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EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF LEAF EXTRACT OF *CHRYSOPHYLLUM ALBIDUM* AGAINST SELECTED ENTERIC BACTERIAL PATHOGENS

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ABSTRACT

This study investigated the antibacterial and antioxidant potentials of *Chrysophyllum albidum* leaf extract against selected enteric bacterial pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi*, *Proteus mirabilis* and *Proteus vulgaris*). Fresh leaves of *Chrysophyllum albidum* were shade air-dried and ground into fine powder. Thereafter, the leaves powder was cold extracted using methanol and sterile distilled water in ratio 3:2(v/v). The mixture obtained was concentrated *in vacuo* using a rotary evaporator and lyophilized. The crude extract was screened for antibacterial, phytochemicals and antioxidant properties. The antibacterial properties were determined using agar well diffusion and agar dilution methods while the antioxidant and phytochemical assay were analyzed using standard methods. The phytochemical screening of the extract revealed the presence of tannins, alkaloids, flavonoids, saponins, steroids, terpenoids, reducing sugar and cardiac glycosides. The zones of inhibition shown by the extract at 10 mg/mL against the bacterial isolates ranged between 10 mm and 22 mm. The highest zone of inhibition (22 mm) was expressed against *Escherichia coli* at a concentration of 10 mg/mL. The MICs ranged between 1.25 mg/mL and 5mg/mL while MBCs ranged between 2.5 mg/mL and 10 mg/mL. The antioxidant assay of leaf extract showed appreciable antioxidant potential when compared with ascorbic acid used as standard. The leaf extract exhibited percentage of 92.03% at a concentration of 500 µg/mL while ascorbic acid exhibited percentage of 96.54% at the same concentration. This study, therefore showed that leaf extract of *Chrysophyllum albidum* exhibited significant antibacterial and antioxidant activities against the test isolates.

Keywords: Antibacterial properties, *Chrysophyllum albidum*, Enteric bacterial pathogens, Phytochemicals, Antioxidant assay

INTRODUCTION

Plants have been used for alleviating human suffering from the very beginning of human civilization, and records of the use of plants are available since about 5000 years ago. The bioactive constituents isolated from medicinal plants have provided leads in the development of several lifesaving drugs, which are in use today. Indigenous system of medicines was developed by different civilizations (Gupta et al., 2010). Several studies conducted by World Health Organization, showed that 80% of the world's population depend on using traditional medicine (WHO, 2014). According to De Ghosh, 2014, there are more than 350,659 medicinal plants globally of which only 2% have been explored and scientifically validated based on their phytochemical's composition and effective bioactivities. These bioactive constituents are used as a starting point for antibiotics synthesis in order to treat infectious diseases (Rahman and Anwar, 2007). They are also a rich source of nutrition, hence can be used as preventive too (Sedighi *et al*., 2017). These bioactive principles in medicinal plants exposed them to human exploitation and these are basically secondary metabolites with proven significant pharmacological properties, produced during the plant's metabolism as a form of defense mechanism against invasion of pathogens, pests and other foreign bodies (Ning *et al*., 2009; Akinpelu *et al*., 2015; Anantaworasakul *et al*., 2017).

The plant secondary metabolites such as flavonoids, terpenoids, alkaloids, reducing sugars, steroids, tannins and cardiac glycosides have been deeply studied on how they are used in modern day medicine in the treatment of diseases such as toothache, wound infections, diarrhea, snakebite, paralysis among others (Sharma and Kaur, 2017). The plants products may possess a new source of antimicrobial agents with possible novel mechanisms of action (Ahmad and Aqil, 2007; Barbour *et al*., 2004). The natural antimicrobial compounds can act alone or in combination with antibiotics to enhance antimicrobial activity against a wide range of microbes (Rios and Reccio, 2005; Savoia, 2012). Among the plant that contains these natural antimicrobials is *Chrysophyllum albidum*.

Chrysophyllum albidum (Family Sapotaceae) is often called the white star apple or African star apple (Idowu *et al.*, 2006). *Chrysophyllum albidum* is widely distributed in Nigeria, Uganda, Niger, Cameroun and Cote d' Voire (Duyilemi and Lawal, 2009, Adebayo *et al*., 2011). The plant is distributed in Nigeria within the southern part of the country especially in the South-West and South-East and its cultivation is highly ecologically specific (Oyebade *et al*., 2011). It is commonly called agbalumo in Yoruba (South western Nigeria), 'Udara' in Igbo (South eastern Nigeria) and agbaluba in Hausa (North eastern Nigeria). *Chrysophyllum albidum* is used in Nigeria for the treatment of different ailments (Idowu *et al*., 2006). The stem barks are used for malaria and yellow fever treatment (Bello and Henry, 2015). The stem barks and root are used in urinary related infections (Florence and Adiaha, 2015). The leaves are used as emollients and for the treatment of skin eruptions, diarrhoea and stomach ache (Adisa, 2000). It is also used for cancer remedy in Cuba (MacDonald *et al*., 2014). In South western Nigeria, the leaves are used for the management of infections or ailments since prehistoric times (Duyilemi and Lawal, 2009). The fruits are known as a natural

source of antioxidants that can reduce free radical mediated diseases such as diabetes, cancer and coronary heart disease (Ibrahim *et al*., 2017). Several researchers have carried out the *in vitro* inhibitory effects of extracts from plants against bacterial isolates. There is paucity of data on the antibacterial and antioxidant potentials of *Chrysophyllum albidum* leaves extracts against enteric bacterial pathogens. Hence, this study was therefore designed to assessed the antibacterial and antioxidant potentials of *Chrysophyllum albidum* leaf extract against selected enteric bacterial pathogens.

MATERIALS AND METHODS

Preparation of test isolates

The test isolates used in this study include clinical and reference strains. The clinical strains were collected from the culture room of Microbiology Laboratory, Aminu Kano Teaching Hospital, Kano, Kano State, Nigeria. These include *Escherichia coli*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus mirabilis* and *Salmonella typhi* while *Escherichia coli* (ATCC 25922), *Shigella dysenteria*e (ATCC 13313), *Klebsiella pneumoniae* (ATCC 13883), *Proteus vulgaris* (ATCC 29905) and *Salmonella typhi* (ATCC 14208) were reference strains. They were obtained from American Type Culture Collection (ATCC), Manassas, Virginia, United States of America. The test isolates identities were confirmed based on cultural, morphological and biochemical laboratory tests. After confirmation, the bacterial isolates were sub-cultured in nutrient broth, incubated at 37ºC for 18 h and then standardized to 0.5 McFarland standard (10^6 cfu/mL) before use.

Culture media used

Nutrient agar medium was used for sub-culturing the test isolates, while Mueller Hinton agar medium was used for the sensitivity testing.

Plant Materials

Fresh leaves of *Chrysophyllum albidum* (G. Don) were collected in Ile-Ife, Osun State, Nigeria in the month of December, 2020. The leaves were identified and authenticated at the Herbarium, Plant Biology Department, Bayero University, Kano, Kano State, Nigeria by Mr Baha'uddeen Said Adam. Voucher specimen was prepared and deposited for reference purposes under herbarium accession number BUKHAN 0522.

Preparation of the Plant extract

The *C. albidum* leaves were washed thoroughly under tap water, shade air dried, milled into powdery form and sieved using filter to obtain fine ground particles. Exactly, 1500 g of powdered leaves were extracted using methanol and sterile distilled water in the ratio of 3:2 (v/v) for four days, with regular agitation at time intervals. The supernatant collected were filtered using number 1 Whatman filter paper and the filtrate was concentrated *in vacuo* using a rotary evaporator to eliminate the methanol. The aqueous residue left was then lyophilized to obtain a 179. 8 g of a dark brown crude extract.

Qualitative phytochemical tests

The *C. albidum* leaf extract was subjected to phytochemical screening using the methods described by Trease and Evans (2002) and Harborne (2006).

Quantitative phytochemical tests

The methods described by Makinde and Obih (1985), Obdoni and Ochuko (2001), Ejikeme *et al*., (2014), Boham and Kocipai-Abyazan (1974) and Indumathi *et al*., (2014) were used to determine the quantity of each bioactive constituent present in the leaf extract.

Determination of Antioxidant Property of *C. albidum* Leaf Extract

The antioxidant property of the leaf extract was assessed on the basis of its ability to reduce the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay as described by Brand-Williams *et al*. (1995). Different concentrations of *C. albidum* leaf extract were prepared using analytical methanol. Ascorbic acid (Vitamin C) was used as an antioxidant standard. Exactly, 1 mL from each extract and 3 mL of methanol were mixed with 0.5 mL of 1.0 Mm DPPH in methanol and allow to react at room temperature for 30 minutes. The absorbance was measured using a spectrophotometer at a wavelength of 517 nm. The blank solution was prepared using the same amount of methanol and DPPH. The DPPH with solvent (without extract) serves as the control. All measurements were made in triplicate and averaged. The ability of the extract to scavenge DPPH radical was calculated using the following equation:

DPPH radical scavenging activity (% inhibition)

$$= \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Where A_{blank} = Absorption of the blank, A_{sample} = Absorption of the extract.

Sensitivity test of leaf extract of *C. albidum* against test isolates

The antibacterial activity of the leaf extract was determined using agar-well diffusion method (Hugo and Russel, 2015) with little modifications. Exactly, 0.1 mL of the standardized test isolates (0.5 McFarland standard) was inoculated into molten Mueller Hinton Agar (Oxoid, UK), poured into sterile Petri dishes and allowed to set. The wells were then bored into the agar medium using a sterile cork borer with diameter of 6 mm. The wells were filled with 0.1 mL prepared solutions of the extract at a concentration of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL and 0.63 mg/mL and care was taken not to allow spillage of the solution on the surface of the medium. The plates were allowed to stand on the laboratory bench for about 1 h before incubating the plates in an upright position at 37ºC for 24 hours. The plates were later observed for zones of inhibition and the diameters of zones of inhibition were measured using millimeter rule. The sensitivity of the isolates to the extracts were compared with that of streptomycin, ampicillin, tetracycline and ceftriaxone at a concentration of 1 mg/mL each which served as positive control. Exactly, 10% Dimethyl sulfoxide (DMSO) served as negative control and the experiment was carried out in triplicates.

Determination of minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations of the leaf extract were determined by agar dilution method following standard procedure (EUCAST, 2000; Akinpelu *et al*., 2015). Two-fold dilution of the leaf extract were prepared in sterile distilled water and 2 mL of different concentrations of the solution was added to 18 mL of sterile molten Nutrient agar to give final concentration regimes of 0.16 mg/mL to 10 mg/mL. The medium was then poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry before streaking with 18 h old standardized bacteria cultures. The plates were later incubated at 37ºC for up to 48 h after which they were examined for the presence or absence of growth. Sterile agar medium plate without the extract served as

control. Minimum inhibitory concentrations were taken as the lowest concentration that prevented the growth of the bacteria.

Determination of minimum bactericidal concentrations (MBCs)

The minimum bactericidal concentrations of the leaf extract were determined by using Akinpelu *et al*. (2015) method. Samples for the MBCs were taken from the line of streaks without visible growth on the MIC plates and sub-cultured onto freshly prepared Nutrient agar plates and later incubated at 37ºC for 48 hours. The MBCs was taken as the lowest concentration of the extract that did not show any growth on a new set of plates.

Statistical analysis

Data were expressed as mean±SD (standard deviation) of three replicates and were statistically analysed using one-way analysis of variance (ANOVA). Values were considered significant at $p \leq 0.05$.

RESULTS

The leaf extract exhibited significant antibacterial potentials against the test isolates used for this study. The leaf extract exhibited antibacterial potentials at different concentrations. The zones of inhibition expressed by the leaf extract against the test isolates ranged between 10 mm and 22 mm (Table 1). The zones of inhibition expressed by *Escherichia coli* at different concentrations ranged between 10 mm and 22 mm, *Salmomella typhi* (10 mm and 19 mm), *Klebsiella pneumoniae* (10 mm and 20 mm), *Proteus vulgaris* (10 mm and 19 mm) and *Proteus mirabilis* (10 mm and 16 mm). The highest zone of inhibition 22 mm was expressed against *Escherichia coli* at a concentration of 10 mg/mL. Some test isolates were resistant to the standard antibiotics used for this study while some were susceptible at a concentration of 1 mg/mL. *Proteus vulgaris* demonstrated 83.3% resistance to streptomycin followed by *Proteus mirabilis* (80%), *Salmonella typhi* (72.7%), *Escherichia coli* (36.4%), *Klebsiella pneumoniae* (36.4%) and *Shigella dysenteriae* (33.3%). High frequency of resistance (83.3%) was observed by *Proteus vulgaris* and *Shigella dysenteriae* against streptomycin and ampicillin. The standard antibiotics, streptomycin, ampicillin, ceftriaxone and tetracycline each at a concentration of 1 mg/mL inhibited the growth of 30, 26, 49 and 44 out of 50 test isolates respectively. The zones of inhibition expressed by the standard antibiotics used as a positive control ranged between 10 mm and 34 mm. The leaf extract did not inhibit the growth of test isolates at a concentration of 0.63 mg/mL (Table 1).

Table 1: Sensitivity patterns exhibited by the leaf extract of *Chrysophyllum albidum* against test isolates

	Zones of inhibition (mm)**									
Isolates code	**10 mg/mL**	**5 mg/mL**	**2.5 mg/mL**	**1.25 mg/mL**	**0.63 mg/mL**	**STP (1mg/mL)**	**AMP (1mg/mL)**	**CFX (1mg/mL)**	**TET (1mg/mL)**	**DMSO (10%)**
EC1	12±0.82	10±0.00	0	0	0	10±0.82	0	26±0.00	24±1.63	0
EC2	19±1.63	15±0.00	12±0.82	0	0	0	18±1.63	24±0.82	22±0.00	0
EC3	12±0.82	10±0.82	0	0	0	24±0.82	22±0.00	28±0.82	26±1.63	0
EC4	14±1.63	12±1.63	0	0	0	23±0.82	0	30±0.82	27±0.00	0
EC5	14±0.00	12±1.63	10±0.00	0	0	24±1.63	12±0.00	25±0.82	21±0.82	0
EC6	12±0.82	10±0.00	0	0	0	0	20±0.82	26±0.00	23±0.82	0
EC7	22±0.00	18±1.63	15±0.82	12±0.00	0	26±0.82	28±0.82	34±0.82	32±0.82	0
EC8	16±0.82	12±1.63	10±0.00	0	0	22±0.82	26±0.00	26±0.82	28±0.00	0
EC9	20±1.63	16±0.00	11±0.82	0	0	0	0	26±0.00	22±0.82	0
EC10	14±0.82	12±0.00	10±0.82	0	0	16±0.82	0	21±0.82	20±0.82	0
EC25922	19±0.82	14±0.00	12±0.82	10±0.82	0	24±0.82	22±0.00	28±0.82	26±0.00	0
ST1	12±0.00	10±0.82	0	0	0	13±0.82	0	27±0.82	20±0.82	0
ST2	19±0.00	15±1.63	12±0.00	0	0	20±0.82	0	24±0.82	22±0.82	0
ST3	14±1.63	12±0.00	10±0.82	0	0	10±0.00	20±0.82	24±1.63	24±0.00	0
ST4	19±0.82	14±0.82	11±0.00	0	0	13±0.82	22±0.82	26±0.82	22±0.82	0
ST5	15±0.82	13±0.82	11±0.00	0	0	0	0	23±0.82	21±0.82	0
ST6	16±0.00	13±0.00	11±0.82	0	0	12±0.00	20±1.63	23±0.82	18±0.82	0
ST7	19±1.63	16±1.63	13±0.00	11±0.00	0	24±0.82	26±0.00	28±0.82	25±0.82	0
ST8	13±0.82	11±0.00	0	0	0	0	22±0.82	25±0.82	22±0.00	0
ST9	14±0.00	12±0.82	0	0	0	0	0	24±0.82	12±1.63	0
ST10	16±0.00	14±0.82	11±0.00	0	0	23±0.82	21±0.82	26±0.00	26±1.63	0
ST14028	14±0.82	12±0.82	10±0.82	0	0	0	0	22±0.82	20±0.82	0
KP1	18±0.82	16±1.63	14±0.00	12±0.82	0	19±0.00	12±0.82	13±0.82	0	0
KP2	12±0.82	10±0.00	0	0	0	16±0.82	0	18±0.82	13±0.82	0
KP3	13±1.63	11±0.00	0	0	0	0	13±0.82	24±0.82	20±0.00	0
KP4	14±0.82	12±0.82	0	0	0	21±0.00	19±0.82	26±0.82	24±0.82	0
KP5	19±0.00	16±0.00	13±1.63	10±0.00	0	24±0.82	22±0.00	29±0.82	26±0.82	0
KP6	12±0.00	10±0.82	0	0	0	0	18±0.82	20±1.63	12±0.82	0
KP7	14±1.63	12±1.63	10±0.00	0	0	20±0.82	0	23±0.82	0	0
KP8	20±1.63	15±0.82	12±0.82	0	0	22±0.82	20±0.82	28±0.82	26±0.82	0
KP9	13±0.00	11±0.82	0	0	0	13±0.82	12±0.00	22±0.82	22±0.82	0
KP10	14±0.82	12±0.00	0	0	0	10±0.82	12±0.00	20±0.82	18±0.82	0
KP13883	14±0.82	12±1.63	0	0	0	18±0.00	0	20±0.00	13±0.82	0
SD1	12±0.82	10±0.00	0	0	0	0	0	18±0.82	22±1.63	0

SD2	14±1.63	12±0.82	0	0	0	21±0.82	19±0.82	24±0.82	24±1.63	0
SD3	12±1.63	10±0.00	0	0	0	25±0.00	0	26±0.82	23±0.82	0
SD4	14±0.82	12±0.82	10±0.00	0	0	18±0.00	0	20±0.00	0	0
SD5	20±0.82	16±0.00	12±1.63	10±0.00	0	26±0.00	0	32±0.82	30±0.82	0
SD13313	12±1.63	10±0.00	0	0	0	0	0	24±1.63	20±0.00	0
PV1	12±0.82	10±0.82	0	0	0	0	0	22±0.82	24±0.00	0
PV2	14±0.82	12±1.63	10±1.63	0	0	0	23±0.00	26±0.82	26±1.63	0
PV3	12±0.00	10±0.00	0	0	0	0	0	21±0.82	19±0.82	0
PV4	12±0.82	10±0.00	0	0	0	21±0.82	0	23±0.82	0	0
PV5	19±1.63	16±0.00	11±0.82	0	0	0	24±0.82	26±0.00	24±0.82	0
PV29905	16±0.82	14±0.82	12±0.00	0	0	0	0	20±0.82	22±1.63	0
PM1	16±0.00	14±0.82	11±0.00	0	0	18±0.82	20±0.00	23±0.82	20±0.82	0
PM2	14±1.63	12±0.82	10±0.00	0	0	0	0	20±0.82	13±0.00	0
PM3	12±1.63	10±0.00	0	0	0	0	0	12±0.82	0	0
PM4	14±0.82	11±0.82	0	0	0	0	0	0	12±0.00	0
PM5	16±0.82	14±1.63	12±0.00	10±0.00	0	0	18±0.82	21±0.82	0	0

Key: EC1-EC10=Strains of *Escherichia coli*, EC11 = *Escherichia coli* (ATCC 25922), ST1-ST10 = Strains of *Salmonella typhi*, ST11 = *Salmonella typhi* (ATCC 14028), KP1-KP10 = Strains of *Klebsiella pneumoniae*, KP11= *Klebsiella pneumoniae* (ATCC 13883), SD1-SD5 = Strains of *Shigella dysenteriae*, SD6 = *Shigella dysenteriae* (ATCC 13313), PV1-PV5 = Strains of *Proteus vulgaris*, PV6 =*Proteus vulgaris* (ATCC 29905), PM1-PM5=Strains of *Proteus mirabilis*, ATCC=American type culture collection, STP= Streptomycin, AMP = Ampicillin, CFX = Ceftriaxone, TET =Tetracycline, 0= Not sensitive, mm* = mean of three replicates, P≤ 0.05, DMSO = Dimethyl sulfoxide

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations(MBCs) exhibited by the leaf extract against the test isolates were shown in Table 2. The MIC exhibited by the leaf extract against the test isolates ranged between 1.25 mg/mL and 5mgmL while the lowest MICs of 1.25 mg/mL was exhibited against all the test isolates except *Proteus vulgaris*. The highest MICs of 10 mg/mL was observed against *Proteus vulgaris* (PV3). The MBCs of the leaf extract against the test isolates ranged between 2.5 mg/mL and 10 mg/mL while the lowest MBCs of 2.5 mg/mL was observed against all the test isolates except *Proteus vulgaris*.

Table 2: The minimum inhibitory and bactericidal concentrations exhibited by the leaf extract against bacterial isolates

Isolates Code	MIC (mg/mL)	MBC (mg/mL)
EC1	5	5
EC2	2.5	5
EC3	5	10
EC4	5	5
EC5	2.5	5
EC6	5	10
EC7	1.25	2.5
EC8	2.5	5
EC9	2.5	5
EC10	2.5	2.5
EC25922	1.25	2.5
ST1	5	10
ST2	2.5	5
ST3	2.5	5
ST4	2.5	5
ST5	2.5	5
ST6	2.5	5
ST7	1.25	2.5
ST8	5	10
ST9	5	5
ST10	2.5	5
ST14028	2.5	5
KP1	1.25	2.5
KP2	5	10
KP3	5	10
KP4	5	10
KP5	1.25	2.5
KP6	5	5
KP7	2.5	5
KP8	2.5	2.5

KP9	5	10
KP10	5	10
KP13883	5	10
SD1	5	5
SD2	5	10
SD3	5	10
SD4	2.5	5
SD5	1.25	2.5
SD13313	5	5
PV1	5	10
PV2	2.5	5
PV3	10	ND
PV4	5	10
PV5	2.5	5
PV29905	2.5	5
PM1	2.5	2.5
PM2	2.5	5
PM3	5	10
PM4	5	10
PM5	1.25	2.5

Key: EC1-EC10 = *Escherichia coli*, EC11 = *Escherichia coli* (ATCC 25922), ST1-ST10 = *Salmonella typhi*, ST11 = *Salmonella typhi* (ATCC 14028), KP1-KP10 =*Klebsiella pneumoniae*, KP11= *Klebsiella pneumoniae* (ATCC 13883), SD1-SD5=*Shigella dysenteriae*, SD6 = *Shigella dysenteriae* (ATCC 13313), PV1-PV5 = *Proteus vulgaris*, PV6 = *Proteus vulgaris* (ATCC 29905), PM1-PM5 =*Proteus mirabilis*, ATCC = American type culture collection, ND = Not determined

The result of qualitative phytochemical screening of the *Chrysophyllum albidum* leaf extract revealed the presence of alkaloids, tannins, flavonoids, saponins, steroids, terpenoids, reducing sugar and cardiac glycosides (Table 3).

Table 3: Qualitative phytochemical composition of the *Chrysophyllum albidum* leaf extract

Chemical test	Results
Tannins	Positive
Alkaloids	Positive
Flavonoids	Positive
Saponins	Positive
Steroids	Positive
Terpenoids	Positive
Reducing sugar	Positive
Cardiac glycosides	Positive

Quantitative phytochemical analysis of the plant indicated that the leaf extract contains the phytochemicals in varying amounts. The bioactive compound with the highest quantity was flavonoids followed by saponins, alkaloids, tannins, phenols and terpenoids respectively as shown in Table 4.

Table 4: Quantitative phytochemical composition of the *Chrysophyllum albidum* leaf extract

Chemical test	Result (%)
Tannins	0.94±0.01
Alkaloids	11.00±0.01
Flavonoids	18.03±0.10
Saponins	16.01±0.01
Phenols	0.72±0.01
Terpenoids	0.40±0.16

The antioxidant property of *Chrysophyllum albidum* leaf extract was measured by the ability to scavenge DPPH free radicals comparing with ascorbic acid (Vitamin C). The scavenging effects of the extracts and the standard on the DPPH radical were shown in Table 5 and Figure 1. According to the result obtained, *Chrysophyllum albidum* leaf extract showed significant antioxidant activity compared to ascorbic acid (Vitamin C) used as standard. For instance, *C. albidum* leaf extract expressed percentage of 92.03% at a concentration of 500 µg/mL while ascorbic acid exhibited percentage of 96.54% at the same concentration.

Table 5: Antioxidant properties of *C. albidum* leaf extract

	DPPH radical scavenging activity (% inhibition)	
Concentration ((µg/ml)	**Leaf extract**	**Ascorbic acid**
15.625	53.32	63.53
31.25	71.18	90.66
62.50	84.70	90.90
125	88.16	95.77
250	91.63	98.89
500	92.03	96.54
1000	94.20	97.34

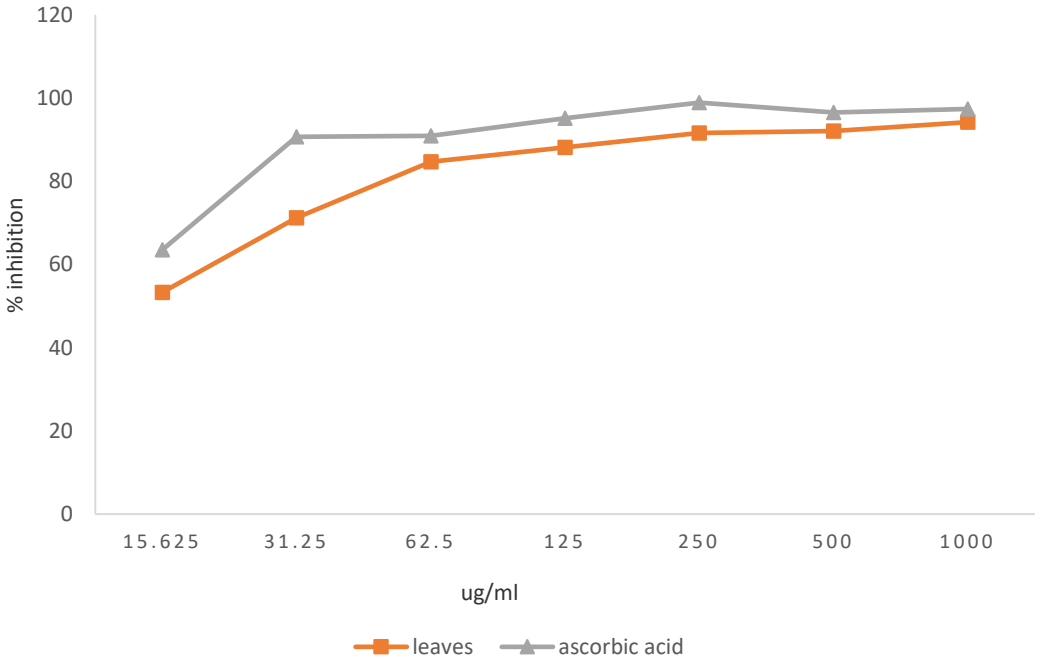

Figure 1: Antioxidant activity of *Chrysophyllum albidum* leaf extract

DISCUSSION

The antibacterial potentials of leaf extract of *Chrysophyllum albidum* were investigated against various strains of enteric bacterial pathogens from clinical samples. These organisms include *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Proteus vulgaris* and *Proteus mirabilis*. The leaf extract demonstrated a significant bioactivity against the test isolates. The *C. albidum* leaf extract at a concentration of 5 mg/mL and 10 mg/mL inhibited the growth of all the test isolates while the leaf extract did not inhibit the growth of test isolates at a concentration of 0.63 mg/Ml. The zones of inhibition demonstrated by the leaf extract against the test isolates ranged between 10 mm and 22 mm. The zones of inhibition expressed by *Escherichia coli* at different concentrations ranged between 10 mm and 22 mm, *Salmomella typhi* (10 mm and 19 mm), *Klebsiella pneumoniae* (10 mm and 20 mm), *Proteus vulgaris* (10 mm and 19 mm) and *Proteus mirabilis* (10 mm and 16 mm). This affirmed the previous work on the antibacterial potentials of this plant (Duyilemi and Lawal, 2009; Adeleye *et al*., 2016; George *et al*., 2018). Thus, the leaf extract of *C. albidum* could serve as a pointer towards the development of drugs of natural origin to combat the infections caused by these pathogens and reduced or prevent death of patients. Also, the susceptibility of these pathogens to the extract further affirms its efficacy in the management of infectious diseases by the people of Southern part of Nigeria as earlier reported by Laurent *et al*., (2012). Also, antibacterial activity of the leaf extract of the plant were found to be concentration dependent as the highest activity/zones of inhibition was at the highest concentration (10 mg/mL) followed by 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL and 0.63 mg/mL (Table 1). On the other hand, streptomycin, ampicillin, ceftriaxone and tetracycline at a concentration of 1 mg/mL inhibited the growth of 30, 26, 49 and 44 out of 50 bacterial isolates respectively. The zones of inhibition ranges between 10 mm and 34 mm.

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the *C. albidum* leaf extract were also investigated. The MICs exhibited by the leaf extract against the test isolates ranged between 1.25 mg/mL and 5mgmL while the lowest MICs of 1.25 mg/mL was exhibited against all the test isolates except *Proteus vulgaris*. The highest MICs of 10 mg/mL was observed against *Proteus vulgaris* (PV3). The MBCs of the leaf extract against the test isolates ranged between 2.5 mg/mL and 10 mg/mL while the lowest MBCs of 2.5 mg/mL was observed against all the test isolates except *Proteus vulgaris* (Table 2). Medicinal plant extract with very low MIC and MBC is known to possess significant antimicrobial potentials (Achinto and Munirrudin, 2009) thus, supported this finding. The antibacterial activity of plant extract is considered significant if the MIC of the extract is less than or equal to 200 mg/mL (Suffredini *et al*., 2006). The MICs exhibited by the leaf extract was below 200 mg/mL as observed in this study. Hence, potent antimicrobial compounds could be developed from this plant for the treatment of infections caused by multi-resistant microorganisms.

The phytochemical analysis of *C. albidum* leaf extract revealed the presence of tannins, alkaloids, flavonoids, saponins, steroids, reducing sugars, terpenoids and cardiac glycosides (Table 3). This agrees with the work of previous researchers (Oladipupo, 2014; Ushie *et al*., 2014; Ojemeleke *et al*., 2018) who reported the presence of these phytochemicals in the crude extracts of *Chrysophyllum albidum*. Quantitative analysis of the pharmacologically important phytochemicals in the plant indicated that the crude extracts contain these phytochemicals in varying amounts in the leaves (Table 4). The phytochemical with the highest quantity was flavonoids, followed by saponins, alkaloids, tannins, phenols and terpenoids respectively (Table 4). Previous phytochemical analysis of the leaves, stem bark and root extracts of *Chrysophyllum albidum* showed large quantities of flavonoids, alkaloids, saponins, tannins, phenol, steroids and Cardiac glycosides (Okoli and Okere, 2010; Owolabi *et al*., 2017, Akanji, 2020), complementing the results of this study. These bioactive compounds are known to be biologically active and contribute to the antimicrobial and antioxidant activities of medicinal plants (Trease and Evans, 2002).

This is an indication that these phytochemicals contributed to the antibacterial potentials of the plant extract against test isolates used for this study (Odewade *et al*., 2021). These bioactive compounds exert antimicrobial activity through different mechanisms; for example, tannins act by forming irreversible complexes with proline-rich proteins (Shimada, 2006) resulting in the inhibition of the cell protein synthesis. Also, tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflammed tissues (Parekh and Chanda, 2007). The pharmacological activities of the saponins include antioxidant, anticancer, anti-inflammatory, antifungal and antibacterial (Ramachandran *et al*., 2014; Mboweni, 2018; Mapiye, 2019).

Saponins which have been ascertained to be responsible for numerous pharmacological properties (Estrada *et al*., 2000) were also present in *C. albidum* leaf extract. They are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects in medicinal plants (Liu and Henkel, 2002).

Alkaloid is one of the phytochemical compounds revealed in the extract of *C. albidum* leaf extract. Alkaloids have been found to possess antibacterial, antiviral, anticancer, antifungal, antimalarial and antihyperglycemic activities (Namadina *et al*., 2019; Thawabteh *et al*., 2019; Casciaro *et al*., 2020). Kamba and Hassan (2011) and Okoli and Okere, (2010) also reported the presence of alkaloids in leaves and stem bark extract of this plant.

Chrysophyllum albidum extract also tested positive for terpenoids. They are known to facilitate membrane disruption using lipophilic compounds and they possess a quite number of medicinal properties such as anti-carcinogenic, antimalarial, antihypertensive, insecticidal, antiviral and anti-ulcer (Saxena *et al*., 2013; Kabera *et al*., 2014; Ndongo, 2017).

Flavonoid was another phytochemical compound detected in *C. albidum* leaf extract. They exhibit a wide range of biological activities which include antimicrobial, anti-inflammatory, antitumor, anti-carcinogenic, anti-aging analgesic, anti-allergic effects, antiviral and antioxidant properties (Hodek *et al*., 2002; Saxena *et al;* 2013; Ndongo, 2017). Okoli and Okere (2010) reported that flavonoids are potent water-soluble super antioxidants and free radical scavengers which prevent oxidative cell damage.

The stability of DPPH free radical method is a sensitive way of determining the antioxidant property of plant extract (Kumar *et al*., 2008). The leaf extract of *C. albidum* exhibited antioxidant activity and this compared favourably with ascorbic acid (Vitamin C) used as standard (Table 5 and Figure 1). This finding supported the work reported by George *et al*., (2018) in which the methanolic leaf and fruit extracts of *C. albidum* had high antioxidant properties similar to vitamin C and E. *Chrysophyllum albidum* leaf extract significantly inhibited hydroxyl radicals produced by DPPH in this study and could serve as a free radical's inhibitor or scavenger using its proton donating ability. This study further supports the usefulness of the *C. albidum* in scavenging hydroxyl radicals (OH^-) formed in the biological systems of humans which has been recognized as inhibitory extremely damaging (Rajeev *et al*., 2011). Medicinal plants that exhibit antioxidant properties are known to possess free radical scavenging ability (Atawodi, 2005) and this antioxidant potential in plants is majorly due to the phenolics components present in them (Pourmorad *et al*., 2006). High antioxidant property observed for *C. albidum* extracts may serve as a pointer towards development of antioxidant drug of natural origin from this plant and such drug could go a long way in healthcare delivery.

CONCLUSION

Chrysophyllum albidum leaf extract demonstrated significant antibacterial and antioxidant properties against selected enteric bacterial pathogens which further affirms its significance as a folklore remedy. It also established that the *C. albidum* leaf extract can serve as a veritable source of bioactive substance of natural origin required to combat increasing resistance of pathogens to the existing antibiotics.

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