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Comparative Study of the Antibacterial and antifungal Spectrum, Phytochemical screening and Antioxidant potentials of *Alchornea laxiofolia* and *Piliostigma reticulatum* Leaf on Pathogenic Isolates

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Abstract *Piliostigma reticulatum* plant found in West Africa. It is an Evergreen shrub with a twisted bole to 9 m high, with a bushy spherical canopy. Bark dark grey to brown, fibrous and corky, slash dark red. Leaves large, thick, leathery, grey-green, 6-12 cm long, Fruit large, long, straight, undulate or twisted and hard, either glabrous or sparsely pubescent, brown, indehiscent, up to 25 cm long, 5 cm wide. *Alchonea laxiofolia* is a Deciduous, erect to straggling shrub 7m tall. Two extracting solvents were used for purpose of extraction, (Ethanol and Aqueous). Six clinical and environmental organisms were used for this research work. The organisms are *Escherichia coli, Salmonella typhi, Candida albicans, Klebsiella pneumonia, Aspergilus flavus, Staphylococcus aureus.* Agar well diffusion method was used to determine the antimicrobial activities of the two medicinal plants. It was observed that all the clinical and environmental organisms were susceptible to the two medicinal plants, both exhibits different degree of antimicrobial zones inhibition. Quantitative and Qualitative phytochemical screening was also determined on the two medicinal plants. It was observed that both plants,*Piliostigma reticulatum and* Alchonealaxiofolia contains various bioactive components like Alkaloids, Cardiac glycoside, Flavonoids, Steroids, Tannin, Saponin and Phenol. Quantitative Analyses of element Present in Plant Extracts (ug/100g) were also determined, Minerals like Sodium, Potassium, Calcium, Magnesium, Zinc, Lead, Manganese, and Phorphorus were present at various percentage compositions by mass. Quantitative Analyses of Anti –nutrients Present in Plant Extracts were also elucidated. Anti-nutrients like Tannin Phenol, Oxalate, Saponin and flavonoids were present in various quantities.Quantitative analyses Of Proximate Nutrient Composition of Plant Extracts of Alchornea laxiflora and Piliostigma reticulatum were also determined. It was observed that the two plants contain Fats, Carbonhydrates, Moisture contents, Crude protein, Fibres and Ash. *In vitro*-antioxidant activities of ethyl acetate leaf extracts of the two plants were also determined. They both contain different antioxidant in various quantities. Antioxidant are Ferric reducing property (FRAP)Azino-bis (3-ethylbenthiazoline-6-sulphuric acid)(ABTS), Superoxide**,** Diphenyl - 2-picryhydrazyl(DPPH), H_2O_2, Flavonoids and Phenols. The purpose of this research work is to determine the antimicrobial activities,elementpresent, Anti-nutrients, Proximate contents and *In vitro*-Antioxidants activities of *Piliostigma reticulatum and Alchonea laxiofolia.*

Keywords Antibacterial, antifungal spectrum, qauntitative methods of analysis; mineral composition; anti-nutrients; proximate method; in-vitro-antioxidant analysis.

Introduction

Herbal medicine has been shown to be effective and about 60% of rural populations depend on it for their primary health care [1-2]. This could be attributed to affordability, accessibility, in the economic sense and socially, an uneven distribution of health personnel between rural and urban areas. Traditional medical practitioners in Nigeria use a variety of herbal preparations to treat different kinds of ailments such as typhoid and paratyphoid fevers, dysentery, malaria, diarrhea, etc. Various research works conducted in recent years have shown that pathogenic microorganisms are becoming increasingly resistant to existing antibiotics at alarming rates [3-7].

Piliostigma reticulatum (DL.) Hochst. (common name; Yoruba: 'abafin', Hausa: 'kalgo', Igbo: okpoatu') belongs to the family Leguminosae - Caesalpiniaceae and is found in the savannah region of Nigeria. It is a tree, occurring up to 30ft in height with an evergreen, dense spreading crown. Evergreen shrub or small tree with a twisted bole to 9 m high, with a bushy spherical canopy. Bark dark grey to brown, fibrous and corky, slash dark red. Leaves large, thick, leathery, grey-green, 6-12 cm long x 4-8 cm wide; split in half in cattle-hoof shape, apex bilobate, obtuse ; 9 palmate conspicuous central nerves. Flowers dioecious, clustered in short, hairy, axillary racemes measuring 4-5 cm. Petals white with pink stripes. Fruit large, long, straight, undulate or twisted and hard, either glabrous or sparsely pubescent, brown, indehiscent, up to 25 cm long x 5 cm wide.

Alchornea laxiflora occurs from Nigeria east to Ethiopia and south to DR Congo and through East Africa to Zimbabwe, Mozambique, north-eastern South Africa and Swaziland. *Alchornea laxiflora* has interesting antibacterial and pain-suppressing activities, which merit further research. The antioxidant activities of the leaves are interesting as well, and more research is needed to elucidate the effectiveness under different conditions [8]. Leaves alternate, simple; stipules linear, 2–8 mm long; petiole 1–7(–9) cm long, thickened at both ends with 2 filiform, 1–3 mm long stipels at top; blade elliptical-lanceolate to oblong-oblanceolate, 5–18 cm × 3–8 cm, base rounded or cuneate, apex acuminate, margins shallowly toothed, glandular at base, sparingly shortly hairy on the veins, base 3-veined, reddish when young. In Nigeria a decoction of the leaves is taken to treat inflammatory and infectious diseases. It is also a common ingredient in herbal antimalarial preparations. In Tanzania the ground leaves are taken in water to treat hernia. The leaf sap and root decoction are drunk to treat pain in neck and shoulders [9]. The ash of the stem pith is applied to a stiff neck. In Nigeria the leaves are used as packing and preservation material for kola nuts. Small branches are used as chew sticks. Straight stems are used as fence poles [10].

Materials and Methods

Plant collection /Source

The leaf, bark and stem of the selected plants were obtained from a location in the southwestern part of Nigeria, in the tropical rainforest of IkareAkoko, Ondo state and Ile Ife, Osun state, Nigeria. The plant was authenticated by a certified botanist at the herbarium unit of Department of Plant science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria and Obafemi Awolowo University, IleIfe, Osunstate, Nigeria. The leaves, bark and stem were washed thoroughly with distilled water, stored in air tight containers and kept at room temperature prior to use [11].

Test organisms

The test bacteria used in this study were *Salmonella typhi, Klebsiellapneumoniae, Escherichia coli and Candida albican*. Candida krusei, and Fusarium Solani. They were obtained from the Adekunle Ajasin University, Akungba Akoko, OndoState , Nigeria and Obafemi Awolowo University,IleIfe.Osunstate, Nigeria They were isolated on sterile nutrient agar slants and taken to the microbiology laboratory of the Adekunle Ajasin University, Akungba Akoko, Nigeria. All slants of test organisms were kept at -4O^{C} prior to bioassay of the extracts. Extensive biochemical tests were carried out to further confirm all the test bacterial strains [12-13].

Preparation of plant extracts for Extraction

All the plant materials obtained were first washed thoroughly with sterile distilled water and air dried at room temperature for about two weeks to ensure that the samples lose most of their moisture content. The following extractions were carried out: aqueous and Ethanol. For each extraction, 250g of each dried plant material was weighed separately into conical flasks containing 750ml each of distilled water and ethanol, The mixtures were

initially shaken rigorously and left for 9 days. All mixtures were filtered using sterile Whatman filter papers and the filtrates were collected directly into sterile crucibles. All filtrates obtained were introduced into sterile reaction tubes and heated continuously in water bath at the following temperatures: 78 °Cfor ethanol extraction, and 105 °C for distilled water. The residues obtained were kept at room temperature [14].

Standardization of Extracts

Using aseptic condition, the extract is reconstituted by adding 1.2g of each extract with 5ml of dimethylsulphoxide (DMSO) and 15ml of sterile distilled water making it 60mg/ml.For each extract, 7.5ml of distilled water is measured into three sterile bijou bottle. In bijou bottle A 7.5ml from 60mg/ml extract was added and in bijou bottle B 2.5ml from 60mg/ml extract was added and bijou bottle C 2.5ml from bijou bottle A was added. A is 30mg/ml, B is 15mg/ml, C is 7.5mg/ml respectively [15].

Standardization of Inoculum

Slants of the various organisms were reconstituted using an aseptic condition. Using a sterile wire loop, approximately one isolated colony of each pure culture was transferred into 5ml of sterile nutrient broth and incubated for 24 hours. After incubation, transfer 0.1ml of the isolated colony using a sterile needle and syringe into 9.9ml of sterile distilled water contained in each test tube and then mixed properly. The liquid now serve as a source of inoculum containing approximately 10^6cfu/ml of bacterial suspension [16].

Antimicrobial assay of plants extracts Using agar well diffusion method.

All antibacterial assays for the plant extracts were carried out by well diffusion technique. All the test organisms were sub-cultured onto sterile Mueller Hinton Agar plates and incubated at 37 °C for 18-24h. Five distinct colonies for each organism were inoculated onto sterile Mueller Hinton broth and incubated for 3-4h. All innocula were standardized accordingly to match the 0.5 McFarland standard and this standard was used for all susceptibility tests. All the extracts were reconstituted accordingly into the following concentrations:60, 30, 15 and 7.5mg/ml; using the Dimethyl Sulphoxide (DMSO). The susceptibility testing was investigated by the Agar well diffusion method.A 0.1 ml of 1:10,000 dilutions (equivalent to 10^6cfu/mL) of fresh overnight culture of the clinical isolates grown in Muller Hinton agar and potato dextrose agar was seeded into 40 mL of Muller Hinton agar, and properly mixed in universal bottles. The mixture was aseptically poured into sterile Petri dishes and allowed to set. Using a sterile cork borer of 4 mm diameter, equidistant wells were made in the agar. Drops of the re-suspended, (2mL per well) extracts with concentrations between 60 to 7.5 mg/mL were introduced into the wells till it was filled. Ciprofloxacin and Metronidazole 2mg/mL were used as the control experiment. The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation at 37^oC for 24 hours. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. All experiments were performed in duplicates [12, 14-15].

Determination of bioactive component (phytochemical screening), minerals, anti-nutrient and proximate composition of plant extracts:

Qualitative Method of Analyses

Preliminary test / Preparation test

Plant filtrate was prepared by boiling 20 g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrate was used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides [17].

(i)Test for Alkaloids

About 0.2 gram was warmed with 2% of H_2SO_4 for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicated the present of Alkaloids.

(ii) Test for Tannins

One milliliter of the filtrate were mixed with 2m1 of FeC1,A dark green colour indicated a positive test for the tannins [18].

(iii) Test for Saponins

One milliliter of the plant filtrate were diluted with 2 ml of distilled water; the mixture were vigorously shaken and left to stand for 10min during which time, the development of foam on the surface of the mixture lasting for more than 10mm, indicates the presence of saponins [18].

(iv) Test for Anthraquinones

One milliliter of the plant filtrate were shaken with 10ml of benzene; the mixture was filtered and 5 ml of 10 % (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test[18].

(v)Test for Anthocyanosides

One milliliter of the plant filtrate were mixed with 5 m1 of dilute HCI; a pale pink colour indicates the positive test.

(vi) Test for Flavonoids

One milliliter of plant filtrate were mixed with 2 m1 of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, I m1 of the plant filtrate were mixed with 2m1 of dilute NaOH; a golden yellow colour indicated the presence of flavonoids [19].

(vii) Test for Reducing Sugars

One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown colour with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars [19].

(viii) Test for Cyanogenicglucosides

This was carried out subjecting 0.5g of the extract 10ml sterile water filtering and adding sodium picrate to the filtrate and heated to boil [19].

(ix) Test for Cardiac glucosides

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydrate plus H_2S0_4.

Quantitative Method of Analyses

(i) Saponins

About 20grams each of dried plant samples were ground and, put into a conical flask after which 100 ml of 20 % aqueous ethanol were added. The mixture were heated using a hot water bath. At about 55°C, for 4 hour with continuous stirring, after which the mixture were filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 rnl of diethyl ether were added and then shaken vigorously. The aqueous layer were recovered while the ether layer was discarded. The purification process was repeated three times. 60 rnl of n-butanol were added. The combined n-butanol extracts were washed twice with 10 m1 of 5% aqueous sodium chloride. The remaining solution were heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material [20].

(ii) Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution were filtered through Whatman filter paper No 42. The filtrate were later transferred into a crucible and evaporated into dryness over a water bath; the dry content were weighed to a constant weigh [20].

(iii) Cardiac glucosides

Legal test and the killer-kilianiwwas adopted, 0.5g of the extract were added to 2ml of acetic anhydrate plus H_2S0_4.

(iv) Tannins

About 500 mg of the plant sample were weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M FeCl in 0.1 M Hcl and 0.008 M potassium ferrocyanide. The absorbance were measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract [21]

(v) Alkaloids

Five grams of the plant sample were weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then be added, the reaction mixture were covered and allowed to stand for 4 hour. This were filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was

added drop-wise to the extract until the precipitation is complete. The whole solution were allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass [22].

(vi) Phlobatannins

About 0.5grams of each plant extracts were dissolved in distilled water and filtered. The filtrate was boiled in 2% HCl, red precipitate show the present of phlobatannins [23].

Total phenol (Spectrophotometric methods)

2 g each of the samples were defatted with 1mL of diethyl ether using a soxhlet apparatus for 2 hours. The fat free samples were boiled with 50 mL of ether for the extraction of the phenolic components for 15 minutes. 5 mL of the extracts were pipetted into 5 mL flask and then 10 mL distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes for color development. This was measured at 505 nm [24].

Determination of Proximate Analysis of Medicinal Plants

The proximate parameters (moisture, dry matter, ash, crude fats, proteins and fibers, nitrogen, carbohydrates and energy values) were determined using Association of Official Analytical Chemists Methods [25].

1. Determination of moisture content was done by drying samples in oven (WiseVen, WON-50, Korea) at 110 °C until constant weight was attained [26].
2. Nitrogen estimation was carried out by the micro-Kjeldahl (BUCHI, KjelFlex K-360, Switzerland) method with some modification [27].
3. The crude proteins were subsequently calculated by multiplying the nitrogen content by a factor of 6.25,Hussain*et al*,.2011 The energy value estimation was done by summing the multiplied values for crude protein,
4. Crude fat and carbohydrate respectively at Water Factors (4, 9 and 4). Crude fats were determined by Soxhlet apparatus using *n*-hexane as a solvent.
5. The ash values were obtained by heating samples at 550 °C in a muffle furnace (Wise Therm, FHP-03, Korea) for 3 h [27].
6. The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash content and crude fat from the total dry matter [26].
7. Crude fiber was estimated by acid-base digestion with 1.25% H_2SO_4 (v/v) and 1.25% NaOH (w/v) solutions [28].

Determination of *in vitro*-antioxidant conpoments of *medicinal plants*

Determination of total phenol

0.2 ml of the extract was mix with 2.5ml of 10% Folinciocalteau's reagent and 2ml of 7.5% Sodium carbonate. The reaction mixture will be subsequently incubated at 45oC for 40mins, and the absorbance was measure at 700nm in the spectrophotometer, garlic acid would be used as standard phenol.

Determination of total flavonoid

The total flavonoid content of the extract was determined using a colourimeter assay. 0.2 ml of the extract was added to 0.3ml of 5% $NaNO_3$ at zero time. After 5min, 0.6ml of 10% $AlCl_3$ was added and after 6min, 2ml of 1M NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

Determination of ferric reducing property

0.25ml of the extract was mixed with 0.25ml of 200mM of Sodium phosphate buffer pH 6.6 and 0.25ml of 1% KFC. The mixture was incubated at 50oC for 20min, thereafter 0.25ml of 10% TCA was also added and centrifuge at 2000rpm for 10min, 1ml of the supernatant was mixed with 1ml of distilled water and 0.1% of FeCl3 and the absorbance was measure at 700nm.

Determination of free radical scavenging ability

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picryhydrazyl). 1ml of the extract was mixed with 1ml of the 0.4mM methanolic solution of the DPPH the mixture was left in the dark for 30min before measuring the absorbance at 516nm.

Determination $Fe2^+$ Chelation

150mM $FeSO_4$ will be added to a reaction mixture containing 168ml of 0.1M Tris-HCl pH 7.4, 218ml saline and extract and the volume is made up 1ml with distilled water. The reaction mixture will be incubated for 5min, before the additional of 13ml of 1, 10-phenantroline the absorbance will be read at 510nm.

ABTS scavenging ability

2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability. The ABTS scavenging ability of the extract was determined and the ABTS was generated by reacting an (7mM).

ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM/l, final conc.) in the dark for 16hours and adjusting the absorbance at 734nm to 0.700 with ethanol 0.2 of the appropriate dilution of the extract was then added to 2.0ml of ABTS solution and the absorbance was read at 732nm after 15mins. The TROLOX equivalent antioxidant capacity was subsequently calculated (264.32g).

Superoxide anion scavenging activity assay

The superoxide anion radicals are produced in 2 ml of phosphate buffer (100 mM, pH 7.4) with 78 µM β-nicotinamide adenine dinucleotide (NADH), 50µM nitro blue tetrazoliumchloride (NBT) and test samples at different concentrations. The reaction mixture is kept for incubation at room temperature for 5 min. It is then added with 5-methylphenazinium methosulphate (PMS) (10 µM) to initiate the reaction and incubated for 5 min at room temperature. The colour reaction between superoxide anion radical and NBT is read at 560 nm. Gallic acid is used as a positive control agent for comparative analysis. The reaction mixture without test sample is used as control and without PMS is used as blank18.

Results

Table 1 shows the Antimicrobial activites of *Alchornea laxiofolia* and *Piliostigma reticulatum* on ethanolic leaf extracts. *Escherichia coli* and *Klebsiella pneumonia has the highest* zones of inhibition at 60mg/ml which ranges between 10mm and 12mm.At 7.5mg/ml, *Salmonella typhi,Candida albican, Klebsiella pneumonia, Aspergilus flavus and Staphylococcus aureus* has the lowest zones of inhibition which ranges between 1.0mm and 0.0mm respectively.

Table 2 shows the Antimicrobial activities of *Alchornea laxiofolia* and *Piliostigma reticulatum on aqueous leaf extracts.* It was observed**,** at 60mg/ml, *Salmonella typhi and Staphylococcus aureus* has the highest zones of inhibition. At 7.5mg/ml *Salmonella typhi ,Candida albican, Klebsiella pneumonia and Aspergilus flavus has the lowest zones of inhibition which ranges between 0.0mm and 1.0mm respectively.*

Table 3 shows the Qualitative analysis of the phytochemical screening of *Alchornea laxiflora* and *Piliostigma reticulatum.* All bioactive components were present in *Alchornea laxiflora*but phenol and steroids were absent and steroids and saponin were also adsent in *Piliostigma reticulatum.*

Table 4 shows the Quantitative analyses of elemental composition of *Alchornea laxiflora,* and *Piliostigma reticulatum* extracts (ug/100g). The crude leaf extracts of *Alchornea laxiflora,* and *Piliostigma reticulatum* contains: Potassium (K^+), and Sodium (Na), Calcium (Ca^{2+}), Magnesium (Mg^{2-}), Zinc (Zn^{2-}), Iron (Fe^{2+}), Manganese (Mn) and Phosphorous (P^+). Lead and Copper were not found in the bark of the *M. lucida*. Lead (Pb) was absent in crude leaf extracts of *M. lucida,* other minerals were present in appreciable quantity.

Table 5 shows the quantitative analyses of anti –nutrients present in *Alchornea laxiflora and Piliostigma reticulatum* extracts**.** It was observed that phenols and oxalate has the highest composition between 15.27 and 16.30 respectivelly. it was also observed that flavonoids and alkaloids has the lowest percentage in both *Alchornea laxiflora,* and *Piliostigma reticulatum* extracts.

Table 6 shows the Quantitative analyses Of Proximate Nutrient Composition of *Alchornea laxiflora and Piliostigma reticulatum* Extracts. Carbohydrate and crude protein has the highest quantity in both plants and fat has a minimal quantity in both plants.

Table 7 shows the *in-vitro* antioxidant Assay of ethanol extracts of *Alchornea laxiflora and Piliostigma reticulatum.* It was observed that at 50mg/ml, both plant exhibit the highest concentration of antioxidant**.**

Table 1: Antimicrobial activities of *Alchornea laxiflora* and *Piliostigma reticulatum* ethanolic leaf extracts

Pathogenic Isolates	Zones of Inhibition-mm									
	60mg/ml		30mg/ml		15mg/ml		7.5mg/ml		30mg/ml	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	Ciprofloxacin	Metronidazole
Escherichia coli	12.0	10.0	7.0	5.0	2.0	1.0	1.0	1.0	20.0	20.0
Salmonella typhi	11.0	13.0	7.0	4.0	3.0	1.0	0.0	0.0	19.0	20.0
Candida albican	10.0	12.0	6.0	14.0	3.0	2.0	0.0	1.0	20.0	22.0
Klebsiella pneumonia	12.0	9.0	10.0	12.0	5.0	2.0	0.0	0.0	28.0	20.0
Aspergilus flavus	9,0	8,0	7.0	5.0	3.0	2.0	0.0	0.0	19.0	20.0
Staphylococcus aureus	10.0	12.0	6.0	8.0	3.0	1.0	0.0	0.0	20.0	19.0

Table 2: Antimicrobial activities of *Alchornea laxiflora* and *Piliostigma reticulatum* ethanolic leaf extracts

Pathogenic Isolates	Zones of Inhibition-mm									
	60mg/ml		30mg/ml		15mg/ml		7.5mg/ml		30mg/ml	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	Ciprofloxacin	Metronidazole
Escherichia coli	9.0	10.0	5.0	5.0	2.0	4.0	1.0	1.0	20.0	20.0
Salmonella typhi	10.0	13.0	7.0	6.0	3.0	2.0	0.0	0.0	19.0	20.0
Candida albican	11.0	10.0	6.0	10.0	3.0	2.0	0.0	1.0	20.0	22.0
Klebsiella pneumonia	12.0	9.0	7.0	6.0	5.0	4.0	0.0	0.0	28.0	20.0
Aspergilus flavus	9,0	7,0	7.0	5.0	3.0	2.0	0.0	0.0	19.0	20.0
Staphylococcus aureus	10.0	11.0	6.0	8.0	3.0	2.0	1.0	1.0	20.0	19.0

Table 3: Qualitative Analysis of the Phytochemical Screening of *Alchornea laxiflora* and *Piliostigma reticulatum* Leaf extracts.

Sample	Alkaloid	Cardiac Glycoside	Steroid	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids
Alchornea laxiofolia	+ve	+ ve	- ve	+ ve	- ve	+ ve	+ ve	+ ve
Piliostigma reticulatum	+ve	+ ve	- ve	+ ve	+ve	+ ve	- ve	+ ve

Table 4: Quantitative Analyses of Elemental composition of *Alchornea laxiflora*, and *Piliostigma reticulatum* LeafExtracts (ug/100g)

Plant	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn	P
Alchornea laxiofolia	10.29	18.56	11.70	20.65	19.55	6.72	ND	0.01	16.32	9.45
Piliostigma reticulatum	11.24	15.46	15.35	15.11	13.34	5.23	ND	0.02	17.23	10.00

Table 5: Quantitative Analyses of Anti –nutrients present in *Alchornea laxiofolia and Piliostigma reticulatum* Leaf Extracts in Percentage (%)

Parameters	*Alchornea laxiofolia*	*Piliostigma reticulatum*
Tannin	3.60	3.57
Phenol	16.30	15.27
Phylate	3.70	3.71
Oxalate	12.89	14.01
Saponin	7.53	8.59
Flavonoid	1.23	2.25
Alkaloids	2.20	2.12

Table 6: Quantitative analyses of Proximate Nutrient Composition of *Alchornea laxiofolia and Piliostigma reticulatum Leaf* Extracts

S/N	Ash	Moisture Content	Crude Protein	Fat	Fibre	Carbohydrate
Alchornea laxiofolia	1032 %	7.10 %	19.45 %	7.10 %	11.99 %	45.20 %
Piliostigma reticulatum	10.78 %	7.00 %	14.68 %	5.19 %	13.23 %	52.99 %

Table 7: *In-vitro* antioxidant assay of ethanol leaf extracts of *Alchornea laxiflora and Piliostigma reticulatum*.

	Alchornea laxiflora				***Piliostigma reticulatum***			
Concentration	**5mg/ml**	**10mg/ml**	**20mg/ml**	**50mg/ml**	**5mg/ml**	**10mg/ml**	**20mg/ml**	**50mg/ml**
Phenol	4.4563	7.74451	14.1644	22.54	5.64418	11.9385	17.56689	20.763
Flavonoids	2.5270	6.4141	8.03525	10.454	3.59874	6.42065	11.6846	16.456
Frap	11.7755	22.8691	33.3195	43.453	20.5439	42.6418	62.6319	71.652
Fe^{2+}	24.8850	45.5697	58.9501	65.432	37.5478	51.5747	63.8352	73.873
H_2O_2%	11.6935	26.0897	47.4396	65.453	40.8668	58.2755	66.3962	69.352
ABTS µ	13.0752	15.7548	19.3585	30.765	15.1277	18.8074	20.9929	31.978
DPPH%	13.5405	19.8648	42.2972	51.354	37.7027	42.7072	58.4594	66.672
SO%	16.9775	33.5206	51.9363	70.863	38.2022	53.8726	57.678	64.463

Key: FRAP=Ferric reducing property; ABTS =Azino-bis (3-ethylbenthiazoline-6-sulphuric acid) (unit = **µ**)
SO=Superoxide, DPPH=Diphenyl -2-picryhydrazyl (unit = %), H_2O_2 = (unit = %)

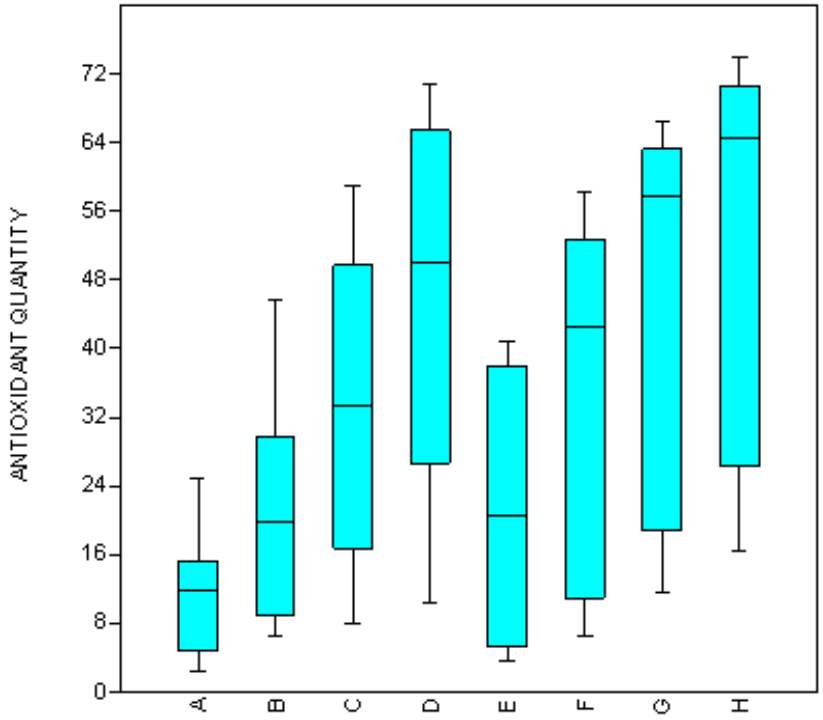

Figure 1: Graphical representation of in-vitro antioxidant assay of ethanol leaf extracts of Alchornea laxiflora and Piliostigma reticulatum

Key: A & E= 5mg/ml, B & F=10mg/ml, C & G = 20mg/ml, D & H=50mg/ml

Discussion

Alchornea laxiflora and *Piliostigma reticulatum* leaf extract exhibited appreciable antibacterial and antifungal activities against the bacteria and fungi used in this study. *Escherichia coli* and *Klebsiella pneumonia* was highly susceptible to ethanolic leaf extracts of *Alchornea laxiflora* compared to that of *Piliostigma reticulatum* while *Salmonella typhi, Staphylococcus aureus, Candida albican* has the highest zones of inhibition at 60mg/ml to *Piliostigma reticulatum*. The results obtained revealed the inhibition of both Gram-positive and Gram-negative bacteria while the fungi used were susceptible to the effects of the plant extract. This is an indication of broad spectrum activities exhibited by *A. laxiflora* and *Piliostigma reticulatum* leaf extract.

Salmonella typhi known to be resistant to many antibiotics was susceptible to the effect of the *Piliostigma reticulatum*aqueous extract compared to that of *Alchornea laxiflora*.*Staphylococcus aureus, Candida albican and Escherichia coli* were also susceptible to *Piliostigma reticulatum* aqueous extract while *Aspergilus flavus* has the least zone of inhibition. All the isolates has high zone of inhibition at conc 30mg/ml of *Piliostigma reticulatum* except *Klebsiella pneumonia* and *Aspergilus flavus* which have low zones. All isolates shows low activity at concentration 7.5mg/ml on both extracts. *Aspergillus* species which was among the fungal isolates used and known to cause aspergillosis mainly bronchopulmonary aspergillosis were susceptible to this plant extract.

The phytochemical analysis of the leaf extract of *A. laxiflora* revealed the presence of alkaloids, cardiac glycosides, Anthraquinone, tannins, flavonoids, saponins while steroids and phenol were absent compared to phytochemical analysis of *Piliostigma reticulatum* where steroids and saponin were absent but all other phytochemicals are presnt. These compounds play important roles in bioactivity of medicinal plants, thus medicinal values of these plants rely on the embedded phytochemicals and as such produce definite physiological actions on human body. Flavonoid one of the phytochemicals resident in *A. laxiflora* leaf extract and *Piliostigma reticulatum*is known to play important roles in cleaning human bodies of oxides generated during metabolism [29]. Tanin were found to be useful to human physiological activities such as phagocyte cell, host mediated activity and a wide range of anti-effective action [30].

The elemental composition of *Alchornea laxiflora*shows that Mg, Zn, K, Mn, Ca and Na are present in large quantity and Fe, Cu, and P in lesser quantity compared to *Piliostigma reticulatum*where Na, Ca and Mn are present in high quantity than in*Alchornea laxiflora*. Fe, Cu and P are also present in lesser quantity in *P. reticulatum*. Pb is not present in any of the plant. Minerals in plants are essential for growth as some serves as protein building block. There are trace and major minerals in plant. The amount of major and trace minerals the body needs is small, but the importance of these nutrients is huge. Humans needs these minerals to maintain health, they transport life-giving oxygen to the body; aid in assimilation of other nutrient; form building blocks such as amino acids, hormones and proteins; Basically, the entire body including your hair, nails, bones, blood and nerve relies on major and trace minerals for it proper function.

Anti-nutrients present in *Alchornea laxiflora*in phenol was very high while the highest in *Piliostigma reticulatum*was oxalate and the least was observed in flavonoids in *Alchornea laxiflora*.Alkaloids was the least in *Piliostigma reticulatum*plants.

The proximate analysis of *Piliostigma reticulatum*showed that ash, fibre and carbohydrate are present in high quantity while moisture content, crude protein and fat, were present in lesser quantity in *Piliostigma reticulatum*, while *Alchornea laxiflora*showed that crude protein, carbohydrate and moisture content has higher quantity and lesser quantity was observed in ash, moisture content and fat. This shows that the plant contain some vital nutrient that can support life and grow healthy. *Piliostigma reticulatum* has high activity in phenol, Ferric reducing property, Azino-bis (3-ethylbenthiazoline-6-sulphuric acid), Superoxide and Diphenyl -2-picryhydrazylcompared to *Alchornea laxiflora*.

Conclusion

This study suggested that *Piliostigma reticulatum* and *Alchornea laxiflora* with unique chemical compounds that inhibit the growth of bacteria and fungi or kill them, are effective in the treatment of infectious diseases and areconsidered as potential candidates for developing new antimicrobial drugs.

Recommendation

The use of local herbs or medicinal plant in general should be encouraged by health practitioners and government agencies.

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