African Journal of Pharmaceutical Research & Development

Vol. 11 No.2; pp. 106-115 (2019)

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACT OF THE LEAVES OF *Hippocratea Welwitschii* OLIV. (CELASTRACEAE)

ONYEKERE PECULIAR FEENNA*[1], ENEBECHI CHARLES KENECHUKWU[1], NNAMANI DANIEL OKWUDILI[1], PECULIAR-ONYEKERE CHIOMA OBIANUJU[2] AND OKONTA ELEJE OBOMA[1]

1. Department of Pharmacognosy & Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. 410001. Enugu State, Nigeria.
2. Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. 410001. Enugu State, Nigeria.

ABSTRACT

The objectives of this study were to investigate the phytochemical constituents, determine the spectrum of activity and evaluate the Minimum Inhibitory Concentrations (MIC) against susceptible microorganisms of the leaf extract of *Hippocratea welwitschii* Oliv. The methanol extract of leaves was obtained by maceration using absolute methanol and concentrated with a rotary evaporator. The phytochemical analysis was carried out using standard procedures. The sensitivity of the methanol extract of *Hippocrateae welwitschii* to selected micro-organisms was determined using the agar well diffusion method. The inhibition zones diameters (IZDs) were measured and recorded. The arithmetic square of the IZDs values in millimeter (mm) were plotted against the logarithmic doses to get the MIC. The phytochemical screening showed that it contained alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids. Antimicrobial tests carried out showed that the crude methanol extract was active against a broad spectrum of microorganisms including gram-positive – *Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus subtilis* and gram-negative – *Escherichia coli* and *Salmonella typhi*, but not *Klebsiella pneumonia*. It also showed antifungal activities against *Candida albicans* and *Aspergillus niger*. The MIC obtained were 14.68, 50.13, 9.92, 10.61, 16.68, 26.73 and 10.8 mg/ml for *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Eshcherichia coli, Salmonella typhi, Candida albicans* and *Aspergillus niger* respectively. The zones of inhibition were significantly different ($p < 0.05$) at 100 mg/ml of the plant extracts when compared to the standard positive controls to the selected tested organisms. The antimicrobial and phytochemical characteristics of the plant support the folkloric use of the plant in the management of infectious diseases.

KEYWORDS: **Phytochemical screening; Antimicrobial; Extraction; Microorganisms.**

INTRODUCTION

Plants are very important sources of drugs that have been used for centuries in the treatment of various microbial infections. Studies have shown that about 33 % of the drugs produced in developed countries are obtained from plant sources and over 60 % of conventional medicines in the global market have been derived either directly or indirectly from natural products, including herbs [1]. About 80 percent of the Africa population depends on traditional remedies as they do not have access to orthodox medicine. Traditional medicines have not only been the bedrock from which newer medicinal agents are launched but also a therapeutic resort for hard-to-cure illnesses. This is the reason why developing countries including Nigeria still rely on them to resolve her health needs, especially in cases where synthetic medicines cannot provide relief from hard-to-cure illnesses [2]. Most of the plant-based drugs when used correctly, pose less or no toxic effect to

***Corresponding author:** peculiar.onyekere@unn.edu.ng, peculiar.onyekere@daad-alumni.de
+2348068068346

the recipients compared to the chemically synthetic drugs [3].

There has been a contest between man and microorganisms from time immemorial. Microbiologists claim that microorganisms have existed on earth for about 3.5 billion years, prior to the evolution of plants and animals from microscopic life forms [4-5]. Antimicrobial agents are agents that kill or inhibit the growth and proliferation of microorganisms. Antimicrobial agents have been used for over 40 years [6]. Issues hinging on antibiotic resistance have constituted a great concern for many researchers; hence the search for potent agents from medicinal plants [7].

Phytochemical constituents represent important sources of biological active products of plants origin from where many herbal products used by human beings were developed over the years [8]. Several plant species have been claimed to possess medicinal properties and employed in the treatment of many ailments. These activities can be traced to the presence of these pharmacologically active constituents.

The plant *Hippocratea welwitchii* Oliv belongs to the family of the Celastraceae (also known as staff vine or bittersweet family) [9]. The family has over 90 genera and 1,300 species of vines, shrubs and small trees. It is important to note that majority of the genera are tropical with the celastrus (the staff vine), Euoymus (the spindles) and maytenus distributed widely in the temperate climates. Common names of some of its genera include canotia (Crucifixion thorn), catcha, celastrus, hippocratea etc [10]. Most of the representatives of this family are shrubs and some as in Hippocratea are like climbers by their branchlets, twisting round their supports. *Hippocratea welwitschii* is a staff vine of the bittersweet family of shrubs native to Liberia, Ghana and Nigeria. The plant is widespread across Africa to Angola, Uganda and Tanganyika (Tanzania), and also a shrub of closed, primary or mature secondary forest or in thickets of secondary shrub from Guinea to western Cameroon [11]. It is an extensive woody climber with glabrous leaves which are broadly elliptical and non-prominent reticulation.

Hippocratea welwitschii is known by different names in different parts of Africa. It is called *'obulumgbede'* by the Igbo tribe of south-eastern Nigeria, '*ijan'* in Yoruba, '*Nya woro urua mbombo'* in Efik, or *'Nya woro urua mbombi'* in Ibibio both of which literally means 'coming out of the fattening room into the market [10, 12]. In Ghana, the plant is called '*Adangme akladefi*' and '*mano gie gbini*' in Liberia [12]. It has been reported to be used in the treatment of ailments like broken bones and anorexia [13]. Also, in Cross-River, south-south Nigeria, the root of the plant is used effectively to manage epilepsy [12]. In Ivory Coast, part of the plant is used to ease labour and delivery at childbirth [11]. Despite the vast local use of *Hippocratea welwitchii,* there is dearth of information on its' effect on antimicrobial activities. To the best of our knowledge there is no previously reported antimicrobial activity of the methanol extract of leaves of *H. welwitschii.* In this study, we report the antimicrobial effect of the methanol extract of the leaves of *H. welwitchii* as novel bioactive antimicrobial compounds.

MATERIALS AND METHOD

Chemicals and Reagents

All materials to be used for this study were of analytical grade. Drugs used for the in-vitro phytochemical and antimicrobial analysis. Chloramphenicol, Gentamycin and Fluconazole, and Antimicrobial discs for comparative *in vitro* studies of anti-microbial activities of the extract were obtained through the Chief pharmacist, Bishop Shanahan Hospital, Nsukka. Methanol (Merck KGaA, Darmstadt, Germany), reagents for antimicrobial studies including MacConkey agar, Mueller Hinton agar, Nutrient agar, Sabouraud Detrose Agar (SDA), Salmonella Shigella Agar (SSA), Peptone water; C.L.E.D, all were also obtained from Lavans Scientific Ltd, Nsukka

Others are absolute methanol, Ferric chloride, iodide solution, ethanol (Merck KGaA, Darmstadt, Germany), dilute ammonia solution, sulphuric acid, naphthol solution in ethanol, potassium mercuric solution (Mayer's reagent), bismuth potassium iodide solution (Dragendorff's reagent), Fehling's solution, Million's reagent, picric acid solution.

Test Microorganisms

Test microorganisms used for the study include: *Escherichia coli, Pseudomonas aeruginosa*, *Salmonella typhi, Staphlococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Candida albicans* and *Aspergillus niger.* All the microbial strains used in this study were pure culture obtained as clinical isolates from the medical laboratory unit of Bishop Shanahan Hospital, Nsukka.

Plant collection and Authentication

Hippocratea welwitschii leaves were collected from the tropical rain forest of Ogrute, Enugu-Ezike in Igbo Eze North Local Government Area of Enugu State.

The plant was authenticated by the plant taxonomist, Mr Nwafor Felix, Department of Pharmacognosy and Environmental medicine, University of Nigeria, Nsukka on 16th July, 2018 with the voucher number: **PCG/UNN/0305** and deposited in the herbarium of Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Plant Preparation and Extraction

The plant was dried under ambient temperature of (25 °C). The dried plant sample was milled and extracted using solvent methanol. Powdered root sample (500 g) of *Hippocratea welwitschii* was macerated with absolute methanol for seventy two (72) hours and extracted using Soxhlet extractor. The mixture containing the extracting solvent and the menstruum was shaken vigorously eight hourly until the extraction process was completed. The mixture was then double filtered with Whatman pre-plated quality filter paper, grade 4V circle of diameter 18.8 mm, thickness 205 µm, and pore size (25 µm). The extract was then filtered and evaporated *in-vacuo* using a rotary evaporator to give the crude extract (49.2 g). Percentage yield of the extract was calculated thus:

$$\text{Yield (\%)} = \frac{crude\ extract\ weight}{initial\ dry\ weight} \times 100 \quad \text{...Equation 1}$$

Antimicrobial Evaluation

The methods of disc diffusion method [14] as described by [5] were used for anti-microbial analysis. The spectrum of antimicrobial activity was determined. Then the minimal inhibitory concentrations (MIC) of the extract were respectively determined.

Sterilization of Materials

In-process sterilization of forceps and inoculating loop were done by dipping into 70% ethanol solution, followed by flaming in the Bunsen burner flame until the material became red hot. The forceps were allowed to cool before reuse. Media sterilization after preparation was obtained by heating in an autoclave up to 121°C for 15 minutes.

Preparation and Inoculation of culture media

A 38 g of Mueller Hinton dehydrated media was suspended in 1000 ml of purified filtered water. The mixture was heated with frequent agitation for one minute. It was then sterilized at 121 °C for 15 minutes, and then cooled to 45-50 °C. The media was formally mixed gently and dispensed into sterile Petri dishes.

Prior to inoculation, Mueller Hinton broth was used to grow 4 – 5 similar colonies and the turbidity was adjusted to 0.5 McFarland standards. The culture plates were inoculated by swabbing the inoculums on the surface three times. Antimicrobial susceptibility discs were placed gently on the surface, and incubated at 35°C for 24 hours.

Microbial culture, Isolation and characterization

The spread plate method was used in culturing the different microorganisms. Each of the samples was diluted serially using different concentrations (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}). Then 0.5 ml of the different concentrations were plated out on Sabouraud Dextrose Agar (SDA) medium supplemented with chloramphenicol to inhibit bacterial growth under strict aseptic conditions. A glass spreader was used to evenly spread the plate. Each plate was incubated at room temperature (28 °C) for 24 - 48 hours. After incubation, the plates were examined for yeast growth. The colonies of yeast that appeared in the culture were sub-cultured on plates of SDA supplemented with chloramphenicol to get pure culture. The representative colonies were picked out of the plate with the help of a wire loop and sub-cultured in SDA slant using a bijou bottle and were incubated at room temperature for 48 hours. The slant cultures were then stored in the refrigerator for further tests. Standard procedures from Barges manual of determinative bacteriology were used for microbial characterization.

Five (5) bacterial strains *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae, and Bacillus subtilis*) and two fungal strains (*Aspergillus niger* and *Candida albicans* were used in this study. These test microorganisms used were clinical isolates obtained from Bishop Shanahan Hospital, Nsukka medical laboratory unit, which were further purified and characterized in the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Preliminary antimicrobial screening (Sensitivity Testing)

The sensitivity of the methanol extract of *Hippocrateae welwitschii* to selected micro-organisms was determined using the agar well diffusion method [14]. The Twenty (20) ml of molten Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (for bacterial and fungal isolates

respectively) were poured into sterile Petri dishes (90 mm) and allowed to set. The size of the cork borer (6mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in duplicate and the mean IZDs calculated and recorded. Standardized inoculums (1-2 x 10^7 CFU/ml 0.5 McFarland standards) were each introduced onto the surfaces of sterile agar plates using a sterile glass spreader. Different concentrations (100, 50, 25, and 12.5 mg/ml) Of the extract were introduced into the hole. 30 µg/ml of Ciprofloxacin and Fluconazole were respectively used as controls for bacteria and fungi. The plates were then incubated at 37°C for 24 hours, and the SDA plates were incubated at room temperature (25 - 27 °C) for 2 - 3 days. The zones of inhibition were measured and recorded.

Determination of Minimum Inhibitory Concentrations (MIC)

The inhibition zones diameters (IZDs) were measured and recorded. This procedure was conducted in duplicate and the mean IZDs calculated and recorded. The arithmetic square of the IZDs values in millimeter (mm) were plotted against the logarithmic doses to get the MIC [15].

Phytochemical Analysis

The phytochemical analyses of the methanol extract of *Hippocratea welwitschii* were carried out according to the methods of [16] to identify the active phytochemical constituents.

Test for Tannins

A 1g of the powdered material was boiled with 20 ml of water, filtered and used for the following tests.
Ferric chloride test: 3 ml of the filtrate were added a few drops of ferric chloride. A greenish-black precipitate indicates the presence of tannins.

Test for Saponin

A 20 ml of distilled water was added to 2 g of the extract and boiled on a hot water bath for 2 minutes. The mixture was filtered while hot and allowed to cool and the filtrate was used for the following tests:
Frothing test: 5 ml of the filtrate was diluted with 15 ml of distilled water and shaken vigorously. A stable froth (foam) upon standing after two minutes indicates the presence of saponins.
Emulsion test: To the frothing solution was added 2 drops of olive oil and the contents shaken vigorously. The presence of an emulsion confirms that saponins are present.

Test for Flavonoids

A 10 ml of ethyl acetate was added to 0.2 g of the powder and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate was used for the following tests.
Ammonium hydroxide test: 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and yellow colour in the ammoniacal layer confirms that flavonoids are present.
1 % Aluminum chloride solution test: Another 4 ml portion of the filtrate was shaken with 1ml of 1 % aluminum chloride solution. The layers were allowed to separate and yellow colour in the aluminum chloride layer indicates the presence of flavonoids.

Test for Glycosides (Combined reducing sugars)

A 5 mL of dilute sulphuric acid was added to 0.1 g of the powder in a test tube, boiled for 15 minutes on a water bath, and then neutralized with 20 % potassium hydroxide solution. 10 mL of a mixture of equal parts of Fehling solution I and II was added and heated for 5 minutes. A denser brick-red precipitate indicates the presence of glycosides.

Test for Steroids

Five mL of acetic anhydride was added to 0.5 g of the powdered sample with 2 mL H_2S0_4. A colour change from violet to blue indicates that steroids are present.

Test for Terpenoids (Salkowski test)

Five mL of the crude extract was carefully mixed in 2 mL of chloroform after which concentrated H_2S0_4 (3 mL) was gradually added. The formation of a reddish-brown colour at the interface indicates the presence of terpenoids.

Test for Alkaloids

A 0.5g of the powdered extracts was stirred in 5 mL of 1% HCl on a steam bath for 5 minutes. The mixture was then filtered using Whatman's no1 filter paper. To the filtrate, two drops of Dragendoff's reagent were added to 1 ml of the filtrate. An orange-red colour was observed indicating the presence of alkaloids.

Test for Carbohydrates (Molisch's Test)

A 0.1g of the sample was boiled with 2 ml of water and filtered. To the filtrate, few drops of naphtol solution in ethanol (Molisch's reagent) were added. Concentrated sulphuric acid was gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicates the presence of carbohydrates.

Statistical Analysis

The experimental data are expressed as mean ± standard error of the mean (SEM). Data were analyzed using the Statistical Package for Social Sciences (SPSS) (version 20.0) software. The statistical difference of the mean zone of inhibition of the extract for individual bacterium was carried out by employing one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test at a significance level of $p<0.05$. The MIC and MBC are analyzed using one-way analysis of variance (ANOVA) using SPSS software.

RESULTS

The results for the antimicrobial study of the methanol extract of *Hippocratea welwitschii* and standard antimicrobial agents – Ciprofloxacin (30 g/ml) and Fluconazole (30 g/ml) - are shown in the Figure 1 and Table 1.

The results of the Minimum Inhibitory Concentrations (MIC) of susceptible organisms were determined by the plot of arithmetic square of the Inhibitory Zone Diameter values against the logarithmic concentrations of the methanol extract of *H. welwitschii*. The results obtained are presented Figure 2 and Table 2

The phytochemical analysis carried out on the aqueous extract and methanol extracts of the leaves of *Hippocratea welwichi* revealed the presence of some secondary plant metabolites which include alkaloids, flavonoids, glycosides, carbohydrates, reducing sugars, tannins, saponins and terpenoids with steroids as the only exception in the methanolic extract and reducing sugar in the dried leaf powder (Table 3).

DISCUSSION

The methanol extract of *Hippocratea welwitschii* leaves was found to possess antimicrobial activities. It demonstrated a broad spectrum against both Gram-positive— *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Bacillus subtilis* and Gram-negative – *Eshcherichia coli,* and *Salmonella typhi,* but not *Klebsiella pneumonia*. It also showed anti-fungal activities against C*andida albicans* and *Aspergillus niger*. From the minimum inhibitory concentration, significant activity was observed on *Staphylococcus aureus* with least effect on *Pseudomonas aeruginosa*. This study confirms the investigations on the ethanol extract of the roots and leaves of *Hippocratea welwitschii*. [10]

The result of the phytochemical analysis of the methanol leaves extract of *Hippocratea welwitschii* showed significant concentration of secondary metabolites which include alkaloids, flavonoids, glycosides, saponins, tannins and terpenoids. These phytochemical compounds are known to play important roles in the biological system. When compared with the crude drug powder, the extract contains higher alkaloidal content. Flavonoids which was found present in large concentration in the plant sample and the methanol extract exhibit a wide range of biological activities including anti-inflammatory, anti-allergic, anti-oxidant, analgesic and antimicrobial properties [17,18]. Tannins have been observed to have remarkable astringent properties, thus herbs that have tannins as their components are astringent in nature and could be deployed in the treatment of intestinal disorders such as diarrhea and dysentery [19]. Tannins have also been observed to have significant activity in cancer prevention and anticancer therapy [20].

The antimicrobial property of the methanol extract of *H. welwitschii* seen in Table 1 could be attributed to the presence of saponins and phenolic compounds (tannins and flavonoids) present in it. This is confirmed in previous studies on antimicrobial activities of known medicinal plants [21]. The result particularly indicated that alkaloids were present in relatively high concentration; flavonoids, glycosides, carbohydrates, terpenoids were present in moderate concentrations whereas reducing sugars, tannins and saponins were present in low concentrations

CONCLUSION

The methanol leaf extract of *H. welwitschii* was found to possess antimicrobial activities against some test microorganisms due to the secondary metabolites present in the plant. This study scientifically validates its folkloric use in traditional medicine. Further fractionation, isolation and purification of the bioactive compounds present in the leaves of *H. welwitschii* are necessary that could lead to the discovery and development of novel antimicrobial agents.

Table 1: Table of Sensitivity of various test organisms to the methanol extract of *Hippocratea welwitschii*

Sample Concentration	Inhibitory Zone Diameter (mm)/Inference							
	E. coli	***S. aureus***	***P. aeruginosa***	***B. subtilis***	***S. typhi***	***K. pneumonia***	***C. albicans***	***A. niger***
100mg/ml	16	13	10	19	14	-	11	15
50mg/ml	12	11	-	15	11	-	9	12
25mg/ml	9	9	-	11	8	-		8
12.5mg/ml	7	-	-	8	-	-	-	-
6.25mg/ml	-	-	-	-	-	-	-	-
Ciprofloxacin 30ug/ml	11	14	13	19	13	11	-	-
Fluconazole 30ug/ml	-	-	-	-	-	-	17	12

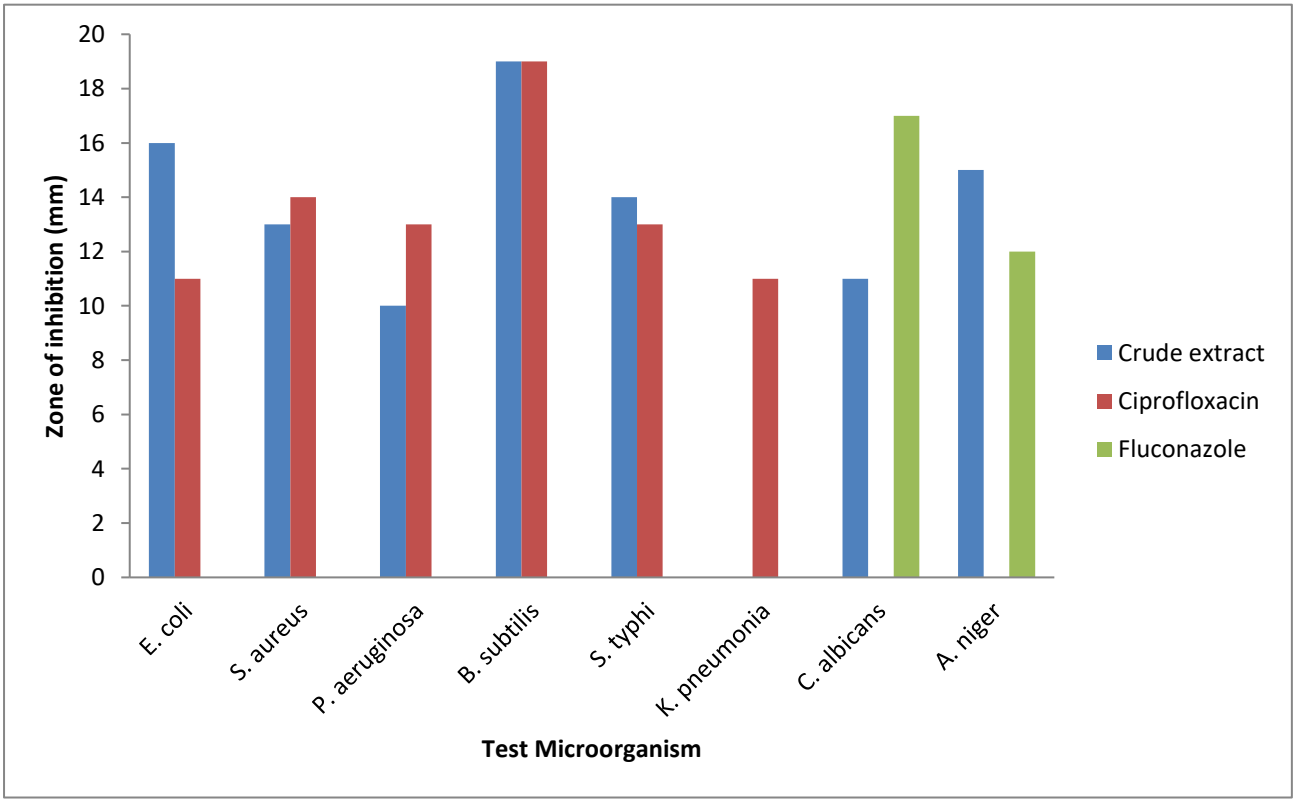

Figure 1: The inhibition zone diameter (IZD) (mm) of crude extract compared to standard antimicrobial agents, Ciprofloxacin and Fluconazole for bacteria and fungi respectively.

Table 2: Table of the relationship between Minimum Inhibitory Concentrations (MIC) and Inhibitory zone diameter (IZD)

Test Organism	Concentration (mg/ml)	Log concentration	IZD (mm)	IZD^2	MIC
E. coli	100	2	16	256	10.61*
	50	1.69	13	169	19.57
	25	1.39	11	121	20.07
	12.5	1.09	9	81	10.61
	6.25	0.79	-	-	-
	0.03 (CIP)	-1.52	11	121	
S. aureus	100	2	13	169	14.68*
	50	1.69	11	121	13.63
	25	1.39	9	81	15.91
	12.5	1.09	-	-	1.17
	6.25	0.79	-	-	14.68
	0.03 (CIP)	-1.52	14	144	
P. aeruginosa	100	2	10	100	50.13*
	50	1.69	-	-	13.33
	25	1.39	-	-	22.67
	12.5	1.09	-	-	1.70
	6.25	0.79	-	-	50.13
	0.03 (CIP)	-1.52	13	169	
B. subtilis	100	2	19	361	9.92*
	50	1.69	15	225	34.63
	25	1.39	11	121	34.50
	12.5	1.09	8	64	0.99
	6.25	0.79	-	-	9.92
	0.03 (CIP)	-1.52	19	361	
S. typhi	100	2	14	196	16.68*
	50	1.69	11	121	17.77
	25	1.39	8	64	21.73
	12.5	1.09	-	-	1.22
	6.25	0.79	-	-	16.68
	0.03 (CIP)	-1.52	13	169	
K. pneumonia	100	2	-	-	
	50	1.69	-	-	
	25	1.39	-	-	
	12.5	1.09	-	-	
	6.25	0.79	-	-	
	0.03 (CIP)	-1.52	-	-	
C. albicans	100	2	11	121	26.73*
	50	1.69	9	81	10.50
	25	1.39	-	-	14.98
	12.5	1.09	-	-	1.43
	6.25	0.79	-	-	26.72
	0.03 (FNZ)	-1.52	17	289	
A. niger	100	2	15	225	10.8*
	50	1.69	12	144	16.53
	25	1.39	8	64	17.09
	12.5	1.09	-	-	1.03
	6.25	0.79	-	-	10.80
	0.03 (FNZ)	-1.52	12	144	

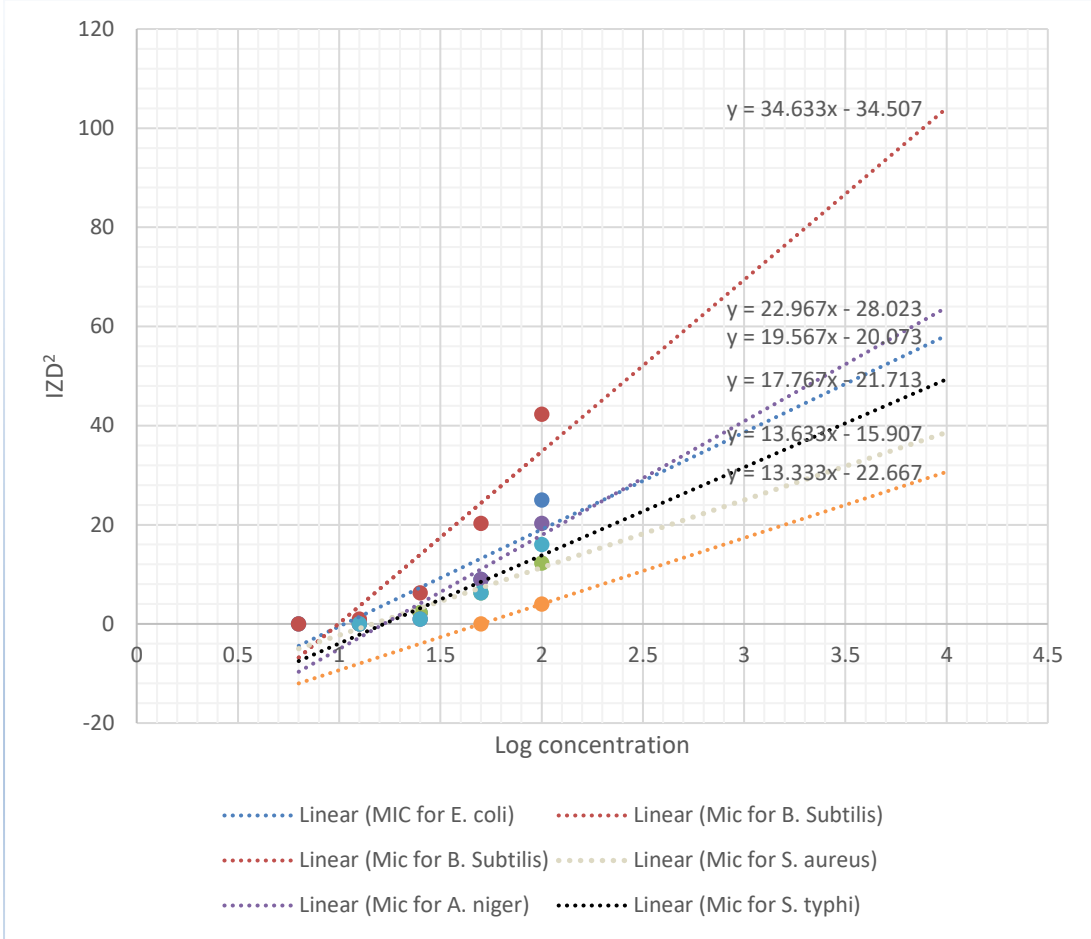

Figure 2: Minimum Inhibitory Concentrations (MIC) of susceptible microorganisms.

Table 3: Phytochemical constituents of *Hippocratea welwichi*

S/No	Phytochemical constituent	Methanol extract	Dried Powder
1	Alkaloids	+++	++
2	Flavonoids	++	++
3	Glycosides	++	+
4	Carbohydrates	++	+
5	Reducing sugars	+	-
6	Tannins	+	++
7	Steroids	-	+
8	Saponins	+	++
9	Terpenoids	++	++

Key: (-): Absent, (+): Present, (++): Moderately present, (+++): Abundantly present.

ACKNOWLEDGEMENTS

We wish to acknowledge the Medical Laboratory Unit of the Bishop Shanahan Hospital, Nsukka for granting us ethical approval and access to obtain clinical isolates from their laboratory. We also appreciate Mr. Felix Nwafor for helping us to source and authenticate the plant leaves of *Hippocratea welwitschii* in our herbarium.

REFERENCES

1. Ji HF, Li XJ, Zhang HY. Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? European Molecular Biology Organization Reports, 10(3), 2009, 194–200.
2. Tripathi AS, Chitra V, Sheikh NW, Mohale DS, and Dewani AP. Immunomodulatory Activity of the Methanol Extract of *Amorphophallus campanulatus* (Araceae) Tuber. Tropical Journal of Pharmaceutical Research. 9 (5), 2010, 451-454.
3. Umar M, Mohammed IB, Oko JO, Tafinta IY, Aliko AA, Jobbi DY. Phytochemical Analysis and Antimicrobial Effect of Lemon Grass (Cymbopogon citratus) Obtained From Zaria, Kaduna State, Nigeria. Journal of Complementary and Alternative Medical Research 1(2), 2016, 1-8.
4. Nester VA, Anderson SR, Roberts MS, and Nester SI. Immunomodulatory Activity of Alcoholic Extract of *Habenaria intermedia* in Mice. International Journal of Pharmacy and Pharmaceutical Sciences, 5, 2009, 406-409.
5. Willey JM, Sherwood L and Woolverton CJ. Pathogenicity and Host Response: Presscott's Microbiology. Boston: McGraw-Hill Higher Education Print Incoporated. 8, 2011, 760 – 815.
6. Zhanel GG, Hoban DJ, Harding GK. The Postantibiotic Effect: A Review of *in vitro* and *in vivo* data. *DICP*- The Annals of Pharmacotherapy. 25, 1991, 153-163.
7. Akinpelu DA, Aiyegoro OA, Okoh AI. *In vitro* antimicrobial and phytochemical properties of crude extracts of stem bark of *Afzelia Africana* (Smith). African Journal of Biotechnology 7 (20), 2008, 3665-3670.
8. Souza MC. *In vitro* and *In vivo* anti *Helicobacter pylori* activity of natural product, peptic ulcer disease: Dr Jianyuan Chai(Ed). 2011. ISBN: 978-953-307-976-9.
9. http://en.wikipedia.org/wiki/Celastraceae Celastraceae 2010.
10. Okwute SK, Okoh-Esene RU and Okogun JI. Phytochemical, Antimicrobial and Anti-Epilepsy Characteristics of Root Extract of Hippocratea welwitschii (Celastraceae) Oliv. International Journal of Pharmacy and Pharmaceutical Research. 11(1), 2017, 1-13.
11. Burkill HM.The useful plants of West Tropical Africa, 2nd ed. The White Friars Press limited, Great Britain. 2, 1985, 352-364.
12. Okoh-Esene RU, Okogun JI, Okwute SK and Thomas SA. Preliminary phytochemical and mineral analysis of the root of Hippocratea welwitschii. Archives of Applied Science Research. 4(1), 2012, 315-322.
13. Okeke EC, Eneobong HN, Uzuegbunam AO, Ozioko AO, Umeh SI, and Kuhnlein H. Nutrient Composition of Traditional Foods and Their Contribution to Energy and Nutrient Intakes of Children and Women in Rural Households in Igbo Culture Area. Pakistan Journal of Nutrition. 8, 2009, 304-312.
14. Bauer AW, Kirby MM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. American Clinical and Climatological Association of Pathologists. 45, 1966, 493-496.
15. Enemor EC, Akagha TN, Ngwoke KG, Gugu TH, Oli AN, Eze CO, Ugwu BC, Ejikeugwu PC and Ugwu MC. Phytochemical Analysis and Antimicrobial Activity of Ethanolic extracts of *Cnestis ferruginea* on Multidrug resistant Isolated From Raw Retail meat sold in Awka, Nigeria. *Journal of Pharmaceutical Sciences & Research* 7(11), 2015, 1044-1049.
16. Harborne JB. Phytochemical methods. London: Chapman and Hall Ltd. 1973, pp 49-188.
17. Ferguson LR. Role of plant polyphenols in genomic stability. Mutation Resource. 475, 2001, 9-111.
18. Hodek P, Trefit P, Stiborova A. Flavonoids potent and versatile biologically active compounds interacting with cytochrome P450 Chemico-Biological Interactions. 139, 2002, 1-21.
19. Dharmananda S. Allnuts and the use of tannins in Chinese medicine. In: Proceeding of Institute for Traditional Medicine, Port-land Oregon. 2003.
20. Li, H., Nang, Z., Liu, Y. (2003). Review in the studies on tannins activity of cancer prevention and anticancer.
21. Romulo A, Ervizal AM, Zuhud JR and Ladislav K. Screening of in vitro antimicrobial activity of

plants used in traditional Indonesian medicine.
Pharmaceutical Biology, 56 (1), 2018, 287-293.