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Original article

Evaluation of the antibacterial potential of ethyl alcohol leaf extract of *Chrysophyllum albidum* (G. Don) against five bacterial isolates

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ABSTRACT

Background: In contributing to solving the global challenge of antimicrobial resistance, an *in- vitro* experiment was conducted to evaluate the antibacterial potential of ethyl alcohol leaf extract of *Chrysophyllum albidum* (G. Don) against five clinical pathogenic microorganisms namely: *Escherichia coli* (EC), *Proteus mirabilis* (PM), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA), and *Salmonella typhimurium* (ST). **Methods:** Agar well diffusion method was adopted and the exhibited zones of inhibition by the isolates were measured, and taken as the antimicrobial potential of the extract. Phytochemical screening of the leaf extract revealed the presence of tannins, saponins, terpenoids, steroids, flavonoids and cardiac glycosides. Sixteen different concentrations (0.003 – 100 mg/mL) of the extract were prepared to establish the minimum inhibitory concentrations (MICs). **Results:** The MICs ranged between 0.006 - 0.195 mg/mL. *Staphylococcus aureus* with MIC of 0.006 mg/mL was the most sensitive among all the isolates to the extract while *E. coli* was resistant. *Pseudomonas aeruginosa* was resistant to all the tested (control) commercial antibiotics but highly sensitive to the extract with MIC of 0.195 mg/mL respectively. **Conclusion:** It was concluded that *C. albidum* is a medicinal plant possessing a broad spectrum antimicrobial activities and hence could be explored for the production of antibiotic by phyto-pharmaceutical establishments.

Introduction

The resistance of many medically important pathogenic organisms to many commercial antibiotics has propelled researchers and scientists to keep on sourcing for alternatives among which medicinal plant with biologically active compounds against many pathogenic microorganisms have come to limelight [1]. The use of plant products as therapeutic agents dated back over five thousand years ago [2]. It was reported by **Friedman et al.** and **Serafino et al.** [3, 4] that the effectiveness of many plant extracts by far outweighs that of synthetic drugs coupled with the fact that medicinal plants have little or no side effects. *C. albidum* (G. Don) has been reported by **Bruits and Bucar** [5] to possess antimicrobial, antioxidant, anti-inflammatory, and anti-cancer properties. The folkloric use of the plant in the treatment of dysentery, wound, sepsis, cough, and infections was reported by **Vade** [6]; hence the choice of *C. albidum* (G. Don) plant in this experiment against five clinical pathogenic microorganisms. *Pseudomonas aeruginosa* infects people with low resistance, such as cystic fibrosis patients, it can invade burns and cause urinary tract infections [7]. Its genome has an unusually large number of genes for catabolism, nutrient transport, the efflux of organic molecules and metabolic

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regulation. This may explain why it is inherently resistant to many antibiotics [8] and the treatment of infections due to the bacterium to be herculean task [9]. This organism has been implicated in several lung diseases and most often do present itself as opportunistic pathogen causing infections in compromised patients [7]. *Staphylococcus aureus* has been found on the skin, skin glands and mucous membranes of warm-blooded animals. Strains of methicillin resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) are among the most threatening antibiotic resistant pathogens. Vancomycin is considered the "drug of last resort", and infections caused by vancomycin-resistant *S. aureus* generally cannot be treated by antibiotic therapy. *S. aureus* is a major cause of food poisoning as it grows in meats, dairy and bakery products and produce enterotoxins [8]. *Salmonella typhimurium* has been found to be resistant to at least five microbial agents. It is the causative agent of typhoid fever, normally invades the epithelium and reaches the lymph-nodes, liver, spleen and gall bladder [8, 10]. *Escherichia coli* generally can be found in association with ingestion of undercooked ground beef and unpasteurized fruit juices. This organism grows in the gut to cause traveller's diarrhoea [8]. According to **Tenaillon et al.** [11], *E. coli* is a fast-growing bacterium and this characteristic has made the bacterium suitable for various studies. *Proteus mirabilis* is well known to be commensal in the gastrointestinal tract of animals and humans [12]. *Proteus spp*. can be naturally resistant to antibiotics, such as benzylepenicillin, oxacillin, tetracycline, macrolides [13], and can acquire resistance to ampicillin through plasmid mediated beta-lactamases, and chromosomal beta-lactamase expression [14]. The nauseating problem of these microbes being resistant to many valued commercial antibiotics has put the scientist on the run-in search for lasting solution like medicinal plants with strong antibacterial activity. This actually underscores the evaluation of the antibacterial potential of *C. albidum* (G. Don) leaf extract against the selected clinical pathogenic microorganisms.

Materials and Methods

Preparation of the extract of *C. albidum* (G. Don)

Chrysophyllum albidum (G. Don) tree was identified in its natural habitat (forest) in Akure, Ondo state, Nigeria. The leaves were harvested and gently rinsed with distilled water to eradicate dust and attached extraneous materials. The leaves were spread on flat platform, air dried for three weeks under the shade and thereafter pulverized using Thomas-Willey® machine. 1kg (1000 g) of the powdered leaves was soaked in 5000 mL (5 litres) ethyl alcohol for 72 hours and well stirred at the intervals of 8 hours for effective extraction. Two different filtrations were carried out using muslin cloth and later with Whatman filter paper No 1(125 mm). The wet extract was later concentrated in rotary evaporator and freeze-dried in bench-top freeze dryer (VaCo2®, 3kgs/24h). The extract was maintained in a sterile plastic container in the fridge with constant electricity supply prior to further activities.

Phytochemical screening of ethyl alcohol leaf extract of *C. albidum* (G. Don)

The qualitative and quantitative phytochemical screening of *C. albidum* (G. Don) was carried out following the procedure described by **Trease et al.** and **Valentine et al.** [15, 16]. The results showed the presence of Tannins (5.17 mg/g), Saponins (13.91 mg/g), Terpenoids (8.52 mg/g), Steroids (9.01 mg/g), Flavonoids (9.45 mg/g) and Cardiac glycosides (30.34 mg/g) as already presented in earlier research on *C. albidum* [17].

Reconstitution of the leaf extract of *C. albidum* (G. Don)

About 1g of the extract was weighed on digital electronic top pan balance Kerro® (series KLS and KLC, BL 3002 made in Taiwan) with 1g accuracy into a universal bottle (McCarteny bottles). 15 mL of Tween 20% was drawn and added into 40 mL of distilled water in another bottle and thoroughly mixed for the extract's reconstitution. 10 mg/mL concentration was prepared and purified using syringe filter (0.2 µm diameter) which was further serial diluted to arrive at sixteen different concentrations (100 mg/mL - 0.003 mg/mL) of the extract for the establishment of MICs according to the method of **Andrews** [18] with slight modifications.

Test organisms

The clinical isolates; *Escherichia coli* (Gram negative), *Proteus mirabilis* (Gram negative), *Pseudomonas aeruginosa* (Gram negative), *Staphylococcus aureus* (Gram positive) and *Salmonella typhimurium* (Gram negative) were obtained from a reputable private Hospital in Akure, Ondo State, Nigeria and maintained on nutrient slants at 4 °C. The bacterial isolates were confirmed by subjecting them to Gram staining test, morphological and biochemical characterization [19, 20]. Thereafter, five broth cultures were prepared on day one and

inoculated with each of the test organisms using peptone water as recommended by the manufacturer. The cultures were incubated for 24 hours at 37 °C. On day two, another five broth cultures were prepared as at day one and 0.2 mL each of day one broth was used to inoculate day two broth culture accordingly. The second day broth cultures were incubated for 3 hours following 0.5 Cfu/mL McFarland Standard and that was adopted for the experiment.

Preparation of agar wells /plates

Sixty-two (62) sterile petri-plates were purchased from a reputable company for this experiment. One petri-plate was prepared per organism, replicated thrice and done in four batches ((5x3x4) + 2) leaving two for positive test control. Mueller Hinton Agar (MHA) was prepared according to specification. Sterilized 6 mm borer was used in boring six equidistant wells (wells 1–4 for different concentrations of extract while wells 5 and 6 for Tween 20% and distilled water respectively to serve as controls) each in the well-set petri-plates. The last two plates for antibiotic sensitivity test were not bored. With the aid of sterile swab, a dip of each of the microbial inoculum was spread on the agar plate per organism and replicated thrice. Sixty (60) µL of the extract was introduced to each of the wells per concentration with the aid of a micro pipette. The plating was done in four batches to arrive at the sixteen different concentrations. Batch 1(100 – 12.5 mg/mL), batch 2 (6.25 – 0.782 mg/mL), batch 3 (0.391 – 0.049 mg/mL) and batch 4 (0.025 – 0.003 mg/mL). Maxi discs for Gram negative and Gram-positive organisms were gently laid on two different plates already filled with sterilised agar. The whole plates were incubated at 37^0C for 18 hours according to **Mukherjee et al.** [21].

Antimicrobial activity of ethyl alcohol leaf extract of *C. albidum* (G. Don)

Antibacterial activity of the extract was assessed based on the observed inhibition zone diameters exhibited by the clinical isolates measured at the expiration of the eighteen hours (18) with the aid of graduated ruler [22,23]. Data from the inhibition zone diameters due to the isolates were tabulated, subjected to one-way analysis of variance (ANOVA) using SPSS [24] while the various means were separated by Duncan's Multiple Range Test [25].

Results

The results of the antimicrobial activities were expressed as inhibition zone diameters' mean ± standard deviation (SD) of triplicate determinations as presented in **tables (1 & 2)**.

Table 1. Antimicrobial activity of ethyl alcohol extract of *C. Albidum* (G. Don) against five clinical pathogenic bacteria.

N	Conc (mg/mL)	Average zone of inhibition (mm) diameters						
		P M	PA	SA	ST	EC	Tween 20 %	DW
1	100	32.00±1.00^{a}	22.33±0.58^{b}	35.33±0.58^{a}	33.55±5.51^{a}	0.00±0.00^{c}	0.00	0.00
2	50	30.00±0.58^{a}	20.00±2.08^{b}	32.00±2.00^{a}	31.00±5.20^{a}	0.00±0.00^{c}	0.00	0.00
3	25	29.00±1.15^{a}	17.67±2.08^{b}	30.00±2.00^{a}	30.33±0.57^{a}	0.00±0.00^{c}	0.00	0.00
4	12.50	26.33±1.53^{b}	16.00±2.00^{c}	28.00±2.08^{ab}	30.00±0.58^{a}	0.00±0.00^{d}	0.00	0.00
5	6.25	25.00±1.15^{b}	14.00±2.65^{c}	26.33±1.53^{ab}	29.00±1.15^{a}	0.00±0.00^{d}	0.00	0.00
6	3.125	24.00±2.00^{b}	13.00±2.00^{c}	24.00±0.58^{b}	26.00±1.15^{a}	0.00±0.00^{d}	0.00	0.00
7	1.563	19.33±1.15^{ab}	12.33±3.21^{c}	22.00±2.00^{a}	18.00±2.00^{a}	0.00±0.00^{d}	0.00	0.00
8	0.782	16.00±0.00^{b}	11.33±2.31^{c}	20.00±2.00^{a}	15.00±1.15^{b}	0.00±0.00^{d}	0.00	0.00
9	0.391	11.33±1.15^{b}	10.00±2.00^{b}	18.00±2.00^{a}	11.33±1.15^{b}	0.00±0.00^{c}	0.00	0.00
10	0.195	7.00±1.15^{b}	5.33±1.15^{b}	16.00±2.00^{a}	7.00±2.31^{b}	0.00±0.00^{c}	0.00	0.00
11	0.098	0.00±0.00^{b}	0.00±0.00^{b}	14.00±2.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00	0.00
12	0.049	0.00±0.00^{b}	0.00±0.00^{b}	9.33±0.58^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00	0.00
13	0.025	0.00±0.00^{b}	0.00±0.00^{b}	7.00±1.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00	0.00
14	0.012	0.00±0.00^{b}	0.00±0.00^{b}	6.00±0.58^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00	0.00
15	0.006	0.00±0.00^{b}	0.00±0.00^{b}	4.33±0.58^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00	0.00
16	0.003	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0.00

Values with different superscript along the same row are significantly different ($p<0.05$) and they are presented as mean ± SD of triplicate determinations minus the wells' diameter. Where Conc = Concentration of the extract, P M = *Proteus mirabilis*, PA = *Pseudomonas aeruginosa*, SA = *Staphylococcus aureus*, ST = *Salmonella typhimurium*, EC = *Escherichia coli*, SD = Standard deviation and DW = Distilled water.

Table 2. Antimicrobial activity of commercial antibiotics against the tested pathogenic bacteria.

Commercial Antibiotics	Bacteria IZ (mm) (Gram negative)				Commercial Antibiotics	SA (Gram positive) IZ (mm)
	PM	PA	ST	EC		
Septrin (30µg)	26	R	R	26	Pefloxacin (10µg)	26
Chloramphenicol l(30µg)	30	R	25	20	Gentamycin (10µg)	18
Sparfloxacin (10µg)	30	R	26	30	Ampiclox (30µg)	22
Ciprofloxacin (10µg)	30	R	30	32	Zinnacef (20µg)	25
Amoxicillin (30µg)	28	R	20	R	Amoxicillin (30µg)	20
Augmentin (30µg)	22	R	R	R	Rocephin (25µg)	25
Gentamycin (10µg)	24	R	20	25	Ciprofloxacin (10µg)	30
Pefloxacin (30µg)	28	R	26	26	Streptomycin (30µg)	22
Tarivid (10µg)	R	R	R	R	Septrin (30µg)	25
Streptomycin (30µg)	R	R	15	24	Erythromycin (30µg)	24

Where R= Resistant, IZ= Inhibition zone, PM = *Proteus mirabilis,* PA = *Pseudomonas aeruginosa,* SA = *Staphylococcus aureus,* ST = *Salmonella typhimurium and* EC = *Escherichia coli*.

Discussion

There was no expressed inhibition zone by the solvents (Tween 20 % and distilled water) used. Highest concentration of the extract (100 mg/mL) demonstrated the maximum bacterial inhibition zone which agrees with **Louis et al.** [26], that plant extracts are not only good antimicrobial agents but also increase in antimicrobial activities as its concentration increases. *E. coli* was resistant to the sixteen (16) prepared concentrations of the extract as shown in **table (1)**. This observation agrees with the reports of **Okoli and Okere** [27], that *E. coli* is highly resistant to the crude leaf extract of *C. albidum* (G. Don). However, *S. aureus* recorded the highest susceptibility value to the extract in this experiment with the MIC of 0.006 mg/mL but resistant to the crude leaf extract of the plant [27]. *Pseudomonas aeruginosa* was resistant to all the tested commercial antibiotics **(Table 2)** and this indeed confirmed that this organism is highly resistant to many antimicrobials [27, 28]. However, the ethyl alcohol leaf extract of *C. albidum* inhibited the growth of this highly resistant and pathogenic micro-organism with the MIC of 0.195 mg/mL. This finding corroborates plants' antibacterial potentials [5,6]. The extract's minimum inhibitory concentrations (MICs) for the test organisms recorded 0.195 mg/mL for *Proteus mirabilis, Salmonella typhimurium* and *Pseudomonas aeruginosa:* that of *Staphylococcus aureus was* 0.006 mg/mL. It is noteworthy that the zone of inhibition exhibited by *S. aureus* in response to ethanolic extract of *C. albidum* (G. Don) was 35 mm **(Table 1)** which was higher than all inhibition zones exhibited by the bacteria experimented with commercial antibiotics. The highest inhibition zone recorded from the bacteria tested with commercial antibiotics was 32 mm (Ciprofloxacin) as shown in **table (2)** and this result agrees with the report of **Serafino et al.** and **Abeer et al.** [4, 29] that most plant extracts are potent and can be very effective than many synthetic antibiotics.

Conclusion

The outcome of this experiment revealed the possibility of exploring potent broad-spectrum antibiotics from *C. albidum* leaf which may probably be attributed to its inherent phytochemicals. This plant can be further investigated by researchers and pharmaceutical industries in the preparation of novel drug for the prevention and treatment of diseases caused by many pathogenic (Gram positive and Gram negative) and antimicrobial resistant bacteria.

Recommendations

Identification and isolation of bio-active elements in the leaf extract of *C. albidum* as well as animals' *in vivo* experiments are hereby recommended to be further studied.

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Competing interests

The authors have no competing interest to declare.

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