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Phytochemical and *In Vitro* Antimicrobial Properties of *Premna hispida* (Verbenaceae) Linn.

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Abstract: *Premna hispida (Verbenaceae) is a traditional medicinal plant useful in folk medicine in treatment of various human diseases like buccal infections, rheumatism, asthma, dropsy, cough, fever, boils, and scrofulous diseases. The present study was carried out to qualitatively and quantitatively determine the phytochemical constituents of P. hispida using standard methods. The antimicrobial activity of P. hispida was also investigated in vitro using the agar diffusion method. The results revealed the presence of bioactive constituents, comprising saponins, tannins, flavonoids and terpenoids in varying degrees in the crude extract and in all the solvent fractions except in n-hexane fraction. Total phenolics, total flavonoids and tannins contents were highest in the methanol extract while ethyl acetate fraction had the least. The assay results revealed that the methanol extract and the solvent fractions showed activity against Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Aspergillus niger with significant inhibition zones diameter (IZD). The MIC of the crude extract and solvent fractions ranged from 0.44 mg / ml to 2.5 mg/ml. The methanol extract and fractions of this plant compared favourably in terms of zones of inhibition and Minimum Inhibitory Concentration with the standard drug disc (tetracycline and fluconazole) against the tested microorganisms. The antimicrobial activity of the plant leaves as reported by the present study is due to the presence of these secondary metabolites in substantial amounts in the plant. The findings suggest that the plant part has the potential for use as an effective medicine in the treatment of various diseases.*

Keywords: *Premna hispida, Microorganisms, Antimicrobial Properties, Minimum Inhibitory Concentration, Zone of Inhibition, Phytochemical analysis.*

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I. Introduction

Plants have been an integral part of life in many indigenous communities for curative purposes (Bussmann, 2006). Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections (Iwu *et al*, 1999). The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years (Cowan, 1999) as ethno-pharmacologists, botanists, microbiologists, and natural-products chemists are surveying the earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. Herbal medicine have therefore provided the best alternative method for the treatment and management of various diseases.

Many traditional medicinal plants including those belonging to *Premna* genus of the family *Verbenaceae* are useful in treatment of various human diseases. One so prominent among the Igbos in the South eastern Nigeria is the species, *Premna hispida,* traditionally known as "*Ogbunje"* which is used extensively in the treatment of infectious diseases. *Premna* genus consists of trees, shrubs and rarely herbs and climbers. The genus belonging to the family *Verbenaceae* was established by Linnaeus (1771) based on *P. serratifolia*. The genus now contains about 200 species worldwide which are mainly distributed in tropical and subtropical Asia, Africa, Australia and the pacific Island (Harley *et al*., 2004).

Previous phytochemical analysis (Rekha *et al.*, 2015) showed that plants of the genus *Premna* are rich sources of glycosides, diterpenoids and flavonoids. Other classes of secondary metabolites such as

sesquiterpenoids, triterpenoids, isoflavones, lignans and xanthones are known to be isolated from different species of the *Premna* genus. These secondary metabolites have been reported to possess antioxidant, antibacterial, antifungal, anti-inflammatory, cytotoxic and hepatoprotective properties (Rekha *et al.*, 2015). The *Premna* species can be used in treating various ailments like rheumatism, asthma, dropsy, cough, fever, boils and scrofulous disease (Dassanayake *et al*., 1980).The leaves and tender shoots of *P. latifolia* are eaten in curries and used for treatment of fevers, liver complaints etc. Leaf is also known for its cardiotonic, anti-coagulant, and hepatoprotective properties (Weiss, 1979).

Fresh root of *P.herbacea* along with ginger are given in asthma, also used in treatment of rheumatism and inflammation from chronic obstructive pulmonary oxidation. The plant is used as a folk remedy in Yunan province of China to reduce inflammation and to cure malaria (Rekha *et al*., 2015). *P. divaricate* is used for treatment of colds and its leaves as poultices are used to treat headaches (Rekha *et al*., 2015).The root infusion of *P. chrysoclada* is used as purgative and to treat fevers. The leaves are pounded and juice dropped into sore eyes (Secoy *et al*., 1983). Latex from *P. mucronata* bark is applied to boils (Rekha *et al*., 2015). It was also reported that the leaves of *P. obtusifola* are used in folklore medicine as carminative. They are also used in the preparation of soup given as stomachic. A decoction of leaves is used in the treatment of flatulence (Burkill, 1966). Decoctions of both *P. seratifolia* and *P. integrifolia* leaves with little, "calamansi", as tea helps loosen up phlegm and effective for coughs. Decoction of fresh leaves are used for relief of virginal irritation. Decoction of leaves can also be used for cold and fevers, cough and bronchitis, fever blisters of the lips and stomach aches (Rekha *et al*., 2015). In some places, a local patent preparation is claimed to benefit tuberculosis. Crushed leaves are applied on the fore head for curing headache. Chewing the roots is believed to have cardiac benefits. Decoction of leaves is used for bathing infants; also used for treatment for beriberi. Extract of leaves are used for cleaning wounds and for ticks and fleas. Leaves applied over the bladder facilitate urination (Kumar and Jain, 2002). *P. lispida* can be used as an eye lotion, (Clarke, 1999) and also as a mouth wash for treating buccal infections (Mabberley, 1997). The present study aims to qualitatively and quantitatively analyze the phytochemicals present in the methanol extract and various solvent fractions of leaves of *Premna hispida* and also evaluate their antimicrobial potentials against selected microorganisms with a view to proposing its suitability for use in the treatment and management of various diseases and ailments.

II. Materials and methods

The chemical reagents and apparatus used for the study are as follows:

Methanol was purchased from Sigma-Aldrich (Germany); Glacial acetic acid, from BDH (England); Gallic acid were purchased from Qualikems (India); n- hexane and ethyl acetate were bought from JHD (China); Folin-Ciocalteu's phenol reagent from Lobal Chemie (India); Electronic balance (JJ2000Y, Capacity: 2000 g, Precision: 90 %), Rotary evaporator (BuchiRotavapor R-200, Germany). All chemicals that were used are of the analytical grade.

2.1. Collection of plant leaves

The leaves of *Premna hispida* (Linn) were collected from Umuahii Obi-Mbieri in Mbaitoli Local Government Area of Imo state. The plant was identified and authenticated by a taxonomist of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. Voucher specimen number Intercedd/16023 was deposited at the herbarium.

2.2. Preparation of plant extracts

The air dried leaves of *P. hispida* were pulverized and one hundred and fifty grams (150g) of the powder macerated in two and a half litres (two thousand five hundred millilitres) (2.5L) of methanol inside clean solvent bottles. The preparation was left on a shaker at room temperature for 24 hours with regular agitation after which the extracts were filtered and squeezed through four layers of muslin cloth. The filtrate was concentrated *in vacuo* at 40^{o}C to obtain the crude extract. The percentage yield of the extract was determined. The dried extract was used for the qualitative and quantitative phytochemical analysis and bioassay studies (Ayogu and Ugwu, 2019).

2.3. Solvent-solvent partitioning of the extract

About seventeen grams (16.80 g) dried methanol extract was titrated with little amount of silica gel and latter was packed into a column containing four hundred grams (400g) of silica gel for solvent partitioning. Three solvents; n-hexane, ethyl acetate and methanol were used for the partitioning according to their polarity to obtain the solvent soluble fractions. The n-hexane was used first to separate the non- polar components. This was followed by ethyl acetate which was used to separate the moderately polar components and finally methanol was used to wash down the polar components. The fraction obtained from each step was concentrated using a rotary evaporator at 50°C and dried by vacuum evaporation, stored separately in specimen bottles and kept in the refrigerator at 4°C until needed for phytochemical analysis and bioassays (Ayogu and Ugwu, 2019).

2.4. Collection of test microorganisms

Standard strains of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Candida albicans* and *Aspergillus niger* were sourced from the stock of the Department of Pharmaceutics and Pharmacological Microbiology of the University of Nigeria Nsukka, Nigeria.

2.5. Preparation of Media/ Inocula

The bacteria strains were tested for sterility on nutrient agar and then grown in nutrient broth at 37ºC for 24 hours while the fungi species was tested on Sabouraud Dextrose Agar and cultured in Sabouraud Dextrose Liquid medium at 25ºC for 24 hours. The overnight cultures were subsequently diluted and standardized spectrophotometrically to give approximately10^6cfu/mL.

2.6. Preparation of stock solution

The antimicrobial stock solution was prepared using *P. hispida* extract and fractions as the active ingredients and Dimethyl Sulphoxide (DMSO) as the solvent. One half gram (0.5g) of the extract and each of the fractions were dissolved in five millilitres (5mL) of DMSO to get a concentration of 100 mg/ml. Serial dilutions of the dissolved samples were done in order to obtain different concentrations of 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL. These different concentrations were prepared for the sensitivity testing (Ayogu and Ugwu, 2019).

III. Antimicrobial Assay

The agar diffusion method of Bookye-Yiadam (1979) was adopted for susceptibility test of *E. coli*, *B. subtilis*, *P. aeruginosa*, and *S. Aureus* to the leaf extract and solvent fractions using Mueller Hinton agar while Sabouraud Dextrose Agar (SDA) was used for that of *C. albicans* and *A. niger*.

Twenty-four hour old broth culture of the test organisms were swabbed onto sterile Mueller Hinton Agar in Petri dishes using sterile cotton swabs. A sterile stainless steel borer of size 6mm in diameter was used to make wells on the plates. Using sterile 2ml syringes, the holes were filled with 0.05ml each of the three concentrated portions (5 mg/mL, 2.5 mg/mL and 1.25 mg/mL) of the leaf extracts and the solvent fractions. Each well was labelled appropriately. Control experiments were also carried out where the holes were filled with Tetracycline as positive control and sterile distilled water served as a negative control. The inoculated Petri dishes were left for an hour at room temperature for the extracts to diffuse before the growth of the organisms commenced. The plates were incubated at 37ºC for 24 hours after which the results were read by measuring the diameters of zones of inhibition around the wells with the aid of a metric ruler. A larger zone of inhibition indicated the test substance was active against the bacteria, a small zone may mean that the substance was only slightly active against the organisms, and no zone may mean no activity, but this might also mean that the substance was unable to diffuse through the agar (Bookye-Yiadam, 1979).

The agar diffusion method previously described for antibacterial test was used in determining the antifungal action of the leaf extract and solvent fractions against *Candida albicans* and *Aspergillus niger*. The plates were poured with the appropriately standardized culture of the organisms and the excess drained off. Wells (6 mm) were bored into the inoculated plates, using sterile cork-borer. The wells were filled with 0.05 mL of the extract and the solvents fractions with the aid of Pasteur pipette. Fluconazole was used as positive control while DMSO served as negative control. The plates were labelled and left at room temperature for one hour before incubation at 30ºC for 48 hours. Diameter of zones of inhibition due to the activity of the leaf extract and solvent fractions were measured after the period of incubation (Arekemase *et al*., 2011).

3.1. Determination of Minimum Inhibitory Concentration (M. I. C)

Minimum Inhibitory Concentration of the extract and fractions were obtained mathematically, from the plot of logarithm of concentration against the square of the Inhibitory Zone Diameter (IZD^2). The anti-logarithm of the intercept becomes the M I C.

3.2. Qualitative phytochemical analysis of the extract and solvent fractions

Qualitative phytochemical analysis of the extract and solvents fractions was done according to standard methods as described by Harborne (Harborne, 1973).

3.3. Quantitative Phytochemical analysis of the extract and solvent fractions

3.3.1. Determination of Total Phenolics Content (TPC)

Folin-Ciocateau's method was used for the determination of total phenolic content of the extract and solvent fractions using Gallic acid as an internal standard with slight modification as previously reported (Agbo, 2015). The extract and fractions (1.0 g) were separately mixed with distilled water (9 mL) in 25 mL volumetric flask. 10 fold dilute Folin-Ciocateau's phenol reagent (FCPR, 1:10) (2.5 mL) was added. After 5 minutes, 7.5 %

(w/v) Na_2CO_3 solution (10 mL) was added to each mixture and was made to the mark with distilled water. Each of the mixture was incubated in the dark for 90 minutes at room temperature. A set of standard solution of Gallic acid (20, 40, 60, 80 and 100 mg/mL) was prepared in the same manner as described for the extracts. The absorbance of the extract, fractions and the standard solution was read against the reagent blank at 760 nm with a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan). The total phenolics content was determined from the calibration curve and expressed as milligrams of Gallic acid equivalent (GAE) per gram. The determination of the total phenolic was carried out in triplicate.

3.3.2. Determination of Total Tannins Content (TTC)

Folin-Ciocateau's method was used for the determination of total tannins content of the extract and solvent fractions using Gallic acid as an internal standard with slight modification as reported previously (Mythili *et al*., 2004). Briefly, the extract and fractions (1.0 g) were mixed with distilled water (7.5 mL) in 10ml volumetric flask. 10 fold dilute Folin-Ciocateau phenol reagent (FCPR, 1:10) (0.5 mL) was added. 35 % (w/v) Na_2CO_3 solution (1.0 mL) was added to the mixture and make to the mark with distilled water. The mixture was incubated in the dark for 30 minutes at room temperature. A set of standard solution of Gallic acid (100, 80, 60, 40 and 20 mg/mL) were prepared in the same manner as described for the extracts. The absorbance of the extract, fractions and the standard solution were read against the reagent blank at 725 nm with a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan). The total tannins content was determined from the calibration curve and expressed as milligrams of Gallic Acid Equivalent (GAE) per gram of the extracts. (Singh, 2012). The determination of the total tannins content was carried out in triplicate.

3.3.3. Determination of Total Flavonoids Content (TFC)

Aluminium-chloride colorimetric assay was used to determine the total flavonoids content in the extract and solvent fractions previously reported (Mythili *et al*., 2004).

The extract and fractions (1.0 g) was mixed with distilled water (4mL) in a 10 mL volumetric flask. Sodium nitrite (0.30 mL, 5 %) was added to the flask. After 5 minutes, $AlCl_3.6H_2O$ solution (0.30 mL, 10 %) was added to the mixture, followed by addition of 1.0 M NaOH (2 mL) after another 5 minutes and diluted to the mark with distilled water. A set of standard solutions of quercetin (100, 80, 60, 40 and 20 m*g*/mL) were prepared in the same manner as described for the extracts. The absorption peaks of the extract and standard solutions were measured against the reagent blank at 510 nm with a UV/Visible spectrophotometer (UV-1800 Shimadzu Corporation, Japan). The total flavonoids content was determined from the calibration curve and expressed as milligram of quercetin equivalent (QE) per gram of extract as reported by Kostić et al. (2014). The determinations of total flavonoid in the extracts and standards were carried out in triplicates.

IV. Results

4.1. Antimicrobial Susceptibility Test

The result of the antimicrobial assay is presented in Table 1. It revealed that, the extract and the fractions inhibited the growth of the test microorganisms; (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Candida albicans* and *Aspergillus niger*) as they showed significant Inhibition Zone Diameter (IZD) values as the standard drugs (tetracycline and fluconazole) which were used as positive control.

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Table 1. Comparison of the Inhibition Zones Diameter (IZD) of different concentrations of extract and solvent fractions; Tetracycline and fluconazole on test organisms (mm).

Extract/solvent Fraction	Concentration (mg/ml)	IZD of test organism (mm) Ec	Pa	Bs	Sa	Ca	An	**Antibiotics**	Concentration (mg/ml)	IZD of test organisms (mm) Ec	Pa	Bs	Sa	Ca	An
MeOH-Extract	5	16	9	18	20	8	16	**Tetracycline**	5	19	25	16	15	NT	NT
	2.5	12	7	16	13	-	11		2.5	13	21	12	14	NT	NT
	1.25	-	-	10	9	-	9		1.25	8	16	8	10	NT	NT
MeOH	5	13	18	15	11	17	14	**Fluconazole**	5	NT	NT	NT	NT	25	25
	2.5	10	9	11	13	7	9		2.5	NT	NT	NT	NT	20	22
	1.25	7	-	-	9	-	8		1.25	NT	NT	NT	NT	17	15
EtOAc	5	11	15	25	13	18	20								
	2.5	-	13	19	8	15	15								
	1.25	-	-	9	-	11	12								

NT = Not Tested ; Bs= *B. Subtilis*; Ec= *E. coli*; Pa = *P.aeruginosa*; Sa= *S. aureus*; Ca= *C. albicans*; An = *A. niger*.

The result of the antimicrobial assay is presented in Table 2. It revealed that, the extract and the fractions inhibit the growth of the test microorganisms, (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Candida albicans* and *Aspergillus niger)* as they show significant Minimum Inhibitory Concentration (MIC)values as the standard drugs (tetracycline and fluconazole) used as positive control.

Table 2. Comparison of the Minimum Inhibitory Concentration (MIC) of the extract and solvent fractions; Tetracycline and fluconazole against test microorganism (mg/ml).

	M.I.C (mg/ml)											
Extract/Fraction	**Ec**	**Pa**	**Bs**	**Sa**	**Ca**	**An**	**Antibiotic**	**Ec**	**Pa**	**Bs**	**Sa**	**Ca**	**An**
MeOH-Extract	1.25	1.25	0.57	0.79	2.50	0.68	**Tetracycline**	0.93	0.03	0.74	0.35	NT	NT
MeOH	1.11	1.25	1.25	0.49	1.2	1.11	**Fluconazole**	NT	NT	NT	NT	0.37	0.51
EtOAc	2.50	1.25	0.97	1.25	0.44	0.56							

NT= Not Tested; Bs= *B. subtilis*; Ec= *E. coli*; Pa = *P. aeruginosa*; Sa = *S. aureus*; Ca= *C. albicans*; As = *A. niger*.

4.2. Preliminary Qualitative Phytochemical Screening of extract and fractions.

The result of the qualitative phytochemical screening of the extract and fractions of *Premnahispida* leaf is shown in Table 3. From the result, the methanol crude extract has low abundance of saponins and moderate abundance of tannins and high abundance of flavonoids and terpenoids. Ethyl acetate (EtOAc) fraction had no saponins, low abundance of tannins, and moderate abundance of flavonoids and terpenoids. Methanol fraction had low abundance of saponins, high abundance of flavonoid and moderate abundance of tannins and terpenoids. Hexane fraction showed negative result for the phytochemicals. Steroids and alkaloids were not detected in any of the samples.

Table 3. Preliminary qualitative phytochemical analysis result for the plant extract and fractions.

Secondary metabolites	**Extract/ Fractions**			
	Extract	**EtOAc**	**Methanol**	**n-hexane**
Saponins	+	-	+	-
Tannins	++	+	++	-
Flavonoids	+++	++	+++	-
Alkaloids	-	-	-	-
Terpenoids	+++	++	++	-
Steroids	-	-	-	-

KEY: - = negative, + = low abundance, ++ = moderate abundance, +++ = high abundance. EtOAc = Ethyl acetate.

4.3. Quantitative Phytochemical analysis of the extract and fractions.

The results of the quantitative phytochemical analysis of the crude methanol extract, Ethyl acetate fraction, Methanol fraction as well as Hexane fractions are shown in Table 4.

The results show the Total Phenolics Content (TPC) expressed in milligram Gallic Acid Equivalent per gram (mg GAE/g), Total Flavonoids Content expressed in milligram Quercetin Equivalent per gram (mg QE/g) and Total Tannins Content (TTC) expressed in mg GAE/g of the extract and fraction. The extract contains more of the TPC, TFC and TTC than the fractions, with the values 731.9±0.9, 722.7±0.2 and 152.35± 0.6 respectively.

Table 4: Total Phenolics, Total Flavonoids and Total Tannins of the extract and fractions

Extract/ Fraction	TPC (mg GAE/g)	TFC (mg QE/g)	Phenolic to Flavonoid ratio	TTC (mg GAE/g)
MeOH- Extract	731.9±1.9	722.7 ±0.2	1.012	152.35 ± 0.6
EtOAc Fraction	146.6±0.6	111.6±0.2	1.314	18.20 ± 0.3
MeOH Fraction	667.6± 0.5	646.9 ± 0.3	1.032	63.86 ± 0.4

Values are expressed as mean ± SD (n=3). GAE = Gallic Acid Equivalent, QE = Quercetin Equivalent. The absorbance against the reagent blank was determined at 760 nm, 510 nm and 725 nm respectively, with an UV/Visible spectrophotometer.

V. Discussion

The results of the qualitative phytochemical screening of the extract and fractions of *Premna hispida* leaf (Table 3) show that, the methanol crude extract has low abundance of saponins and moderate abundance of tannins and high content of flavonoids and terpenoids. Ethyl acetate (EtOAc) fraction had no saponins, low abundance of tannins, and moderate abundance of flavonoids and terpenoids. Methanol fraction had low abundance of saponins, high abundance of flavonoid and moderate abundance of tannins and terpenoids. Hexane fraction showed negative result for the phytochemicals. Steroids and alkaloids were not detected in any of the samples. This is in agreement with the findings of Dia et al. (2007) who reported the presence of the flavonoids-Apigenin and tannins in *P. fulva* (Dai *et al*., 2007). The present finding also corroborates the findings of Chen et al. (2010). In their report, they reported the abundance of the Triterpene- Betulin in *P. pyramidata* (Chen *et al*., 2010). The results of the quantitative phytochemical analysis of the crude extract, Ethyl acetate fraction and Methanol fractions (Table 4) show that the Total Phenolics Content (TPC) expressed in milligram Gallic Acid Equivalent per gram was highest in the Methanol extract (mg GAE/g) (731.9±1.9), followed by Ethyl acetate fraction (667.6± 0.5) and Methanol fraction (146.6±0.6). Total Flavonoids Content expressed in milligram Quercetin Equivalent per gram (mg QE/g) was high in the Methanol extract (722.7 ±0.2), Methanol fraction had (646.9 ± 0.3) while the Ethyl acetate fraction had (111.6±0.2). Total Tannins Content (TTC) expressed in mg GAE/g of the methanol extract was (152.35 ± 0.6), the methanol fraction had (63.86 ± 0.4) while the Ethyl acetate fraction had (18.20 ± 0.3). This shows that the extract contains more of the TPC, TFC and TTC than the fractions, with the values 731.9±0.9, 722.7±0.2 and 152.35± 0.6 respectively.

Tannins are dietary anti-nutrients that are responsible for the astringent taste of foods and drinks (Chikezie *et al*., 2008). The presence of tannin in *Premna hispida* implies it may have astringent properties and in addition, could quicken the healing of wounds and burns (Farquar, 1996). This justifies the usage in herbal medicine as antibiotics.

Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties (Eleazu *et al*., 2010) and bitterness (Sodipo *et al.*, 2000). The low amounts of saponin in the plant investigated suggested that it may not be deleterious to the user. The large amounts of flavonoids in the investigated plant infer that the plant could have biological functions such as protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumour (Okwu, 2004).

Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect the body against the different levels of carcinogenesis (Okwu, 2004). The antioxidant potentials of plants have been linked to their flavonoids' contents. In addition, the therapeutic potentials of plants have been linked to their antioxidant potentials (Akinmalodun *et al*., 2007; Eleazu *et al*., 2011). Flavonoids are known to possess significant antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherischia coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger* (Okwu and Nnamdi, 2011; Akinpelu, 2000). Thus the high tannin and flavonoid contents of this plant explain its therapeutic use in herbal medicine especially in the treatment of infectious diseases, wounds, burns and ulcers. The presence of these useful phytochemicals shows the plant has possible medicinal values.

Antimicrobial assay revealed that the methanol extract as well as the methanol fraction and ethyl acetate fraction of *Premna hispida* inhibited the growth of the entire test microorganisms in varying degrees with zones of inhibition of16, 9, 18, 20, 8 and 16 (mm) for the (5 mg/mL) Methanol extract; 12, 7, 16, 13, -, 11 (mm) for the (2.5 mg/mL) Methanol extract; 13, 18, 15, 11, 17 and 14 (mm) for the (5 mg/mL) Methanol fraction;10, 9, 11, 13, 7 and 9 (mm) for the (2.5 mg/mL) Methanol fraction; 11, 15, 25, 13, 18 and 20 (mm) for the (5 mg/mL) Ethyl acetate fraction; and 13, 19, 8, 15 and 15 (mm) for the (2.5 mg/mL) Ethyl acetate fraction against *E. coli, P. aeruginosa, B. subtilis, S. aureus, C. albicans* and *A. niger* respectively. The *in vitro* antimicrobial activities of the extract and most solvent fractions showed inhibition of 25.0 and 20.0 mm against *B. subtilis* and *A. niger* respectively. The ethyl acetate fraction showed marginal activity (25.0 mm) against *B. subtilis.*

The methanol extract, methanol fraction and ethyl acetate fraction compared favourably in terms of zones of inhibition with the standard anti biotic disc (tetracycline and fluconazole) against the tested microorganisms (Table 1). The methanol extract showed greater activity against *Bacillus subtilis* (MIC value of 0.57 mg/mL) than Tetracycline (MIC value 0.74mg/mL) (Table 2). This favourably agrees with the findings of Xu et al., (2010) who had reported that methanol extract of *Premna microphylla* showed antimicrobial activity against *S. aureus*, *B. subtilis* and *E. coli* but showed no activity towards *P. aeruginosa* and *S. dysentriae* (Xu *et al*., 2010). The result also lend credence to the findings of Rajendran and Basha, (2010). In their research, Rajendran and Basha investigated the antimicrobial and anti-inflammatory effects of root extract of *Premna serratifolia* and had reported that hexane, ethyl acetate, ethanol and aqueous extracts showed great antimicrobial activity towards some bacteria such as *S. aureus, E. coli, P. aeruginosa, S. typhi, S. paratyphi, V. cholerae* and the fugi; *C. albicans, A. flavus* and *A. niger* with moderate to high zones of inhibition (10-25mm) at MIC towards all tested microorganisms (Rajendran and Basha, 2010).

Also, the ethyl acetate fraction compared favourably with the standard antifungal drug (Fluconazole) against *Candida albicans* and *Aspergillus niger* with MIC values of 0.44 mg/mL and 0.56 mg/mL respectively as compared with 0.31 mg/mL and 0.51 mg/mL respectively of the fluconazole. The methanol extract shows the highest activity against all the tested bacteria and should be the suitable solvent for extraction of this plant for the purposes of treating bacterial infections and ethyl acetate which shows the highest activity against the tested fungi is an ideal solvent for extraction for treatment of fungi diseases.

VI. Conclusion

This study revealed the abundance of secondary metabolites and indicated the antimicrobial potential of the methanol extract and solvent fractions of the leaves of *Premna hispida*. From the results of the phytochemical analysis and antimicrobial assay, it could be agreed that the different fractions of *Premna hispida* aerial parts exhibited appreciable antibacterial and antifungal activities against many strains tested in this study. The antimicrobial activity of leaves of *Premna hispida* could be attributed to the abundance of flavonoids, tannins, saponins and terpenoids in the crude extracts and solvent fractions. This supports the use of the plant in folk medicine for treatment of various diseases. Further work on the types of phytochemical constituents and purification of individual groups of bioactive components is recommended as it may give a profound insight on the many potentials of the plant to inhibit several pathogenic microbes and encourage the development of some novel broad spectrum antimicrobial formulations in the future.

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Conflict of interest

The authors declare that there is no conflict of interest associated with this work

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Supplementary figures

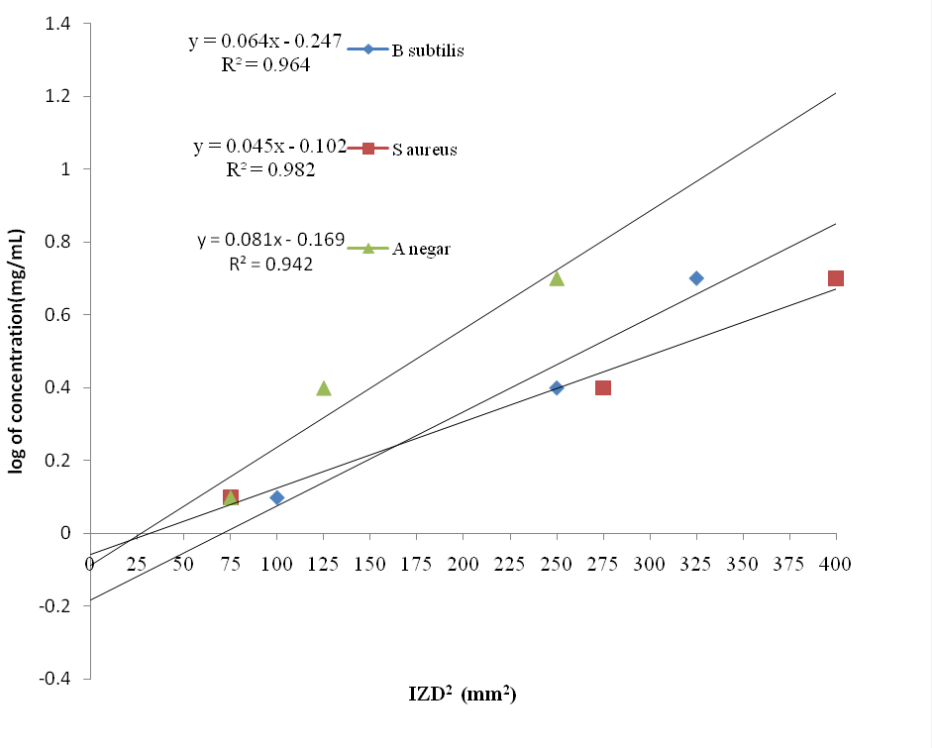

Figure 1. A plot to determine the MIC of extract against *B. subtilis, S. aureus and A. niger.*

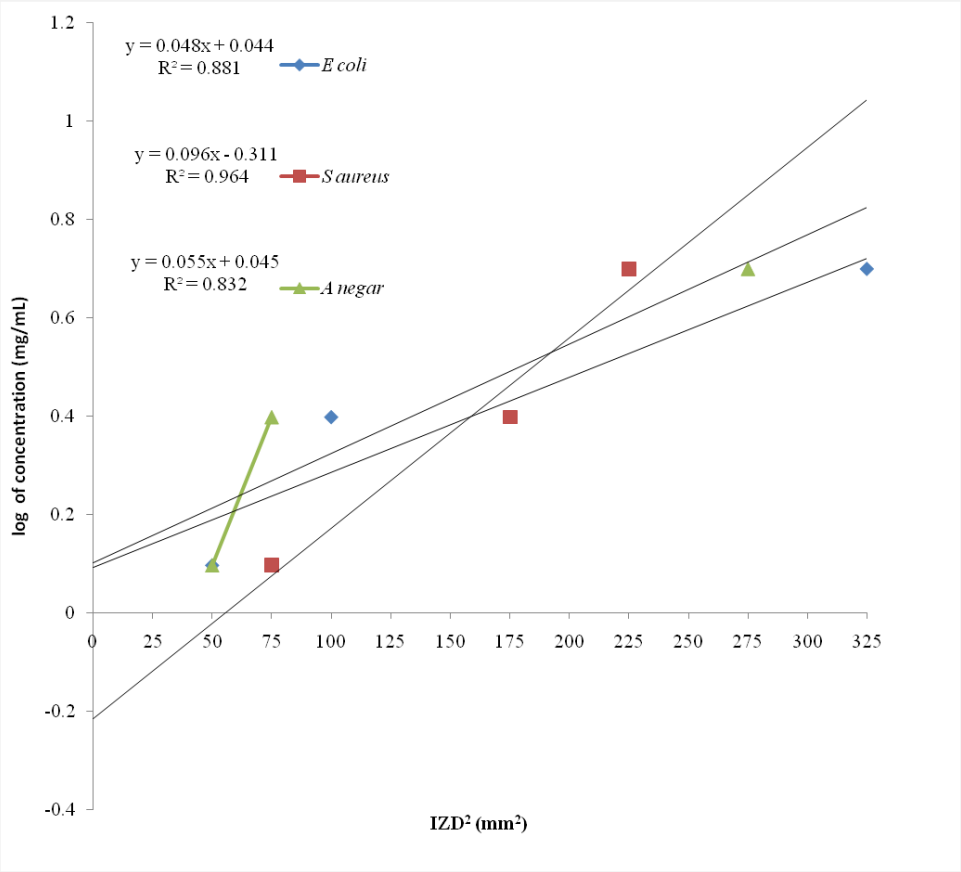

Figure 2. A plot to determine the MIC of MeOH fraction against *E. coli*, *S. aureus and A. niger*

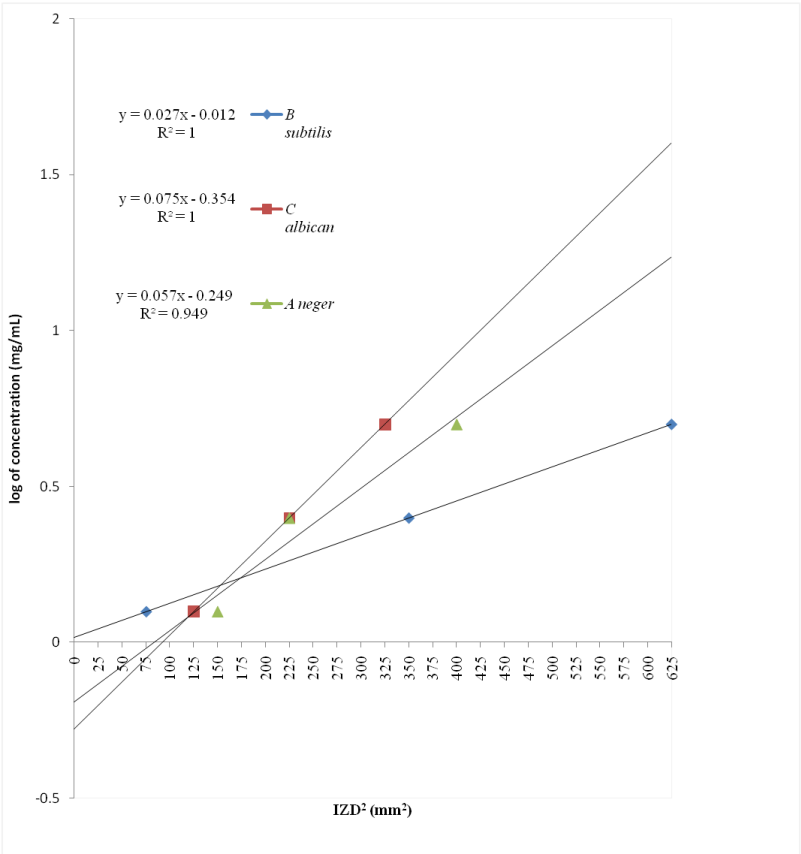

Figure 3. A plot to determine the MIC of EtOAc fraction against *B. subtilis*, *C.albicans* and *A. niger*.

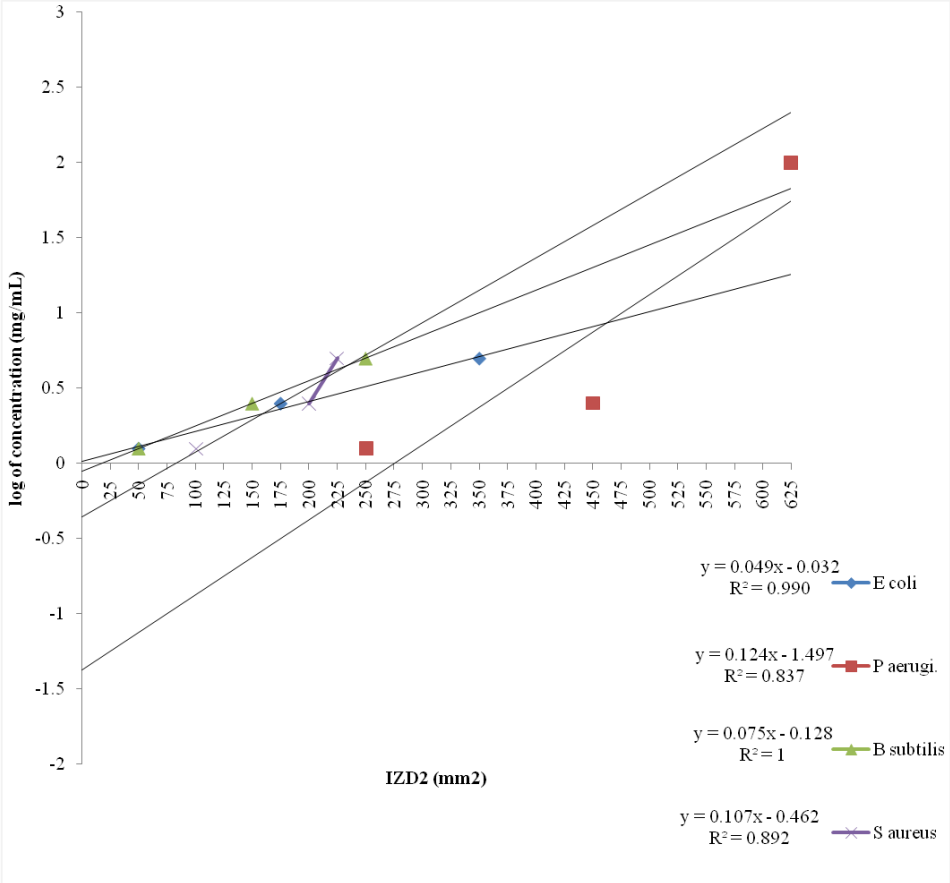

Figure 4. A plot to determine the MIC of tetracycline against *E. coli, B. subtilis, S. aureus and P. aeruginosa*

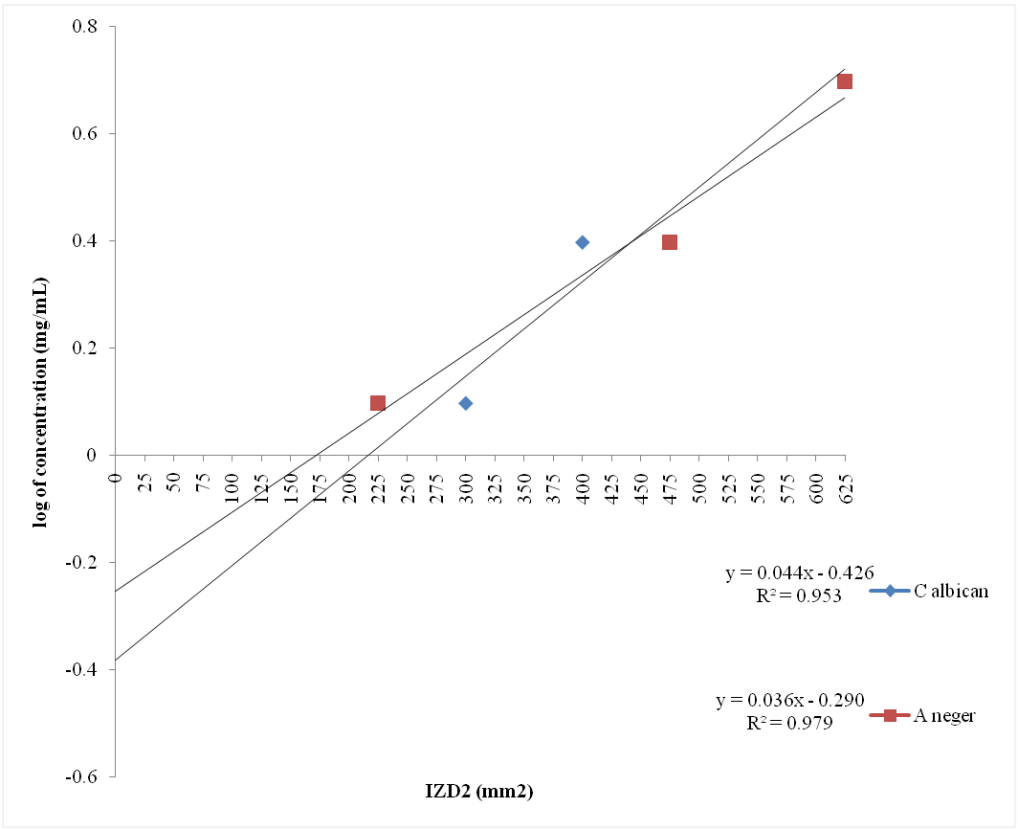

Figure 5. A plot to determine the MIC of fluconazole against *C. albicans* and *A. niger*

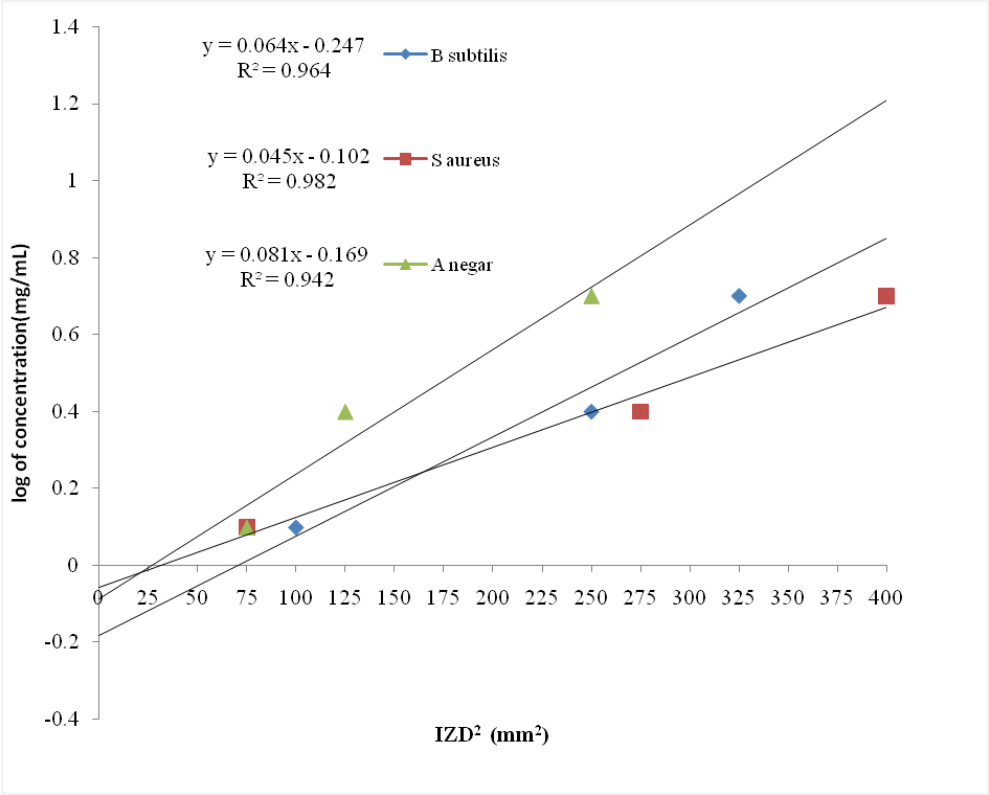

Figure6. A plot to determine the MIC of extract against *B. subtilis, S. aureus and A. niger.*

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