Phytochemical screening, Mathematical analysis and Antimicrobial activity of methanolic seed extract of *Hunteria umbellata*

ABSTRACT

Aim: The study evaluates the in-vitro antimicrobial activity of *Hunteria umbellata* against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus sp.*

Place and Duration of Study: The study was carried out for three months in 2019 in Biochemistry Laboratory, Department of Chemical Sciences (Biochemistry unit), School of Pure and Applied Sciences, Lagos State Polytechnic, Ikorodu, Lagos- Nigeria.

Methodology: The qualitative and GC-MS analysis of *Hunteria umbellata* methanolic seed extract were determined using standard procedure. The antimicrobial activity was evaluated by the disc diffusion method and agar well diffusion method. The experimental data was resampled 1000 times to allow for higher degrees of freedom in carrying out t-test to test for the difference of the effect of *in-vitro* antimicrobial activity of *H. umbellata* against *E. coli*, *S. aureus* and *Streptococcus sp* using mathematical software R language (3.6.1 version). Line plots, histogram, and t-test are used to explain the effect of antimicrobial activity of *H. umbellate* on the selected bacteria. MIC and MBC were determined using standard methods.

Results: The Phytochemical analysis of methanolic seed extract of *Hunteria umbellata* showed the presence of secondary metabolites like saponins, tannins, flavonoids, steroids, phenol among others. GC-MS assay of the *H. umbellata* seed extract revealed the presence of eight different compounds. Agar well diffusion method was characterized by inhibition zones of 18.36±0.87, 19.13±1.03 and 21.62±2.53 mm for *E.coli, S. aureus* and *Streptococcus sp* respectively at 300 $mgml^{-1}$ and 21.70± 1.60, 23.83± 2.64 and 28.57± 1.52 for *E.coli, S. aureus* and *Streptococcus sp* respectively at 500 $mgml^{-1}$. The results of the analysis show that there is a significant difference between the effects of *in-vitro* antimicrobial activity of *H. umbellate* on 300 $mgml^{-1}$ and 500 mg/ml on each bacteria tested at 5% level of significance. *E.coli, S. aureus* and *Streptococcus sp* were tested against 12 standard antimicrobial agents, of which six was sensitive and another six was resistance to *S. aureus*, seven was sensitive, and five was resistance to *S. aureus* while four was resistance and eight sensitive to *Streptococcus sp*. The minimum inhibitory concentration (MIC) for *E.coli, S. aureus,* and *Streptococcus sp* was 250, 125 and 31.25 $mgml^{-1}$ while their

minimum bactericidal concentration (MBC) was 500, 250 and 125 respectively. MIC and MBC tests showed that *H. umbellata* methanolic seed extract had noticeable bactericidal effects with MBC/MIC values ranging between 2 to 4. The extract has strong potency against these microorganisms with *Streptococcus sp* being the most susceptible.

Conclusions:

Hunteria umbellata has potential as natural therapeutic agents against *E. coli*, *S. aureus* and *Streptococcus sp* and they may prevent pathogenic diseases.

Keyword

Phytochemicals*,* GC-MS, *Hunteria umbellata* seed extract, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sp,* MIC and MBC.

INTRODUCTION

Plants constitute the major source of medications for human, since they have the ability to synthesize a lot of secondary metabolites which serve as plants defense mechanisms against micro and macro-organisms [1]. Examples of some of the most important phytochemicals found in medicinal plants are flavonoids, alkaloids, tannins, and phenolics [2]. Several studies have demonstrated that different medicinal plant extracts possess numerous biological properties such as antimicrobial, antioxidant, anti-inflammatory, anticancer and anti-diabetic activities [3-8].

Escherichia coli is a gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals. Most *E. coli* are harmless, but some are known to be pathogenic, causing severe intestinal diseases in man [9]. *Escherichia coli* is a severe infectious disease associated with high rates of mortality and morbidity [10]. *Staphylococcus aureus* is a gram-positive bacterium; it is an important pathogen of animals and humans and is implicated in various infections. It is a pathogen of greater concern because of its virulence [11], its causes a diverse array of life-threatening infections and it can adapt to different environmental conditions. *Streptococcus* species are bacteria that are associated with diseases in both humans and animals. Examples of human diseases are: neonatal sepsis, arthritis, pneumonia and meningitis while in animals they mainly cause mastitis [12].

Antibiotics are the most effective drugs used against microbial infections, and they are recently losing their efficacies as most microorganisms have developed resistance [13]. The intensive use of antibiotics has led to the emergence of what is known as multidrug resistant (MDR) bacteria

which are now raising public health threat [14]. World Health Organization (WHO) estimated that a large population depended on traditional medicinal plants for the treatment of different illnesses and many people have begun to use medicinal plants as an alternative therapy to modern medicines [15]. New antibacterial agents from plants have been develop since most of the recent drugs are initially obtained or semi-synthesized from plants. Cowan, [1] study shows the antibacterial activity of many medicinal plants.

Hunteria umbellata (K. Schum) Hallier belongs to the family Apocynaceae. The plant is a small tree measuring about 2 - 5 feet in girth and 25 - 40 feet high and grows very well in tropical West African forest groove [16]. The leaves of the plant are greenish, measuring 11-23 cm long, 5-9 cm broad [16]. *Hunteria umbellata* is locally called "Abeere" among the Yoruba (South-West Nigeria), nkpokiri in Ibo and Osu in Edo. Oluwemimo and Usifoh, [17] study show that different parts of the plant have been used in herbal medicine for the treatment of helminthic infection. Studies have shown that *H. umbellata* seed can be used in the treatment of diabetes [18-19]. The present study reports the GC-MS and the antibacterial properties of methanolic seed extract of *H. umbellata* against three clinical strains (*S. aureus, E. coli* and *Streptococcus sp).*

Materials and methods

Collection and identification of plant material

The seeds of *Hunteria umbellata* were obtained from Ikorodu market in Lagos State, Nigeria and were authenticated by a botanist from the Department of Biological Science (Environmental Biology Unit), Lagos State Polytechnic Ikorodu, Lagos, Nigeria.

Preparation of *Hunteria umbellata* methanolic seed extract

Hunteria umbellata seeds were air dried under a shade at 28 °C in the laboratory. The dried seeds were grounded into a fine powdered form using an industrial machine. Extraction was carried out by dispersing 150g of the grounded seed material into 1L of 80% methanol, and the maceration was done for 72 h. The solution was filtered by passing through cotton wool, and the filtrate was

concentrated with the help of a rotary evaporator at a temperature not exceeding 40°C. The concentrated extract was later dried to complete dryness in an aerated oven at 40°C for 48 hours. The concentrate was later stored in a refrigerator at 4°C.

Phytochemical analysis of *Hunteria umbellata* methanolic seed extract solution

Determination of saponins

Froth test

2 ml of *H. umbellata* methanolic seed extract solution was shaken vigorously with distilled water to form froth and was then allowed to stand for 10–15 min. The persistent froth was considered as the presence of saponins.

Determination of tannins

Two ml of the *H. umbellata* methanolic seed extract solution was stirred with equal volume of distilled water. A few drops of 2% $FeCl_3$ solution were added. The formation of a green precipitate indicated the presence of tannins.

Determination of alkaloids

Mayer's test

The seed extract of *H. umbellata* solution was mixed with HCl and then filtered. The filtrate was treated with Mayer's reagent. The formation of a yellow coloured precipitate indicates the presence of alkaloids.

Dragendroff's test

The seed extract of *H. umbellata* solution was mixed with HCl and then filtered. The filtrate was treated with Dragendroff's reagent. The formation of a red precipitate indicates the presence of alkaloids.

Determination of flavonoids

Shinoda's test

A piece of magnesium ribbon and HCl were added to *H. umbellata* methanolic seed extract solution . The formation of Red colour confirmed the presence of flavonoids.

Determination of steroids

Two ml of *H. umbellata* seed extract solution was mixed with 2 ml of chloroform and 2 ml of concentrated H_2SO_4. The formation of red colour indicates the presence of steroids.

Determination of carbohydrates and reducing sugars

Fehling's test

An equal volume of Fehling A and Fehling B reagents were mixed together and 2 ml of it was added to the seed extract of *H. umbellata* solution and gently boiled. The formation of a brick-red precipitate at the bottom of the test tube indicated the presence of reducing sugars.

Determination of cardiac glycosides

Liebermann's test

Two ml of the seed extract was mixed with 2 ml of chloroform and 2 ml of acetic acid and the solution was cooled on ice. H_2SO_4 was then added carefully. The colour change from violet to blue to green and this indicates the presence of a steroidal nucleus that is a glycone portion of glycoside.

Determination of antraquinone

0.5 ml of the aqueous garlic extract was boiled in 10% HCl for 5 mins, filtrate and allowed to cool. An equal volume of $CHCl_3$ with few drops of 10% NH_3 was added to 2ml of the filtrate. The formation of rose-pink colour indicates the presence of anthraquinones.

Determination of simple phenolics

One ml of *H. umbellata* seed extract solution was mixed with 1–2 drops of 1% $FeCl_3$. The development of blue-green colouration indicates the presence of phenol.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Methanolic seed Extract of *Hunteria umbellata*

GC-MS analysis of the plant extract was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD) as described by Momoh et al. [20]

Detection of Components

Analysis of mass spectrum GC-MS was conducted by the database of the National Institute Standard and Technique (NIST) which contained more than 62,000 patterns. The spectrum of the unidentified compound was compared with the spectrum of the identified compounds stored in the National Institute Standard and Technique library. The names, molecular weight, structure of the compounds in the test material were ascertained.

Test Organisms

To study the antibacterial activity of methanolic seed extract of *H. umbellata* plant, we used three bacterial strains obtained from the Microbiology Department, University of Lagos, Nigeria. The organisms are *Escherichia coli* a gram-negative bacterium, *Staphylococcus aureus* and *Streptococcus sp* gram positive bacteria. The microorganisms were maintained at 4°C on

Nutrient Agar slant in the Department of Chemical Scsiences (Biochemistry Unit), School of Pure and Applied Science, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria and fresh subcultures were made before use.

Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml of peptone water, incubated at 37°C for 4 hours. This actively growing bacterial suspension was then adjusted with peptone water to obtain turbidity visually comparable to that of 0.5 McFarland standard as described by Momoh et al. [21]. The 0.5 McFarland standard was prepared by mixing 0.5ml of 1.75% (w/v) barium chloride dehydrate ($BaCl_2$. $2H_2O$) with 99.5 ml of 1% (v/v) tetraoxosulphate (vi) acid (H_2SO_4). This turbidity was equivalent to approximately 1 x 10^8 colony forming units per ml (CFU/ml).

Determination of diameter of zone of inhibition (mm) using agar well diffusion method

Agar well-diffusion method was employed to determine the antimicrobial activity. Eighteen hours of broth culture of the test microorganisms were suspended into the sterile nutrient broth. It was standardized according to National Committee for Clinical Laboratory Standard [22] by gradually adding 9% normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1x10^8 colony forming units per ml (CFU/ml). Briefly, Petri-dishes were prepared by loading about 25 ml of an autoclaved nutrient agar on sterile plates and left to solidify. Then, the surface of each plate was drilled using a sterile cork borer (6 mm) and 3 wells were punched out on each plate. A total of 100 µL of a standardized culture (adjusted to 0.5 McFarland) of test organisms was added into the agar plate followed by loading of 100 µL of the *Hunteria umbellate* seed extract in the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37ºC for 18-24 hours for bacterial pathogens. The diameters of the inhibition zone (mm) were measured. The susceptibility of the different organisms to *H. umbellata* methanolic seed extract was assayed using the method described by Momoh et al. [21]. The experiment was repeated thrice, for each replicate, the readings were taken in three different fixed directions and the average values recorded.

Antibiotic susceptibility testing

The susceptibility of organisms to different antibiotics were tested using the disk diffusion method as described [21, 23]. On freshly prepared Mueller Hinton agar and standardized by the method of Famuyide et al. [24] and National Committee for Clinical Laboratory Standard (NCCLS) [22] using some selected antibiotics namely: tetracycline (30µg), pefloxacin (10µg),

ampiclox (30μg), streptomycin (30μg), gentamicin (10μg), erythromycin (10μg), ciprofloxacin (10μg), amoxicillin (30μg), penicillin G (10μg), septrin (30μg) metronidazole (20 μg/disk) and imipenem (10 μg/disk). For each combination of the antibiotics and the bacterial strains, the experiment was performed in triplicate.

Minimum Inhibitory Concentration (MIC)

The minimum inhibition concentration (MIC) is the lowest seed extract concentration that inhibited the growth of the test organisms as indicated by the absence of visible turbidity in the tube compared with the control tubes. The MIC of *Hunteria umbellata* seed extract was determined according to the method described by Chung et al. [25] and Momoh et al. [21]. MIC of the *H. umbellate* seed extract was assayed using the tube serial dilution method. Briefly, a total of 1 ml of Mueller-Hinton broth was poured to a set of different test tubes and autoclaved. Subsequently, 1 ml of 100% *H. umbellata* seed extract (2g/ml) was poured to the 1st test tube to make a concentration of 50%, and two-fold serial dilutions were made by transferring 1 ml from one tube to another to get the following series: 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78% among others. Then, an overnight broth culture of the test organism was adjusted to McFarland turbidity standard and 100 µl of the cell suspension was added to each of the tubes. The tubes were incubated aerobically at 37°C for 18 hours. Negative control was made by pouring 1 ml of physiological saline instead of *H. umbellata* seed extract. The lowest concentration of the dilution without bacterial growth was considered as MIC.

Minimum Bactericidal Concentration (MBC)

The MBC of the *H. umbellate* seed extract was prepared by modification of the method of Spencer and Spencer, [26] and Momoh et al. [21]. 0.1 ml aliquots of samples taken from the non-turbid tubes of the MIC assay tubes were sub-cultured onto nutrient agar plates. The resulting plates were then incubated aerobically at 37°C for 24 hours. The lowest concentration of the seed extract at which no colonies of *E. coli, S. aureus and Streptococcus sp* were seen was taken as the MBC. The results were compared with that of control using sterilized distilled water. The experiment was performed in triplicate. The MBC was taken as the concentration of the seed extract that did not show any growth on a new set of agar plates. The lowest MIC that revealed no visible growth was regarded as MBC. The MBC/MIC was also calculated as bactericidal or bacteriostatic.

Data Analysis

All analyses were carried out in triplicate and results were expressed as mean ± SD. The data analysis was done using the Graph Pad prism computer software version 5.01. A P-value < 0.05 was considered significant.

Phytochemical screening of methanolic seed extract of *Hunteria umbellata*

Phytochemical screening of the *H. umbellata* methanolic seeds extract shows the presence of secondary metabolites like saponins, tannins, alkaloids, flavonoids, steroid, phenolic compounds anthraquinones among others (Table 1).

Table 1: Phytochemical screening of *Hunteria umbellata* methanolic seed extract

Phytochemical constituent	Test performed	Inference
Reducing sugar	Fehling's test	+
Saponins	Froth test	+
Flavonoids	Shinoda test	+
Anthocyanine	Sodium hydroxide test	
Tannins	Ferric chloride test	+
Alkaloids	Dragondorff`s test Mayer`s test	+ +
Anthraquinone	Borntrager's test	+
Steroids and sterol		+
Phenolic compounds	Ferric chloride test	+
Cardiac glycoside	Liebermanns test	+

(+) present

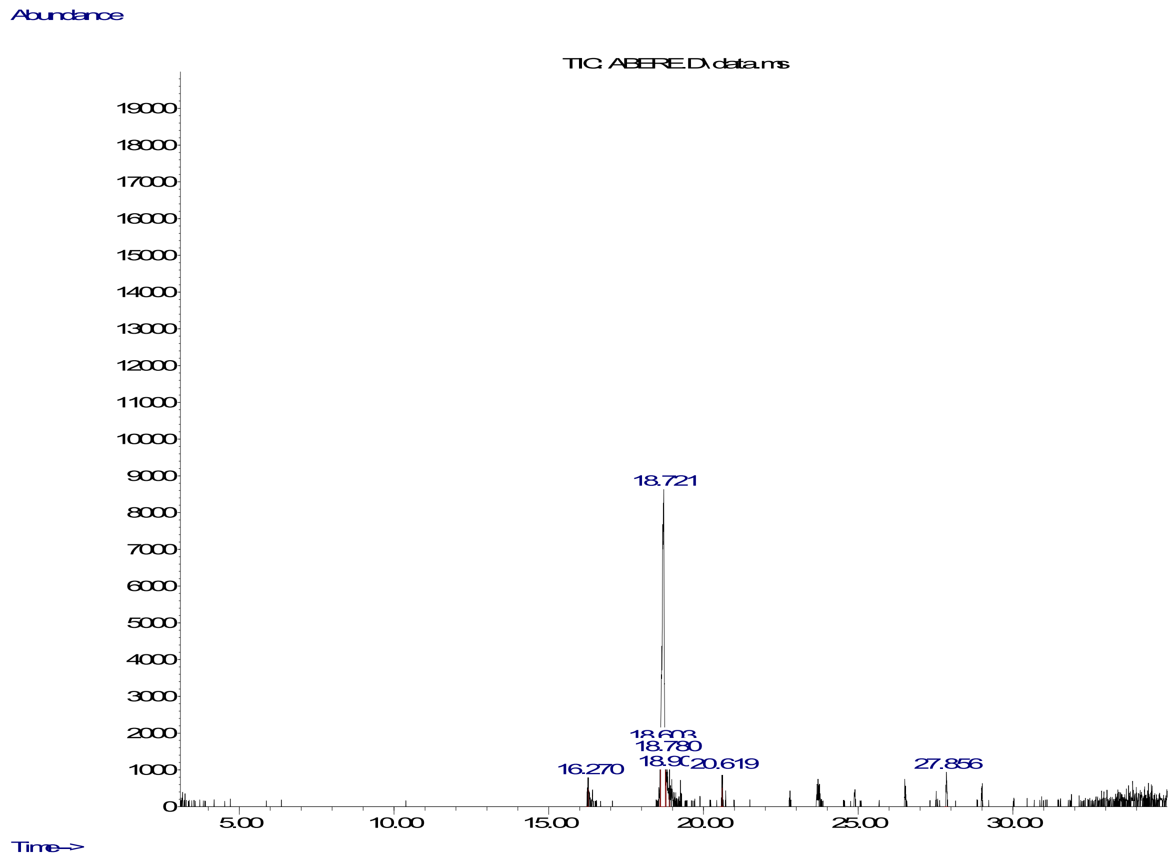

Figure 1. **Gas chromatography-mass spectrometry chromatogram of *Hunteria umbellata* methanolic seed extract**

Table. 2. Chemical composition of *Hunteria umbellate* methanolic seed extract identified by Gas Chromatography-Mass Spectrometry.

PK no	Retention Time	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area (%)
1	16.270	2-Propenamide	C_3H_5NO	71.078	1.83
2	18.605	Acetonitrile, 2,2'-iminobis-	$C_4H_5N_3$	95.1026	3.80
3	18.719	4-Cyclohepten-1-amine	$C_7H_{13}N$	111.18	83.13
4	18.782	5-Azabicyclo[2.2.0]hex-2-en-6-one	C_5H_5NO	95.1	2.81
5	18.903	2-Furanmethanamine	C_5H_7NO	97.1152	1.63
6	20.596	2-Butanamine, (S)-	$C_4H_{11}N$	73.1368	1.21
7	20.619	Acetamide, N-2-propynyl-	C_5H_7NO	97.12 g	2.15
8	27.857	2-Hydroxyskatole	C_9H_9NO	147.17	3.43

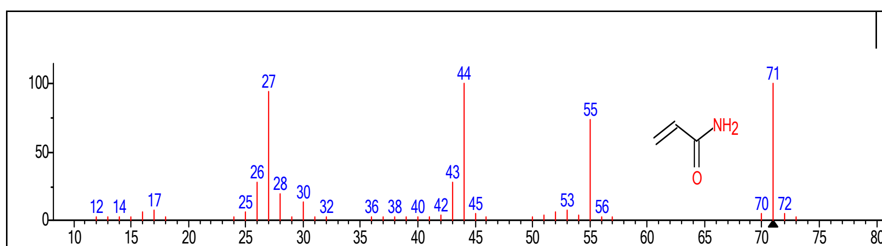

Fig. 2a. Mass spectrum of 2-Propenamide **structure (**1.83**%, RT** 16.270 **)**

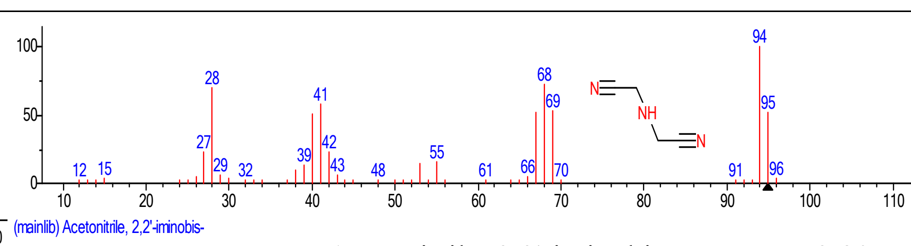

Fig. 2b. Mass spectrum of Acetonitrile, 2,2'-iminobis- **structure (**3.80**%, RT** 18.605**)**

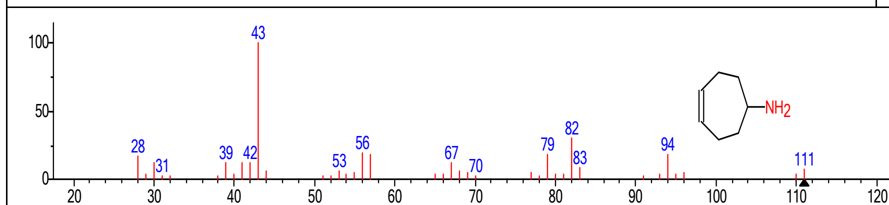

Fig. 2c. Mass spectrum of 4-Cyclohepten-1-amine **structure (**83.13**%, RT** 18.719**)**

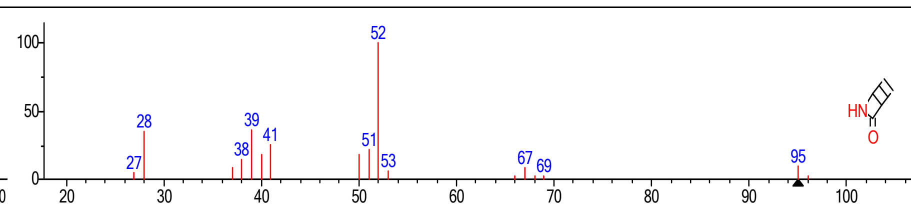

Fig. 2d. Mass spectrum of 5-Azabicyclo[2.2.0]hex-2-en-6-one **structure (**2.81**%, RT**18.782**)**

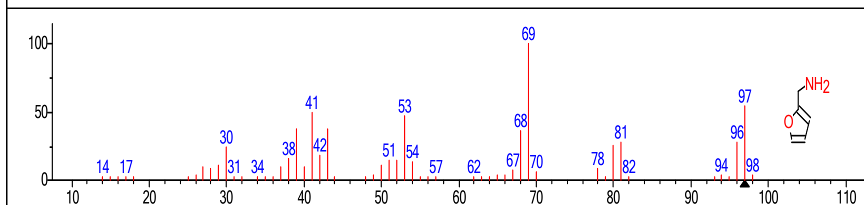

Fig. 2e. Mass spectrum of 2-Furanmethanamine **structure (**1.63**%, RT**18.903 **)**

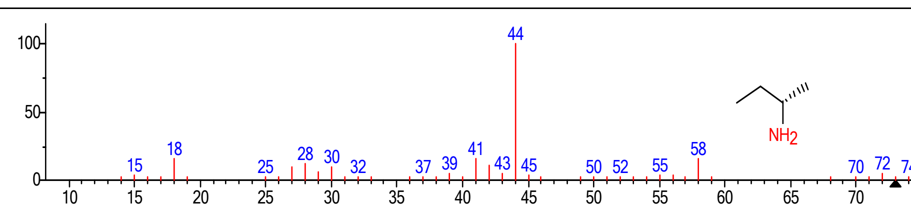

Fig. 2f. Mass spectrum of 2-Butanamine, (S)- **structure (**1.21**%, RT**20.596**)**

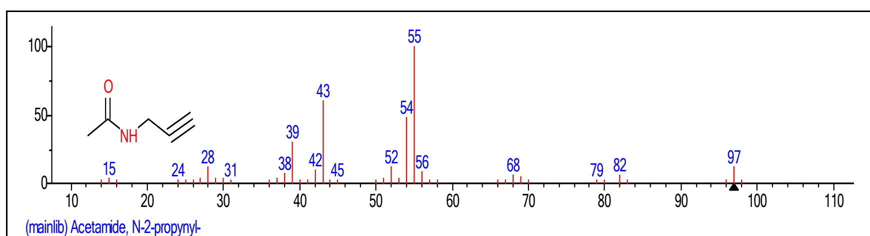

Fig. 2g. Mass spectrum of Acetamide, N-2-propynyl- **structure (**2.15**%, RT**20.619**)**

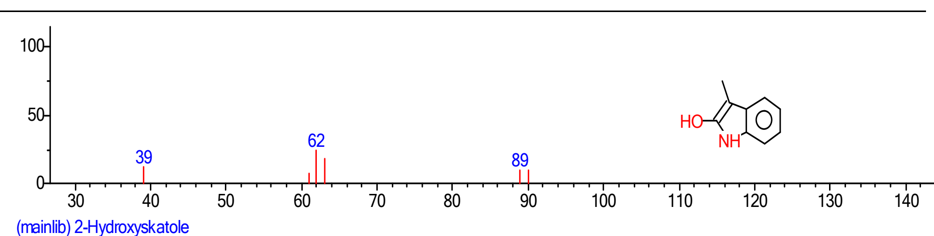

Fig. 2h. Mass spectrum of 2-Hydroxyskatole **structure (**3.43**%, RT**27.857**)**

Figure 2. Mass spectrum of eight different compounds obtained during GC-MS analysis with their peak area and retention time.

 Zone of inhibition at 300 mg/ml concentration of the *H. umbellate* methanolic seed extract against *E. coli*	 Zone of inhibition at 500 mg/ml concentration of the *H. umbellate* methanolic seed extract against *E. coli*
 Zone of inhibition at 300 mg/ml concentration of the *H. umbellate* methanolic seed extract against *S. aureus*	 Zone of inhibition at 500 mg/ml concentration of the *H. umbellate* methanolic seed extract against *S. aureus*
	 Zone of inhibition at 500 mg/ml concentration of the

Zone of inhibition at 300 mg/ml concentration of the *H. umbellate* methanolic seed extract against *Streptococcus sp.*	*H. umbellate* methanolic seed extract against *Streptococcus sp.*

Figure 3. Zone of inhibition of *H. umbellate* methanolic seed extract against *E. coli* , *S. aureus and Streptococcus sp* at 300 and 500 mg/ml concentration.

Table 3. Zone of inhibition of *Hunteria umbellate* methanolic seed extract against *Escherichia* ***coli,*** *Staphylococcus* ***aureus*** **and *Streptococcus sp***

Test organisms	Concentration (mg/ml)	Zone of inhibition (300 mm)	Concentration (mg/ml)	Zone of inhibition (500 mm)	Interpretation
Escherichia coli	300	18.36±0.87	500	21.70± 1.60	Sensitive
Staphylococcus aureus	300	19.13±1.03	500	23.83± 2.64	Sensitive
Streptococcus sp	300	21.62±2.53	500	28.57± 1.52	Sensitive

Table 4: T-test difference between the zone of inhibition of 300 and 500 mg/ml of *Hunteria umbellata* against three different organisms

Test organisms	Zone of inhibition (300 mm)	Zone of inhibition (500 mm)	t-stat.	P-value	Decision
Escherichia coli	18.36 ± 0.87	21.70 ± 1.60	-58.436	2.2e-16	Significant
Staphylococcus aureus	19.13 ± 1.03	23.83 ± 2.64	-53.256	2.2e-16	Significant
Streptococcus sp	21.62 ± 2.53	28.57 ± 1.52	-76.432	2.2e-16	Significant

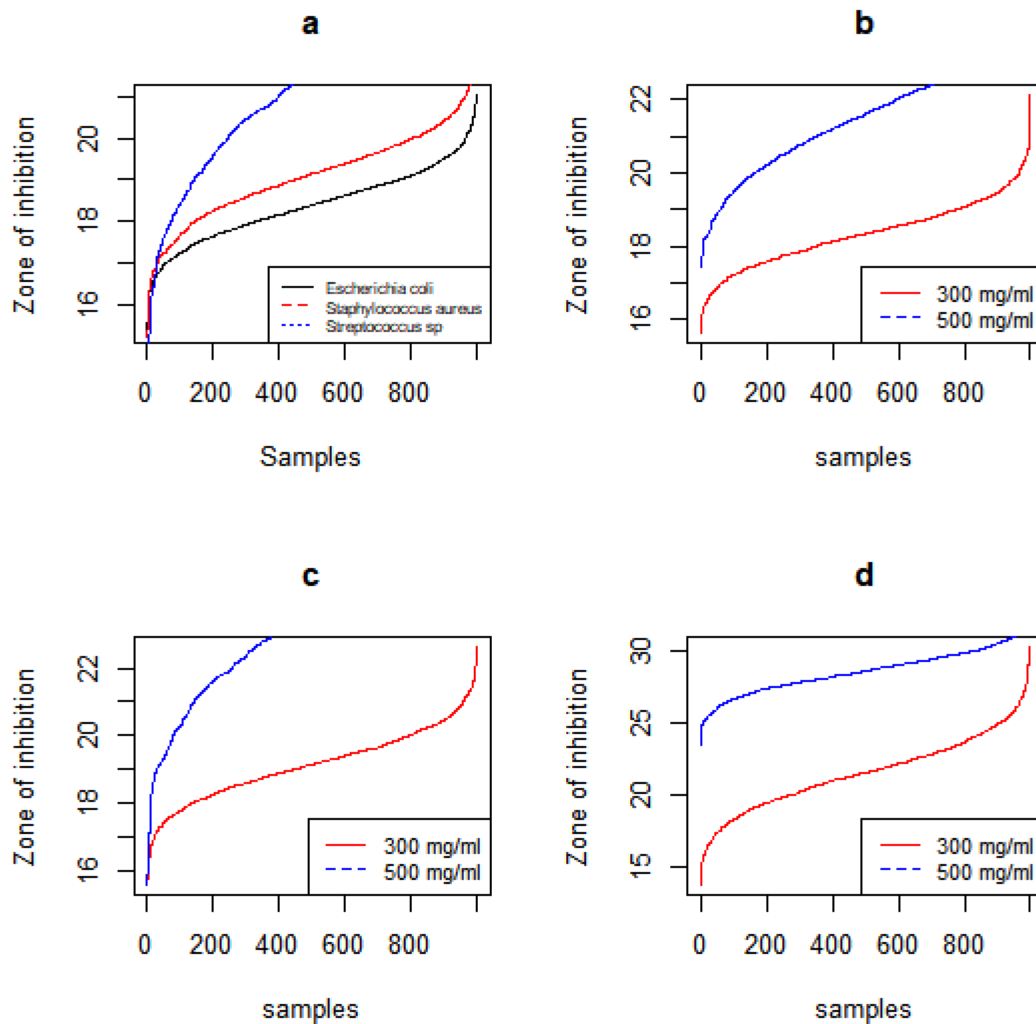

Figure 4: Comparing the zone of inhibition of *Escherichia coli*, *Staphyloccus aureus* and *Streptococcus sp* at concentration of 300 and 500 mg/ml.
a= Sensitivity of *H. umbellata* on the different microorganisms at different concentration.
b= Sensitivity of *H. umbellata* on *E. coli* at different concentration.
c= Sensitivity of *H. umbellata* on *S. aureus* at different concentration.
d= Sensitivity of *H. umbellata* on *Streptococcus sp* at different concentration

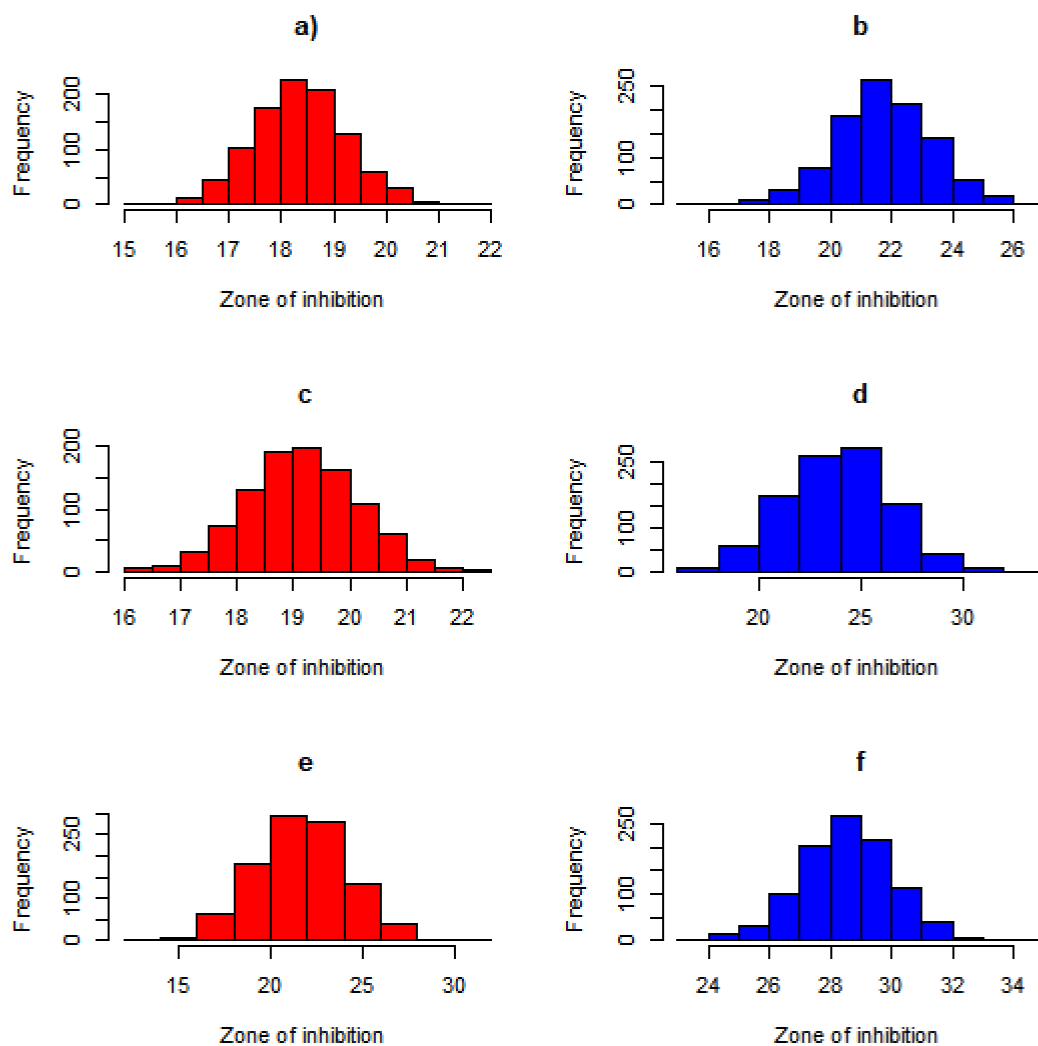

Figure 5: Mode of the zone of inhibition of different organisms at different concentrations. a and b = Mode of *E. coli* zone of inhibition at a concentration of 300 and 500 mg/ml respectively. c and d = Mode of *S. aureus* zone of inhibition at a concentration of 300 and 500 mg/ml respectively. e and f = Mode of *Streptococcus sp* zone of inhibition at a concentration of 300 and 500 mg/ml respectively

Table 5. Antimicrobial susceptibility pattern of standard antibiotics agent against *Escherichia coli*

Antibiotic sensitive disc	Concentration (µg)	Diameter of zone of inhibition (mm)	Interpretation
Tetracycline (TET)	30	18.9 ± 3.7	**S**
Pefloxacin (PEF)	10	18.6 ± 2.1	S
Penicillin G (PG)	10	14.1 ± 1.8	R
Gentamicin (CN)	10	18.7± 1.2	S

Ampiclox (APX)	30	15.7± 2.4	R
Metronidazole (MZ)	20	12.1± 1.6	R
Amoxacillin (AM)	30	16.6± 2.8	R
Ciprofloxacin (CPX)	10	21.4± 2.1	S
Streptomycin (S)	30	19.8± 1.7	S
Imipenem; (IMI)	10	19.2± 1.1	S
Septrin (SXT)	30	16.5 ± 1.7	R
Erythromycin (E)	10	14.1± 2.6	R

Key: S = Sensitive (zone diameter of bacterial inhibition $\geq$ 18mm)
R = Resistant (zone diameter of bacterial inhibition < 18mm).

Table 6. Antimicrobial susceptibility pattern of standard antibiotics agent against *Staphylococcus aureus*

Antibiotic sensitive disc	Concentration (µg)	Diameter of zone of inhibition (mm)	Interpretation
Tetracycline (TET)	30	15.8 ± 0.3	R
Pefloxacin (PEF)	10	19.5 ± 0.8	S
Penicillin G (PG)	10	15.9 ± 1.7	R
Gentamicin (CN)	10	14.4± 2.9	R
Ampiclox (APX)	30	16.4± 0.6	R
Metronidazole (MZ)	20	18.2 ± 3.5	S
Amoxacillin (AM)	30	19.7± 2.3	S
Ciprofloxacin (CPX)	10	23.8± 3.1	S
Streptomycin (S)	30	20.09 ± 2.8	S
Imipenem; (IMI)	10	21.4 ± 1.8	S
Septrin (SXT)	30	18.35± 3.2	S
Erythromycin (E)	10	11.9± 1.6	R

Key: S = Sensitive (zone diameter of bacterial inhibition $\geq$ 18mm)
R = Resistant (zone diameter of bacterial inhibition < 18mm).

Table 7. Antimicrobial susceptibility pattern of standard antibiotics agent against *Streptococcus sp*

Antibiotic sensitive disc	Concentration (µg)	Diameter of zone of inhibition (mm)	Interpretation
Tetracycline	30	16.12 ± 0.9	R
Pefloxacin (PEF)	10	18.85 ± 1.4	S
Penicillin G (PG)	10	18.03 ± 0.8	S
Gentamicin (CN)	10	13.34 ± 1.3	R
Ampiclox (APX)	30	19.26 ± 1.6	S
Metronidazole (MZ)	20	19.48 ± 2.2	S
Amoxacillin (AM)	30	18.56 ± 1.5	S
Ciprofloxacin (CPX)	10	21.18 ± 1.4	S
Streptomycin (S)	30	19.26 ± 1.9	S
Imipenem; (IMI)	10	22.42 ± 2.6	S
Septrin (SXT)	30	16.79 ± 1.4	R
Erythromycin (E)	15	15.62 ± 0.6	R

Key: S = Sensitive (zone diameter of bacterial inhibition ≥ 18mm)
R = Resistant (zone diameter of bacterial inhibition < 18mm).

Table 8: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *Hunteria umbellate* methanolic seed extract against *Escherichia coli, Staphylococcus aureus and Streptococcus sp*

ORGANISMS	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
Escherichia coli	**250**	**500**	**2.00**
Staphylococcus aureus	**125**	**250**	**2.00**
Streptococcus sp	**31.25**	**125**	**4.00**

DISCUSSION

Several studies have shown the presence of different secondary metabolite like: alkaloids, volatile oils, tannins, flavonoids and saponins etc in the different extracts of *H. umbellata* [27-29]. Studies have shown that phytochemicals among others. flavonoids, saponins, tannins and other secondary metabolites in plant play some major roles in the inhibition of malaria parasites in infected animals [30, 31].

The different compounds identified with their functions from each of the mass spectra fragmentation patterns are listed in Table 2. A total of 8 compounds were identified consisting of one (1) major compound and seven (7) minor compounds. The one major compound and the percentage of abundance is 4-Cyclohepten-1-amine and 83.13% respectively. The compound has a retention time of 18.719. The other minor compounds and their percentage abundance are: 2-Propenamide (1.83%), Acetonitrile, 2,2'-iminobis- (3.80%), 5-Azabicyclo[2.2.0]hex-2-en-6-one (2.81%), 2-Furanmethanamine (1.63%), 2-Butanamine, (S)-(1.21%), Acetamide, N-2-propynyl- (2.15%), 2-Hydroxyskatole (3.43%). The different compounds which were identified by GC-MS may be responsible for the activity of *H. umbellata* as antimicrobial agent against gram-negative and gram-positive bacteria.

The search for new antimicrobial drugs from plant materials is essential to curb the menace of multiple antibiotics resistant pathogens since plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines [32]. Antibiotics are naturally occurring or synthetic organic compounds which inhibit or destroy selective bacteria, generally at low concentrations [33]. Boyejo et al. [34] study shows that for standard conversion,

as little as 30µg of antibiotics is equivalent to 10 mg of plant extract, the *H. umbellata* methanolic seed extract is used in this study is in the crude form.

Escherichia coli, Staphylococcus aureus and *Streptococcus sp* were selected and the antibiotics profile showed that they could be considered as multidrug resistant (MDR) bacteria based on the definition that MDR bacteria are resistant to three or more antibacterial classes [35]. The zones of inhibition for the methanolic seed extract of *H. umbellata* were 18.36±0.87 mm and 21.70±1.60mm for *E. coli* at 300 and 500 mg/ml respectively, 19.13±1.03 and 23.83±2.64 mm for *S, aureus* at 300 and 500 mg/ml and 21.62±2.53 and 28.57± 1.52 for *Streptococcus sp* at 300 and 500 mg/ml respectively. (table 3). The seed extract exhibited strong potency against the micro-organisms used with *Streptococcus sp* being the most susceptible. The result of this analysis is similar to the report of Ajayi and Ojelere, [36] study that shows that *H. umbellata* has antimicrobial activity against *S. aureus* and *E. coli* with a zone of inhibition of 18 mm and 19 mm at concentration of 200 mg/ml respectively. Boyejo et al [34] study shows that methanolic and ethanolic extract of *H. umbellata* is sensitive to *Escherichia coli* (ATCC 29929). The ethanolic extract of the plant was sensitive to *Salmonella typhi* (ATCC 14028) while both extracts are resistant to *Staphilococcus aureus* (ATCC 29293), *Shigella dysentariae* (ATCC 23354), *Pseudomonas aeruginosae* (ATCC 27953) and *Klebsiella Pneumoniae* (ATCC 4252) respectively. Anibijuwon et al. [37] study shows that ethanol and methanol seed extracts of *H. umbellata* have a zone of inhibition of 30 and 17 mm respectively against *Streptococcus* sp. Akinrotoye et al. [28] study shows that the agar diffusion assay of *H. umbellate* extract-Azithromycin combination had the least zones of inhibition ≥21.00±1.92 mm in 75% of all isolates tested.

The line plot in Figure 4a shows that *in-vitro* antimicrobial activity of *H. umbellate* is most active on Streptococcus sp, followed by *S. aureus* than *E. coli* on the average with concentration of 300 mg/ml and 500 mg/ml respectively. Figure 4b shows that *in-vitro* antimicrobial activity of *H. umbellata* is more active on *E. coli* at 500 mg/ml concentration compared to 300 mg/ml. Figure 4c shows that the *in-vitro* antimicrobial activity of *H. umbellata* is more active on *S. aureus* at 500 mg/ml concentration compared to 300 mg/ml. Figure 4d shows that *in-vitro* antimicrobial activity of *H. umbellata* is more active on *Streptococcus sp* at 500 mg/ml concentration compared to 300 mg/ml. The histograms in Figure 5 shows that the mode of *E. coli* for 300 mg/ml concentration lies between 18.0-18.5 and that of 500 mg/ml lies between 21.0-

22.0; the mode of *S. aureus* for 300 mg/ml concentration lies between 19.0-19.5 and that of 500 mg/ml lies between 23.0-24.0; while the mode of *Streptococcus sp* for 300 mg/ml concentration lies between 20.0-21.0 and that of 500 mg/ml lies between 28.0-29.0. The t-test shows that there is a significant difference between the effects of in-vitro antimicrobial activity of *H. umbellate* on 300 mg/ml and 500 mg/ml on each bacteria tested at 5% level of significance. The study shows that 500 mg/ml concentration is significantly ($P < 0.001$) different from the 300 mg/ml concentration. This difference is most in *Streptococcus sp* than in *E. coli.* The difference is least in *S. aureus*.

The study shows that *Staphylococcus aureus* was susceptible to amoxacillin (AM), streptomycin (S), pefloxacin (PEF), septrin (SXT), ciprofloxacin (CPX), and imipenem (IMI) since they have a zone of inhibition greater than 17 mm. The organism also shows high resistance to tetracycline (TET), penicillin G (PG), gentamicin (CN), erythromycin (E), ampiclox (APX) and metronidazole (MZ). *Escherichia coli* was susceptible to tetracycline (TET), pefloxacin (PEF), gentamicin (CN), imipenem (IMI) streptomycin (S) and ciprofloxacin (CPX) since they have a zone of inhibition greater than 17 mm. *E. coli* shows resistance to ampiclox (APX), metronidazole (MZ), amoxacillin (AM), septrin (SXT), penicillin G (PG) and erythromycin (E). On the other hand, *Streptococcus sp* was susceptible to amoxacillin (AM), streptomycin (S), ampiclox (APX), pefloxacin (PEF), metronidazole (MZ), penicillin G (PG), ciprofloxacin (CPX), and imipenem (IMI) and resistance to septrin (SXT), gentamicin (CN), tetracycline (TET) and erythromycin (E).

The antibacterial activity of *H. umbellata* against *E. coli*, *S. aureus and Streptococcus sp* pathogens were investigated for their MIC and MBC values. MIC or MBC is the lowest concentration of an antimicrobial agent necessary to inhibit bacterial growth or kill bacteria, respectively. MIC is important in the laboratory to confirm the resistance of microorganisms to an antimicrobial agent and also used to monitor the activity of new antimicrobial agents [21]. The methanolic seed extract of *H. umbellta* has an MIC of 250 125 and 31.25 mg/ml for *E. coli, S. aureus* and *Streptococcus sp* respectively. The seed extract has an MBC of 500, 250 and 125 mg/ml for *E. coli, S. aureus* and *Streptococcus sp* respectively (Table 8). The result of our study showed that gram-negative bacteria (*E. coli*) was less susceptible to the extract when compared to the gram-positive bacteria (*S.aureus* and *Streptococcus sp*). Studies have shown that gram-negative bacteria are more resistant to regular antibiotics especially some nosocomial strains

such as *Acinetobacter baumannii, P. aeruginosa* and *Klebsiella pneumonia* because of their peptidoglycan layer [38, 39]. Ching et al. [27] study shows that methanol extract of *H. umbellata* has an MIC value of 150 mg/ml against *S. aureus* and *E. coli* respectively. Anibijuwon et al. [37] study shows that ethanol seed extract of *H. umbellata* has MIC and MBC values of 20 and 40 mg/ml respectively against *Streptococcus* sp.

Study has shown that calculated MBC/MIC ratio is deemed as bactericidal if the values of MBC/MIC ratio are less than or equal to 4 and deemed as bacteriostatic if the MBC/MIC ratio is greater than 4 [40]. Although *Streptococcus sp* showed bactericidal effects (MBC/MIC value of 4), *S. aureus* and *E. coli* had more noticeable bactericidal activity (MBC/MIC value of 2). *Hunteria umbellata* showed remarkable bactericidal effects on the three organisms tested.

CONCLUSION

Hunteria umbellata have potential as natural therapeutic agents against *E. coli*, *S. aureus* and *Streptococcus sp* and they could prevent pathogenic diseases.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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