. <https://doi.org/10.1016/j.foodchem.2020.127776>
34. Obasuyi O, McClure J, Oronsaye FE, Akerele JO, Conly J, Zhang K (2020) Molecular characterization and pathogenesis of *Staphylococcus aureus* isolated from Benin-City, Nigeria. *Microorganisms* 8(6):912. <https://doi.org/10.3390/microorganisms8060912>
35. Oboh G, Adebayo AA, Ademosun AO (2017) Erection-stimulating, anti-diabetic and antioxidant properties of *Hunteria umbellata* and *Cylicodiscus gabunensis* water extractable phytochemicals. *J Complement Integr Med*. <https://doi.org/10.1515/jcim-2016-0164>
36. Okaiyeto K, Oguntibeju OO (2021) African herbal medicines: adverse effects and cytotoxic potentials with different therapeutic applications. *Int J Environ Res Public Health* 18(11):5988. <https://doi.org/10.3390/ijerph18115988>
37. Oseyomon JO, Erhirhie EO, Ugwu CB, Ilodigwe EE (2023) Toxicological evaluation of methanol extract of *Hunteria umbellata* seed on sperm and hematological parameters in male wistar rats. *J Appl Sci Environ Manag* 27(11):2487–2493. <https://doi.org/10.4314/jasem.v27i11.18>
38. Ozioma JEO, Chinwe NOA (2019) Herbal medicines in African traditional medicine. *Herb Med London* 10:191–214. <https://doi.org/10.5772/intechopen.80348>
39. Peter AK, Paul A, Olabisi L, Clement A (2020) Synergistic evaluation of *Moringa oleifera*, *Hunteria umbellata* and *Azadirachta indica* with antibiotics against environmental MRSA isolates: an in-vitro study. *Am J BioSci* 8(4):91–98. <https://doi.org/10.11648/jajbio.20200804.11>
40. Rufai Y, Isah Y, Isyaka MS (2016) Comparative phyto-constituents analysis from the root bark and root core extractives of *Cassia ferruginea* (schrad D. C) plant. *Scholars J Agric Vet Sci* 3(4):275–283. <https://doi.org/10.21276/SJAVS.2016.3.4.1>
41. Salami TO, Ladokun OA (2021) Extraction and analysis of phytochemicals from *Hunteria umbellata*. *GA J Sci* 79(1):18
42. Salisu TF, Okpuzor JE, Jaja SI (2019) Identification, characterization and quantification of chemical compounds in selected edible wild leafy vegetables. *IJS* 21(1):215–227. <https://doi.org/10.4314/ijss.v21i1.19>
43. Salisu TF, Yahaya TO, Fowora MA, Fagbayi TA, Bolarinwa KA, Owagboriaye FO, Onajobi IB, Ademola LA (2023) In vitro ethno-toxicology of *Hunteria umbellata* methanol seed and stem extracts against *Vibrio parahaemolyticus* and its molecular implication on swarming and adhesion genes. *Bull Natl Res Cent* 47(1):1–10. <https://doi.org/10.1186/s42269-023-01074-1>
44. Schug AR, Bartel A, Scholtzek AD, Meurer M, Brombach J, Hensel V, Séamus F, Schwarz S, Andrea TF (2020) Biocide susceptibility testing of bacteria: Development of a broth micro dilution method. *Vet Microbiol*. <https://doi.org/10.1016/j.vetmic.2020.108791>
45. Shukla S, Mehta A, Bajpai VK (2013) Phytochemical screening and anthelmintic and antifungal activities of leaf extracts of *Stevia rebaudiana*. *J Biol Active Prod Nat* 3(1):56–63. <https://doi.org/10.1080/22311866.2013.782695>
46. Silva V, Hermenegildo S, Ferreira C, Manaia CM, Capita R, Alonso-Calleja C, Carvalho I, Pereira JE, Maltez JL, Igrejas G, Poeta P (2020) Genetic characterization of methicillin-resistant *Staphylococcus aureus* isolates from human bloodstream infections: detection of MLS_B resistance. *Antibiotics* (Basel, Switzerland) 9(7):375. <https://doi.org/10.3390/antibiotics9070375>
47. Tamai M, Yamazaki Y, Ito T, Nakagawa S, Nakamura Y (2023) Pathogenic role of the staphylococcal accessory gene regulator quorum sensing system in atopic dermatitis. *Front Cell Infect Microbiol* 13:1178650. <https://doi.org/10.3389/fcimb.2023.1178650>
48. Upadhyay A, Upadhyaya I, Kollanoor-Johny A, Venkitanarayanan K (2014) Combating pathogenic microorganisms using plant-derived

antimicrobials: a minireview of the mechanistic basis. *Biomed Res Int* 2014;761741. <https://doi.org/10.1155/2014/761741>

49. Xie MZ, Shoukamy MI, Salem AM, Oba S, Goda M, Nakano T, Ide H (2016) Aldehydes with high and low toxicities inactivate cells by damaging distinct cellular targets. *Mutat Res* 786:41–51. <https://doi.org/10.1016/j.mrfmmm.2016.02.005>
50. Xie C, Wang S, Cao M, Xiong W, Wu L (2022) (E)-9-Octadecenoic acid ethyl ester derived from lotus seedpod ameliorates inflammatory responses by regulating MAPKs and NF- κ B signaling pathways in LPS-induced RAW2647 macrophages. *eCAM*. <https://doi.org/10.1155/2022/6731360>
51. Xu Z, Gao P, Liu D, Song W, Zhu L, Liu X (2022) Chemical composition and in vitro antioxidant activity of *Sida rhombifolia* L. volatile organic compounds. *Molecules* (Basel, Switzerland) 27(20):7067. <https://doi.org/10.3390/molecules27207067>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.