Phytochemical Screening, Mathematical Analysis and Antimicrobial Activity of Methanolic Seed Extract of Hunteria Umbellata

Oluwaseun Raphael Aderelle1*, Adekunle Kareem Rasaq1 and Johnson Oshiobugie Momoh2

1Department of Mathematics/Statistics, School of Pure and Applied Sciences, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria.
2Department of Chemical Sciences (Biochemistry Unit), School of Pure and Applied Sciences, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria.

Authors’ contributions

This work was carried out among all authors. Authors ORA and JOM designed the study, wrote literature review, discussion and prepared the manuscript. Authors ORA and AKR carried out the mathematical and statistical analysis. Author JOM carried out the methodology in the laboratory. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i1630325

Editor(s):
(1) Dr. Naseem A. Qureshi, National Center of Complementary and Alternative Medicine, Saudi Arabia.
(2) Marcello Iriti, University of Milan, Italy.

Reviewers:
(1) Rejane Magalhães de Mendonça Pimentel, Universidade Federal Rural de Pernambuco, Brasil.
(2) Pawan Kaushik, Kurukshetra University, India.

Complete Peer review History: http://www.sciencedirect.com/science/article/pii/S108941642030325X

Original Research Article

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. umbellata 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 mg/ml 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 mg/ml. The results of the analysis show that there is a significant difference between the effects of in-vitro antimicrobial activity of H. umbellata on 3001 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 E .coli, 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 were 250, 125 and 31.25 mg/ml while their minimum bactericidal concentration (MBC) were 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.

Keywords: Phytochemicals; GC-MS; Hunteria umbellata seed extract; Escherichia coli; Staphylococcus aureus; Streptococcus sp.; MIC and MBC.

1. 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 mechanism 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].

The organism 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 developing 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 grove [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*).

2. MATERIALS AND METHODS

2.1 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.

2.2 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.

2.3 Phytochemical Analysis of *Hunteria umbellata* Methanolic Seed Extract Solution

2.3.1 Determination of saponins

2.3.1.1 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.

2.3.2 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% FeCl₃ solution were added. The formation of a green precipitate indicated the presence of tannins.

2.3.3 Determination of alkaloids

2.3.3.1 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.

2.3.3.2 Dragendorff’s test

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

2.3.4 Determination of flavonoids

2.3.4.1 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.

2.3.4.2 Determination of steroids

Two ml of *H. umbellata* seed extract solution was mixed with 2 ml of chloroform and 2 ml of concentrated H₂SO₄. The formation of red colour indicates the presence of steroids.

2.3.5 Determination of carbohydrates and reducing sugars

2.3.5.1 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.
2.3.6 Determination of cardiac glycosides

2.3.6.1 Liebermann’s test

Two ml of the seed extract was extracted with 2 ml of chloroform and 2 ml of acetic acid and the solution was cooled on ice. H₂SO₄ 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.

2.3.6.2 Determination of anthraquinone

0.5 ml of the *H. umbellata* extract was boiled in 10% HCl for 5 mins, the filtrate were allowed to cool. An equal volume of CHCl₃ with few drops of 10% NH₃ was added to 2ml of the filtrate. The formation of rose-pink colour indicates the presence of anthraquinones.

2.3.6.3 Determination of simple phenolics

One ml of *H. umbellata* seed extract solution was mixed with 1–2 drops of 1% FeCl₃. The development of blue-green colouration indicates the presence of phenol.

2.3.7 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]

2.3.8 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.

2.3.9 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 Sciences (Biochemistry Unit), School of Pure and Applied Science, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria and fresh subcultures were made before use.

2.3.10 Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml of peptone water, incubated at 37°C for 4 hrs. 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 a 1.75% (w/v) barium chloride dehydrate (BaCl₂ 2H₂O) with 99.5 ml of 1% (v/v) tetraoxosulphate (vi) acid (H₂SO₄). This turbidity was equivalent to approximately 1 x 10⁸ colony forming units per ml (CFU/ml).

2.3.11 Determination of diameter of zone of inhibition 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 1 x 10⁸ 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 umbellata* seed extract in the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37°C for 18-24 hrs 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.

2.3.12 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.

2.3.13 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. umbellata* 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 normal saline instead of *H. umbellata* seed extract. The lowest concentration of the dilution without bacterial growth was considered as MIC.

2.3.14 Minimum Bactericidal Concentration (MBC)

The MBC of the *H. umbellata* 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 hrs. 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.

2.3.15 Mathematical analysis

*in-vitro* antimicrobial activity of *H. umbellata* against *E. coli*, *S. aureus* and *Streptococcus sp* were determined 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. umbellata* on the selected bacteria.

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   | (+)       |
| Anthraquinone             | Mayer's test         | (+)       |
| Steroids and sterol       | Borntreger's test    | (+)       |
| Phenolic compounds        | Ferric chloride test | (+)       |
| Cardiac glycoside         | Liebermanns test     | (+)       |

(+) present
2.4 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.

2.4.1 Phytochemical screening of methanolic seed extract of *Hunteria umbellata*

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

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

| PK no | Retention Time | Name of the compound | Molecular Formula | Molecular Weight | Peak Area (%) |
|-------|----------------|----------------------|------------------|------------------|---------------|
| 1     | 16.270         | 2-Propenamide        | C₃H₅NO           | 71.08            | 1.83          |
| 2     | 18.605         | Acetonitrile, 2,2'-iminobis- | C₄H₇N₂       | 95.10            | 3.80          |
| 3     | 18.719         | 4-Cyclohepten-1-amine | C₇H₁₃N          | 111.18           | 83.13         |
| 4     | 18.782         | 5-Azabicyclo[2.2.0]hex-2-en-6-one | C₇H₁₀NO       | 95.10            | 2.81          |
| 5     | 18.903         | 2-Furanmethanamine   | C₅H₆NO           | 97.12            | 1.83          |
| 6     | 20.596         | 2-Butanamine, (S)-   | C₅H₁₀N           | 73.14            | 1.21          |
| 7     | 20.619         | Acetamide, N-2-propynyl- | C₇H₁₃N         | 97.12            | 2.15          |
| 8     | 27.857         | 2-Hydroxyskatole     | C₉H₉NO           | 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%, RT20.619)

Fig. 2h. Mass spectrum of 2-Hydroxyskatole structure (3.43%, RT27.857)

Fig. 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. umbellata* methanolic seed extract against *E. coli*

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

Zone of inhibition at 500 mg/ml concentration of the *H. umbellata* methanolic seed extract against *S. aureus*

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

Zone of inhibition at 500 mg/ml concentration of the *H. umbellata* methanolic seed extract against *Streptococcus sp.*

**Fig. 3.** Zone of inhibition of *H. umbellata* 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 umbellata* 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 |
Fig. 4. Comparing the zone of inhibition of *Escherichia coli*, *Staphylococcus 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.

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 ≥ 18mm)  
R = Resistant (zone diameter of bacterial inhibition < 18mm).
Fig. 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 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              |
| Amoxicillin (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              |
| Septin (SXT)              | 30                | 18.35 ± 3.2                        | S              |
| Erythromycin (E)          | 10                | 11.9 ± 1.6                         | R              |

Key:  
S = Sensitive (zone diameter of bacterial inhibition ≥ 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 ± 1.2                        | S              |
| Amoxicillin (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              |
| Septin (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 umbellata* 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    |

#### 3. 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 like: 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-Hydroxyxskatole (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 microorganisms 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 Staphylococcus aureus (ATCC 29293), Shigella dysenteriae (ATCC 23354), Pseudomonas aeruginosa (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. Akintoye et al. [28] study shows that the agar diffusion assay of H. umbellata extract-Azithromycin combination had the least zones of inhibition ≥21.00±1.92 mm in 75% of all isolates tested.

The line plot in Fig. 4a shows that in-vitro antimicrobial activity of H. umbellata is most active on Streptococcus sp, followed by S. aureus than E. coli on the average with concentration of 300 and 500 mg/ml respectively. Fig. 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. Fig. 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. Fig. 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 Fig. 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. umbellata at 300 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 amoxicillin (AM), streptomycin (S), pefloxacin (PEF), metronidazole (MZ), 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) 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. umbelita 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.

4. CONCLUSION

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

ACKNOWLEDGEMENT

This research work was financially supported by Tertiary Education Trust Fund (TETFUND) from Nigeria. The authors are grateful to the Management Staff of Lagos State Polytechnic Ikorodu, Lagos, Nigeria for their support.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-82.
2. Purkayastha S, Dahiya P. Phytochemical analysis and antibacterial efficacy of babchi oil (Psoralea corylifolia) against multi-drug resistant clinical isolates. Int Conf Biosci Biochem Bioinf. 2012;31: 64-68.
3. Amrani A, Mecheri A, Bensouici C, Boubekri N, Benaiissa O, Zama D, Benayache F, Benayache S. Evaluation of anti-diabetic, dermato-protective, neuroprotective and antioxidant activities of *Chrysanthemum fontanesii* flowers and leaves extracts. Biocalysis and agricultural biotechnology. 2019;20:101-209.
4. Ahmed AF, Altitia FAK, Liu Z, Li C, Wei J, Kang W. Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum*) plants. Food Science and Human Wellness. 2019;8(3):299-305.
5. Tuama AA, Mohammed AA. Phytochemical screening and in vitro antibacterial and anticancer activities of the aqueous extract of *Cucumis sativus*. Saudi Journal of Biological Sciences. 2019;26(3):600-604.
6. Wang YZ, Fu SG, Wang SY, Yang DJ, Wu Y, Chen YC. Effects of a natural antioxidant, polyphenol-rich rosemary (*Rosmarinus officinalis* L.) extract, on lipid stability of plant derived omega-3 fatty-acid rich oil. LWT – Food Science and Technology. 2018;89:210-216.
7. Cai M, Lv H, Cao C, Zhang L, Cao R, Xu B. Evaluation of antimicrobial activity of *Pterocarpus* Industrial Crops and Products. 2019;140:111668.
8. Olaokun OO, Alaba AE, Ligege K, Mkolo NM. Phytochemical content, antidiabetes, anti-inflammatory antioxidant and cytotoxic activity of leaf extracts of *Elephantorrhiza elephantina* (Burch.) Skeels. South African Journal of Botany. 2020; 128: 319-325.
9. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. Nat Rev Microbiol. 2004; 2:123–140.
10. Glode MP, Sutton A, Robbins JB, McCracken GH, Gotschlich EC, Kajiser B, Hanson LA. Neonatal meningitis due of *Escherichia coli* K1. J. Infect. Dis. 1977; 136:S93–S97. [PubMed].
11. Chambers HF. Community associated MRSA-resistance and virulence converge. New Engl. J. Med. 2005;352:1485-1487.
12. SW Hung, SL Wang, CY Tu et al. Antibiotic susceptibility and prevalence of erythromycin ribosomal methylase gene, erm(B) in *Streptococcus spp*, “*De Veterinary Journal*, vol. 176, no. 2, 197–204, 2008.
13. Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases...
using nanomaterials in the antibiotics resistant era. J Control Release. 2011;156(2):128-45.

14. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18(3): 268-281.

15. World Health Organization. WHO monographs on selected medicinal plants. Geneva: World Health Organization; 1999. p. 183-94 [Online] Available from: Available:http://apps.who.int/mediaindex/docs/pdf/s2200e/s2200e.pdf [Accessed on 18th July, 2016]

16. Adeneye AA, Adeyemi OO. Hypoglycaemic effects of the aqueous seed extract of Hunteria umbellata in normoglycaemic and glucoseand nicotine-induced hyperglycaemic rats. International Journal of Applied Research in Natural Products. 2009; 2(1):9-18.

17. Oluwemimo A, Usifoh CO. The antihelminthic activity of Hunteria umbellata K. Schum. (Fam. Apocynaceae) extracts. Pak J Pharm Sci Ind Res, 2001; 44: 286-290.

18. Longe AO, Momoh J, Adepoju PA and Akoro SM. Hyperglycemic Effects of the Methanolic Seed Extract of Hunteria umbellata (Abeer e ) and Its Effect on Liver, Hematological and Oxidative Stress Parameters in Alloxan-Induced Diabetic Male Albino Rats Int. J. Curr. Res. Biosci. Plant Biol. 2015, 2(6): 27-34. ISSN: 2349-8080.

19. Raman A, Mallam V. Enhanced in vitro activity of glucokinase enzyme in the presence of Hunteria umbellata seeds, a traditional Nigerian treatment for diabetes. J Pharm Pharmacol, 1994; 46: 1046.

20. Momoh JO, Damazio OA, Ajetunmobi AO, Babalola AO, Adekunle OM, Busari NO, Musa AA. Phytochemical analysis and antiplasmodial (curative) activities of methanolic leaf extract of Morinda lucida (Ewe Orunu) in male Swiss mice infected with Plasmodium berghei NK65. IJTDH. 2019;37(1):1-13. Article no.IJTDH.47956. DOI: 10.9734/IJTDH/2019/v37i130156.

21. Momoh J, Oluremi NO, Odetunde SK Antimicrobial and Antioxidant Properties of Aqueous Garlic (Allium sativum) Extract against Staphylococcus aureus and Pseudomonas aeruginosa. British Microbiology Research Journal. 2016;14(1):1-11 Article no.BMRJ.24095 ISSN: 2231-0886, NLM ID: 101608140.

22. National Committee for Clinical Laboratory Standards (NCCLS). Performance standard for antimicrobial susceptibilitytesting, 10th information supplement approved standard. 2000;M100-S10. Wayne P.A.

23. Cheesbrough M. District Laboratory practice in Tropical Countries Part 2: Cambridge University Press. UK. 2002; 136-142.

24. Famuyide IM, Aro AO, Fasina FO, Elloff JN, McGaw LJ. Antibacterial activity and mode of action of acetone crude leaf extracts of underinvestigated Syzygium and Eugenia (Myrtaceae) species on multidrug resistant porcine diarrhoeagenic Escherichia coli. BMC Vete Res. 2019; 15:162.

25. Chung PY, Navaratnam, Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against Staphylococcus aureus strains. Ann Clin Microbiol Antimicrob. 2011; 10(25).

26. Spencer ALR, Spencer JFT. Public health microbiology: Methods and protocols. Human Press Inc. New Jersey. 2004;325-327.

27. Joseph GC, Ching FP, Fidelia O. Evaluation of the antibacterial activity and preliminary phytochemical analysis of the stem, bark and leaves extract of Hunteria umbellata k SCHUM, Apocynaceae. IRJP 2011, 2(9): 85-87.

28. Akinrotoyo KP, Akinduti P, Lanlokun O, Adetogun C. Synergistic Evaluation of Moringa oleifera, Hunteria umbellata and Azadirachta indica with Antibiotics against Environmental MRSA Isolates: An In-vitro Study, American Journal of BioScience. 2020;8(4)91-98. doi: 10.11648/j.ajbio.20200804.11.

29. Abubakar AN, Saidu AN, Akanya HO, Egwim EC. Antioxidants and hypoglycemic effect of some medicinal plants. GSC Biological and Pharmaceutical Sciences, 2019, 08(02), 070–080. DOI: https://doi.org/10.30574/gscbps.2019.8.2.01 24

30. Igile GO, Oleszek W, Jurzysta M, Burda S, Fafunso M, Fasanmade AA. Flavonoids from Vernonia amygdali and their antioxidant activities. Journal of Agricultural and Food Chemistry. 1994;42(11):2445-2448.

31. Udensl E, IJeh I, Ogbonna U. Effect of traditional processing on the phytochemical
and nutrient composition of some local Nigerian leafy vegetables. Journal of Science and Technology. 2002;8:37–40.

32. Nair R, Kalariya T, Chanda S. Antibacterial Activity of Some Selected Indian Medicinal Flora. Turkish Journal of Biology. 2005; 29: 41-47.

33. Brooks GF, Carroll KC, Butel JS, Morse SA. The growth and death of microorganism. In Jawetz, Melnick and Adelberg's Medical Microbiology 24th ed. 2007; McGraw-Hill Toronto, 57p.

34. Issah A.O, Azeez I.A, Boyejo A.O, Owolabi S.L, Buhari O.A, Ikeola M.F. Antibacterial Activities of Some Commonly Used Medicinal Plants against Bacteria Isolates. American Journal of Medical and Biological Research, 2020, Vol. 8, No. 1, 1-11. DOI:10.12691/ajmbr-8-1-1.

35. Wang M, Wei H, Zhao Y, Shang L, Di L, Lyu C. Analysis of multidrug resistant bacteria in 3 223 patients with hospital-acquired infections (HAI) from a tertiary general hospital in China. Bosn J Basic Med Sci. 2019; 19(1): 86-93.

36. Ibironke AA, Olusola O. Evaluation of the Antimicrobial Properties of the Ethanolic Extracts of some Medicinal Plant Seeds from South-West Nigeria. (IOSR-JPBS). e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 9, Issue 4 Ver. I (Jul-Aug. 2014), PP 80-85

37. Anibijuwon I.I, Abioge YA. and Onifade AK. Comparative antimicrobial activities of some plant extracts and commercial antibiotics against some selected pathogens of food origin, Int. J. Med. Med. Sci. 2011; 3(8):268-272. ISSN 2006-9723 ©2011 Academic Journals.

38. Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner C, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis. 2006; 6(9): 589-601.

39. Abdallah EM, Ahamed F, Al-Omari AS. Antibiotic susceptibility patterns of some clinical isolates from Al-Rass general hospital. Int J Biosci. 2015;6(9):47-54.

40. Djesssi DE, Noumedem JA, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, et al. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complement Altern Med. 2013;13:164.

© 2020 Aderele et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/61248