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Antimicrobial and GC-MS Analyses of the Ethyl Acetate Extract of *Chrysophyllum albidum* Fruits' Mesocarps

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Abstract
Emergence of multi-drug resistant bacteria has rekindled interest in plant derived compounds as substitute for synthetically produced antimicrobial agents. This study investigated the antimicrobial activity and chemical composition of ethyl acetate extract of Chrysophyllum albidum fruit (cherry) mesocarps. Crude extract obtained by maceration of the mesocarps in ethyl acetate was tested for antimicrobial activity against five bacterial isolates and two fungi using agar-well diffusion method. Gas Chromatography-Mass Spectroscopy (GC-MS) was used to determine the chemical constituents of the extract. The extraction process gave a yield of 14.44%. Phytochemical constituents derived include steroids, glycosides, terprenoids, tannins, phenolics and saponins. The extract inhibited the growth of all bacteria with inhibition zone diameters ranging from 19.50 - 13.00 mm, but showed no activity against the fungal isolates, at test concentration of the extract. GC-MS identified thirty-three phytochemicals. Data from this study revealed the possible antimicrobial potentials and chemical constituents of Chrysophyllum albidum fruit mesocarps.

Keywords: *Chrysophyllum albidum,* ethyl acetate, mesocarp, antimicrobial, GC-MS

INTRODUCTION
The burden resulting from human diseases and other related ailments have led man to discover ways by which these conditions could be better managed (Vos *et al*., 2020). The recourse to plants as dependable sources of remedy dates back to time immemorial (Bhat, 2021). Plant derived medicines are culturally acceptable because of their effectiveness, affordability, availability, low toxicity and few side effects on the human body (Akharaiye *et al*., 2010; Welz *et al*., 2018). It has been estimated that 14-28% of higher plant species are used medicinally and 74% of pharmacologically active plant derived components have ethno

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medicinal use (Pandey and Kumar, 2013). About 80% of the world population relies chiefly on traditional medicines for their primary health care needs (Mishra *et al.,* 2013).

Existing literatures have shown that bioactive natural compounds exhibiting antimicrobial activities have been isolated mainly from cultivatable microbial strains (Abdel-Razek *et al*., 2020). Thus there is need to exploit the untapped natural resources of plants in producing biologically active metabolites to alleviate the current healthcare challenges (Atanasov *et al*., 2021). Such challenges include increasing cost of chemotherapy, emergence of multi-drug resistant strains and unmet clinical needs. The use of plant extracts and phytochemicals can be of great significance in therapeutic management and could help curb the problem of these multi-drug resistant organisms (AlSheikh *et al*., 2020).

Cinnamon, clove, thyme and lanata extracts have been shown to inhibit the growth of multidrug resistant *Pseudommas aeruginosa* (Pandey *et al.,* 2010). Out of over 250,000 plant species on the planet, only about 10% have been screened for phytochemicals and biological activity (Verpoorte, 2000). This provides an avenue for newer search among plant kingdom for alternative therapies. The importance of the active ingredients of plants in medicine has stimulated significant scientific interest in the biological activities of these substances (Moghadamtousi *et al*., 2013). Study on medicinal plants is essential to promote the proper use of herbal medicine in order to determine their potential as sources of new drugs (Ekor, 2014). *Chysophyllum albidum* is a plant which has been used in traditional alternative medicine in Nigeria to treat health related problems. Various parts of this plant have been proven to have a wide range of therapeutic effects (Adekanmi and Olowofoyeku, 2020). Generally, the roots, barks and leaves of *C. albidum* are widely used as an application to sprains, bruises and wounds in southern Nigeria (Osuntokun, 2021). Literature shows a lack of data regarding extracts from the mesocarp of *C. albidum* with respect to its antimicrobial activityies hence this study was conducted to determine the antimicrobial activities and chemical constituents of *C. albidum* mesocarp using ethyl acetate as solvent for extraction.

MATERIALS AND METHOD

Materials

Nutrient agar and nutrient broth (Titan Biotech, India), Sabouraud dextrose agar (Lifesave Biotech, USA), ethyl acetate and dimethyl sulphoxide (DMSO) (JHD Science Tech Co. Ltd., China), ciprofloxacin powder (Sigma Aldrich, Germany) and fluconazole (Biochemika, India) were employed in this study.

Processing of *Chrysophyllum albidum* fruits

The fresh fruits of *Chysophyllum albidum* were purchased from a local market in Benin City, Edo State, Nigeria. The fruits were washed thoroughly with tap water and the seeds manually separated from the fruits. The mesocarps were separated from the fruits backs (exocarps) by scooping with a spoon and then air dried in trays for two weeks to a constant weight. The dried mesocarps were pulverized into powder using an electric kitchen blender (Eurosonic, China). The powder was weighed and stored in a dessicator containing silica prior to use.

Phytochemical analysis

About 5.0 g of the powdered *C. albidium* mesocarps was boiled with 75 mL of distilled water for 30 minutes. The solution was filtered hot and allowed to cool down. The filtrate obtained was used to test for the presence of tannins, saponins, terpenoids, glycosides, flavonoids, alkaloids, anthraquinones and phenolic compounds using standard procedures (Evans, 2002).

Preparation of crude extract
A 500 g quantity of the powdered plant material (mesocarp) was macerated with 1.5 L of ethyl acetate in a maceration jar at room temperature for 72 hours. Using a glass rod, the content of the jar was stirred intermittently. The jar content was first filtered with cheesecloth and the resulting filtrate was re-filtered using a Whatman filter paper (12.5 mm). The filterate was then concentrated using a rotatory evaporator set at 44 °C and a rotation of 50 rpm (Stuart RE400, UK). The resulting sem-solid extract was weighed and stored in a sterile capped bottle at 4.0 °C in a refrigerator.

Standardization of microbial inocula
Clinical isolates obtained from the stock cultures of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Edo State, Nigeria were used for the study. These isolates include; *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pyrogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albidans*.

The test bacterial isolates were sub-cultured onto freshly prepared nutrient agar plates and incubated at 37 °C for 24 hours while the fungal isolates were sub-cultured onto Sabouraud dextrose agar plates and incubated at 25 °C for 72 hours (Black, 2020).

Some sections of the grown bacterial colonies were transferred into test tubes containing sterile nutrient broth (8.0 mL) and incubated at 37 °C for 24 hours while sections of the fungal colonies were transferred into sterile Sabouraud broth containing test tubes and incubated at 25 °C for 72 hours. The suspension turbidity from the bacterial and fungal growth was compared to a freshly prepared barium sulphate solution (0.5 mL of 1.0% barium chloride to 99.5 mL of 1.0% sulphuric acid (0.36 normal) of standard opacity. The turbidity was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standards (10^8 CFU/mL). A 1:100 dilution was carried out on the adjusted inocula to give inoculum size of approximately 10^6 CFU/mL using the M07-A10 approved guideline (Clinical and Laboratory Standards Institute (CLSI), 2015).

Preparation of stock solutions
Stock solutions of ciprofloxacin was prepared by dissolving 0.5 mg in 5.0 mL of 10% dimethyl sulphoxide (DMSO) solution and making up to 10 mL volume thus giving a concentration of 0.5 µg/mL while 20 mg of fluconazole was dissolved in similar qantity of 10% DMSO solution to give a concentration of 2.0 µg/mL. Working concentrationd of the extract were prepared with 1.0 g of the extract in 2.0 mL 10% DMSO solution to give concentrations of 500 mg/mL (Babaiwa *et al*., 2020).

Antimicrobial susceptibility test
Antimicrobial susceptibility test was carried out using agar well diffusion method (Murray *et al.,* 2009) with some modifications. Petri dishes containing 30 mL each of sterile Mueller Hinton agar were allowed to set and then dried at 40 °C for 10 minutes in a hot air oven. The dishes were incurbated for 24 hours to rule out contamination and then streaked with standardized bacterial inocula (200 µL) using sterile swab sticks. A sterile cork borer (10 mm) was used to bore four (4) wells in each agar plate. The agar disks from the wells were removed with a flame sterilized forcep and the base of the wells sealed with molten agar. Using a micropipette, two wells on each plate were each filled separately with 200 µL of the ciprofloxacin stock solution, equivalent to 0.1 µg of ciprofloxacin while the other two wells were also individually filled with 200 µL of the extract stock solution, equivalent to 100 mg of the extract. Using similar procedures, Sabouraud dextrose agar (SDA) was used in culturing

the fungal isolates with fluconazole as test anti-fungal. Here, two wells were each filled with 200 µL (equivalent to 0.4 µg) of the fluconazole stock solution while the other two wells received 200 µL each of the extract stock solution.

Negative control using 10% DMSO solution (without the extract and test drugs) and positive control (viability test for organisms used) were carried out for each set of experiment. The bacterial inoculated plates were incurbated in an upright position at 37 °C for 24 hours while the fungal inoculated plates were incubated at 25 °C for 72 hours. After incubation, inhibition zone diameter (IZD) was measured in millimeters (mm) as an index of the killing or inhibitory action of the test agent against a given organism. All experiments were carried out under aseptic conditions.

Determination of minimum inhibitory concentration (MIC)

The agar dilution method of Afolayan and Meyer (1997) was used in determining the minimum inhibitory concentration (MIC) of the test extract, ciprofloxacin and fluconazole on susceptible isolates. Double strength nutrient and Sabouraud agar were prepared at 50 °C in a hot water bath, in line with the manufacturer's instruction. The molten agars were used to carry out a two-fold serial dilution of the test extract, ciprofloxacin and fluconazole. Volumes ranging from 0.25 - 8.0 mL of the test extract, 0.40 - 12.8 µL of ciprofloxacin and 0.10 - 6.6 µL of fluconazole were taken from their various stock solutions and introduced into the molten agar to give concentrations of 12.5, 25, 50, 100, 200 and 400 mg/mL of the extract, 0.0125, 0.025, 0.05, 0.10, 0.20 and 0.40 µg/mL of ciprofloxacin and 0.0032, 0.0064, 0.0128, 0.0256, 0.0512, 0.1024 and 0.2048 µg/mL of fluconazole.

The agar-extract and agar-ciprofloxacin/fluconazole mixtures were poured into separate sterile plates, allowed to set and then dried at 40 °C for five minutes. Using a sterile wire loop, the test organism innocula were streaked unto the surface of each agar plate. Negative and positive controls were set up for each of the experiment. All plates were incubated for 24 hours at 37 °C except for *Aspergillus niger* and *C. albicans* which was incubated at 25 °C for 72 hours. After incubation, the plates were visually examined for growth in the inoculated spots. The MIC was defined as the lowest concentration of extract or ciprofloxacin/fluconzole that inhibited growth of test microorganisms (Andrews, 2001).

Determination of minimum bacteriocidal/fungicidal concentration

The minimum bacteriocidal/fungicidal concentration (MBC/MFC) was determined from the MIC plates that showed no growth by swabbing onto fresh nutrient and Sabouraud broth containing neither the extract nor the antimicrobial agents, in order to neutralize any carryover effect of the test extract and antimicrobial agents. After eight (8) hours, each broth content was appropriately streaked onto fresh nutrient and Sabouraud agar plates.The plates were then incubated at 37 °C for 24 hours for bacteria and at 25 °C for 72 hours for the fungi. After incubation, the plates were visually examined for growth and the lowest concentration of the extract that showed no visible growth in the bacterial and fungal plates were considered as the MBC/MFC, respectively.

Gas chromatography - mass spectrometry analysis (GC-MS)

GC-MS analysis was carried out on GCMS-QP2010 SE apparatus (Shimadzu, Japan) with an Agilent 6890N gas chromatograph and Agilent Technologies 5973 Network Mass Selective Detector. Capillary column parameters: 5.0% phenylmethylsiloxane (stationary phase), 0.25 × 30 mm (internal diameter) and 1.0 µm (film thickness). Carrier gas was helium at a flow rate of 1.2 mL/min and an injection volume of 1.0 µL. The inlet temperature was maintained at

230 °C while the oven temperature was initially held at 50 °C for 5 minutes, then increased upto 300 °C at a rate of 10 °C/min within 25 minutes and total run time was 45 minutes.

The mass spectroscopy (MS) transfer line was maintained at a temperature of 300 °C with the source temperature at 230 °C and the MS Quad at 150 °C. Electron ionization mode was at 70.0 eV over a scan range of m/z 1428. Total ion chromatogram (TIC) was used in evaluating compound identification and quantization. The spectrum of the separated compound was compared with the spectral database of known compound in the NIST02 Reference Spectra Library. Data analysis and peak area measurement was carried out using Agilent Chemtation and Pherobase Software in comparison with data reported in literature.

Statistical analysis

Data was reported as mean ± standard error from triplicate determination for inhibition zone diameters and inferential statistics was used to report phytochemical constituents.

RESULTS

The ethyl acetate extract of *Chrysophyllum albidum* fruit mesocarp was an aromatic yellowish oily extract with a yield of 14.48%. Results from the phytochemical analysis of the powdered fruit mesocarps are presented in Table 1. The powdered mesocarps were found to contain tannins, terpenoids, glycosides, phytosterol, flavonoids, phenolics and saponin but devoid of alkaloid and anthraquinones.

Table 1: Phytochemical constituents of powdered *C. albidum* mesocarps

Phyto-constituents	Occurrence
Tannins	+
Flavonoids	+
Phenolics	+
Terpenoids	+
Glycosides (cardiac)	+
Saponins	+
Phytosterol (steroids)	+
Alkaloids	-
Anthraquinone	-

(+) Present, (-) Absent

Susceptibility profile of test microorganisms

Preliminary antimicrobial activity of the crude extract of *C. albidum* mesocarp is shown in Table 2. The extract showed activity against all test bacteria; *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pyrogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but showed no inhibitory activity against test fungi; *Aspergillus niger* and *Candida albican*, at the concentration (500 mg/mL) of the crude extract used in this study.

Results showed that *Staphylococcus aureus* was most susceptible to the extract with inhibition zone diameter (IZD) of 19.50 ± 0.17 mm, followed by *Klebsiella pyrogenes* (19.00 ± 0.00 mm) and *Bacillus subtilis* (16.00 ± 0.00 mm). *Pseudomonas aeruginosa* showed the least susceptibility to the extract with IZD of 13.00 ± 0.00 mm. At the concentrations of the antimicrobial agents used, all the bacterial isolates showed susceptibility to ciprofloxacin with IZD ranging from 32.00 - 34.00 mm while the fungal isolates were susceptible to fluconazole with IZD ranging from 23.00 - 34.00 mm.

Table 2: Inhibition zone diameters of the *C. albidum* mesocarp extract

Test microorganisms	Ethyl acetate extract (mm)	Ciprofloxacin (mm)	Fluconazole (mm)
Bacilli subtilis	16.00 ± 0.00	22.00 ± 0.50	NA
Esherichia coli	13.50 ± 0.20	32.00 ± 0.50	NA
Klebsiella pyrogenes	19.00 ± 0.00	25.00 ± 0.90	NA
Pseudomonas aeruginosa	13.00 ± 0.00	32.00 ± 0.50	NA
Staphylococcus aureus	19.50 ± 0.17	34.00 ± 0.94	NA
Aspergillus niger	0	NA	34.00 ± 0.50
Candida albican	0	NA	23.00 ± 0.50

Key: NA - (not applicable), mm - (millimetre), values ± standard error

Minimum inhibitory concentrations of the test extract and standard drugs

The minimum inhibitory and bacteriocidal/fungicidal concentrations (MIC and MBC/MFC) of the ethyl acetate test extract and standard antimicrobial agents against susceptible microorganisms are shown in Table 3. *Klebsiella pyrogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed the highest susceptibility to the extract at MIC of 25 mg/mL and MBC of 100 mg/mL except *Staphylococcus aureus* with MBC of 200 mg/mL. *Bacillus subtilis*, *Escherichia coli* and *Aspergillus niger* showed susceptibility at MICs and MBCs of 200 mg/mL of the extract while *Candida albican* showed the least susceptibility at MIC and MBC of 400 mg/mL of the extract.

Pseudomonas aeruginosa showed the highest resistance to the standard antibacterial agent with MIC of 0.16 µg/mL of ciprofloxacin while *Escherichia coli* followed with MIC of 0.04 µg/mL of ciprofloxacin. *Bacillus subtilis*, *Klebsiella pyrogenes* and *Staphylococcus aureus* showed highest susceptibility at MIC less that 0.02 µg/mL of ciprofloxacin. *Aspergillus niger* and *Candida albican* exhibited MICs ranging from 0.102 - 0.2048 µg/mL of fluconazole.

Table 3: Minimum inhibitory concentration/Minimum bacteriocidal concentration of the test extract and standard antimicrobial drugs

Test microorganisms	Ethyl acetate extract (mg/mL)		Ciprofloxacin (µg/mL)		Fluconazole (µg/mL)	
	MIC	MBC/MFC	MIC	MBC	MIC	MFC
Bacillus subtilis	200	200	< 0.02	ND	ND	ND
Escherichia coli	200	200	0.04	ND	ND	ND
Kliebsella pyrogenes	25	100	< 0.02	ND	ND	ND
Pseudomonas aeruginosa	25	100	0.16	ND	ND	ND
Staphylococus aureus	25	200	< 0.02	ND	ND	ND
Aspergillus niger	200	200	ND	ND	0.21	ND
Candida albicans	400	400	ND	ND	0.10	ND

ND = Not determined

Gas Chromatography – Mass Spectrometry (GC-MS)

The GC-MS chromatogram obtained from the extract analysis showed various peaks with their corresponding retention times and areas in percentages (Figure 1). A total of thirty-three (33) chemical constituents were identified on comparison with NIST14 reference spectral library (Table 4). They include 4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5- (29.80%), acetic acid (24.44%), betulin (9.77%), cis-9-hexadecenal (6.78%), silane (5.40%), beta-sitosterol (5.40%), stigmasterol (4.10%), n-hexadecanoic acid (3.81%), ergost-5-en-3-ol (2.55%), 17-octadecynoic acid (1.62%) and 2,3-dihydrothiophene (1.22%). Other chemical components in the extract accounted for a total of 7.02%.

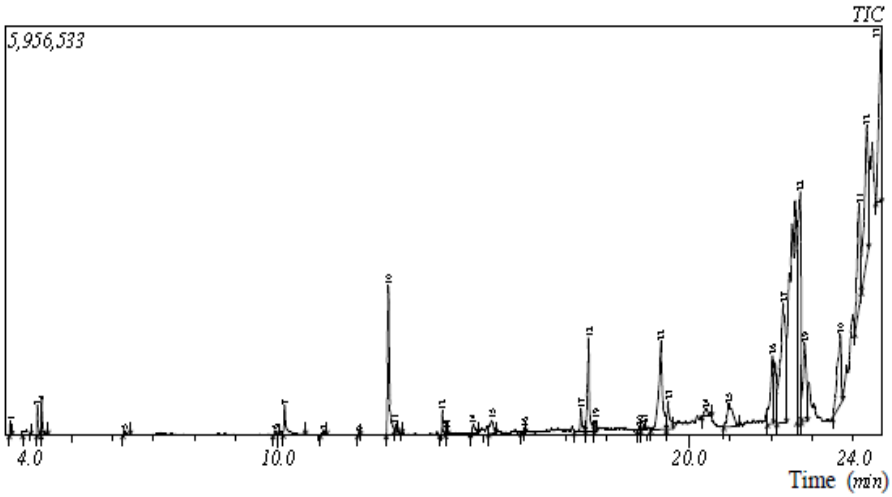

Figure 1: GC-MS spectra of *Chrysophyllum albidum* fruit mesocarp

Table 4: Phytochemicals identified in GC-MS.analysis of the ethyl acetate extract of *C. albidum* fruit mesocarp

Peak	Compound name	Retention time	Area (%)
1.	Propanoic acid ethyl ester	1.511	0.19
2.	Formamide	3.833	0.06
3.	2,3-butanediol	4.178	0.74
4.	2,3-butanediol, [S-(R*,R*)]	4.178	0.58
5.	1H-imidazole	6.276	0.24
6.	2,3-dihydro	9.945	0.16
7.	Thiophene, 2,3-dihydro-	10.184	1.22
8.	Butanoic acid, 3-methyl-2-phenylethyl ester	11.127	0.19
9.	Cyclopentane, 1,1,3-trimethyl	11.992	0.04
10.	Benzene ethanol, 4-hydroxyl	12.880	0.12
11	3,6,9,12-tetraoxahexadecan-1-ol	12.880	0.12
12	Tyramine, N-formyl	14.014	0.57
13	1-hexadecanol	14.117	0.10
14	1-decanoic acid, silver (1+) salt	14.765	0.61
15	2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene	15.210	0.96
16	1-hexadecanol	16.004	0.10
17	Palmitoleic acid	17.383	0.90
18	N-hexadecanoic acid	17.566	3.81
19	Ethyl-9-hexadecanoate	17.745	0.34
20	9,12-octadecadienoic acid (Z,Z)-	18.913	0.17
21	Phytol	18.913	0.17
22	Cis-9-hexadecenal (Palmitole aldehyde)	19.323	6.78
23	17-octadecynoic acid	19.502	1.62
24	1-hepatriacotanol	20.425	0.66
25	Ergost-5-en-3-ol, (3.beta.24R)-	20.980	2.55
26	Stigmasterol	22.033	4.10
27	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5-	22.740	14.90
28	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5-	22.714	14.90
29	Silane, (3-beta)-lanosta-9(11),24-dien-3-yl-	22.816	5.40
30	Beta-sitosterol	23.684	5.40
31	Acetic acid, 3-hydroxyl-6-isopropenyl-4-8a-6-	24.140	6.92
31	Acetic acid, 3-hydroxyl-6-isopropenyl-4-8a-6-	24.140	6.92
32	Acetic acid, 3-hydroxyl-6-isopropenyl-4- 8a-6-	24.327	10.60
33	Betulin	24.692	9.77

DISCUSSION

Phytochemical analysis of the powdered mesocarps of *Chrysophyllum albidum* fruits revealed the presence of metabolites that are of great importance in the development of phytomedicines Aside alkaloid and anthraquinone, cardiac glycosides, steroids, phenols, tannins, saponins, terpenods and flavonoids were detected. Antimicrobial properties of plant based foods have largely been attributed to their wide range of phytochemicals (Hochma *et al.*, 2021).

The presence of these metabolites supports the ethno medicinal use of these fruit mesocarps in the treatment of many diseases.Tannins are polymeric phenolic substances found nearly in all plant parts and also important sources of natural antioxidants with remarkable activity in cancer prevention (Orijajogun *et al.,* 2014). Tannins have been known to display different biological activities including antifungal, antibacterial and antiviral (Bhalodia and Shukla, 2011; Kaczmarek, 2020). Saponins exhibit hypocholesterolemic properties through the formation of insoluble complexes with cholesterol and consequently slowing down its absorption (Imaga *et al*., 2013). Also the presence of cardiac glycoside in *C. albidum* makes it a possible candidate in the management of congestive heart failure (Awuchi, 2019). Terpenoids form the largest group of natural compounds. They have been found to possess diverse therapeutic properties such as antibacterial, antiinflammatory, antioxidant, anticancer, antiseptic, antiplasmodial, and so on. (Cox-Georgian *et al*., 2019; Guimarães *et al*., 2019).

The ethyl acetate extraction process gave a yield of 14.48%, which was high when compared with yields of 7.09 and 11.67% from ethanol and water maceration of the seeds of the plant reported by Egharevba *et al*. (2015). Similar high yields of 13.0 and 16.8% have been reported from studies using acetone in combination with sonification on seeds and petroleum ether on the leaves of the plants (Oguntoyinbo *et al*., 2015; Akinmoladun *et al*., 2022). Extraction yields from crude plant material source do vary and this could be as a result of environmental conditions such as soil type or condition, variation in climate, plant species or age, part of the plant used and the extraction method.

The susceptibility of a test microorganism to an antimicrobial agent is evident by the presence of growth inhibitory zones on agar plate. This zone is measured as an index of the killing or inhibitory zones on agar plate. This zone is measured as an index of the killing or inhibitory action of the extract against the test micro organisms. Extract are considered active at inhibition zone diameter > 10.0 mm (Usman and Haruna, 2005). Thus, the test extract were active against test microorgasnisms with IZD between 13.00 - 19.50 mm except *Aspergillus niger* and *Candida albicans* which showed no zones of inhibition at the 500 mg/mL of the extract. This finding is similar to the values reported from the antimicrobial activity of ethyl acetate extract of *C. albidum* fruit exocarps against *Escherichia coli*, *Staphylococcus spp.* and *Klebsiella spp.* (Orijajogun *et al*., 2014).

Also, the extract showed a range of MIC values between 25 - 400 mg/mL for the test organisms. This variation in their MIC values may be largely due to genetic differences between the strains of test micro-organism. On the other hand, the test antimicrobial agents gave very low MIC values (< 0.02 - 0.21 µg/mL) when compared with the crude extract and this may be due to the purity of the antimicrobial agents compared to the extract which was still in its crude form and containing extraneous constituents that tend to dilute the active principle and reduce its activity. The same values of MIC and MBC observed against *Escherichia coli* and *Bacillus subtillis* suggest a bactericidal activity of the extract against these bacteria, while the increased MBC values of *Pseudomonas aeruginosa, Klebseiella pyrogenes* and

Staphylococcus aureaus is suggestive of a bacteriostatic mechanism of action. Though the MIC and MFC values of the extract for *Aspergillus niger* were lower than those of *Candida albican*, the fact that these values were the same for each fungus indicates a fungicidal mechanism of action of the extract against these fungi.

Furthermore, among the compounds identified by GC-MS analysis, twenty-three have been reported to poseses biological activities. But only two groups (fatty acids and terpenes) of these compounds are worthy of note in their actimicrobial properties. The most abundant among the fatty acids are betulin (9.77%), cis-9-hexadecenal (6.78%), silane (5.40%), n-hexadecanoic acid (3.81%) and 17-octadecynoic acid (1.62%). Fatty acids have been reported to exhibit antifungal or antibacterial with their mode of action being the targeting of cell membrane causing the disruption of the electron transport chain and oxidative phosphorylation (Desbois and Smith, 2010; Yoon *et al*., 2018). It has also been reported that they inhibit enzymatic activity, thereby impairing nutrient uptake, generation of peroxidation and auto-oxidation degradation products (Bhattacharyya *et al*., 2014; Tan *et al*., 2018). The biomedical exploitation and potential commercialization of these antibacterial free fatty acids from plant sources is of importance as this could curtail the rising challenge of multi-drug resistant isolates in our clinics (Desbois and Smith, 2010).

The second group of terpene compounds in order of abundance in the extract includes 4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5- (29.80%), beta-sitosterol (5.40%), stigmasterol (4.10%) and ergost-5-en-3-ol (2.55%). In general, terpenes are among the very promising source of new antimicrobials agents that have shown to have activity against viruses, bacteria, fungi and protozoa (Guimarães *et al*., 2019). Their mode of action has been traced to the rupturing of cell membrane by disrupting the ion channels in the cell membrane, leading to increased permeability and release of essential intracellular componenets (Oz *et al*., 2015). Also, possession of hydroxyl groups makes it very reactive in forming hydrogen bonds with target enzymes in cell membrane, causing their inactivation and subsequent lysis of the cell membrane (Chauhan and Kang, 2014).

CONCLUSION

Data from this study revealed the possible antimicrobial potentials and chemical constituents of *Chrysophyllum albidum* fruit mesocarps. The observed antimicrobial activities of the extract may be due to the abundance of terpene (terpernoids) and fatty acids, thus justifying its ethno-medicinal use. Apart from establishing a scientific basis for its traditional use in treatment of infections, the study has also laid down a platform in the search for a lead molecule that could be a potential antibacterial and antifungal agent of natural origin.

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REFERENCES

Abdel-Razek, A.S., El-Naggar, M.E., Allam, A., Morsy, O.M. and Othman, S.I. (2020). Microbial natural products in drug discovery. *Processes*. 8(4): 470.

Adekanmi, D.G. and Olowofoyeku, A.E. (2020). African star apple: Potentials and application of some indigenous species in Nigeria. *Journal of Applied Sciences and Environmental Management*. 24(8): 1307-1314.

Afolayan, A.J. and Meyer, J.M. (1997). The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. *Journal of Ethnopharmacology*. 57: 177-181.

Akharaiye, F.C. and Boboye, B. (2010). Antibacterial and phytochemical evaluation of three medicinal plants. *Journal of Natural Products*. 3: 27-34.

Akinmoladun, A.C., Falaiye, O.E., Ojo, O.B., Adeoti, A., Amoo, Z.A. and Olaleye, M.T. (2022). Effect of extraction technique, solvent polarity and plant matrix on the antioxidant properties of *Chrysophyllum albidum* G. Don (African Star Apple). *Bulletin of the National Research Centre*. 46: 40.

AlSheikh, H.M.A., Sultan, I., Kumar, V., Rather, I.A., Al-Sheikh, H., et al. (2020). Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. *Antibiotics* (Basel). 9(8): 480.

Andrews, J.M. (2001). Determination of minimum inhibitory concentrations. *The Journal of Antimicrobial Chemotherapy*. 48(1): 5-16.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., The International Natural Product Sciences Taskforce and Supuran, C.T. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery*. 20: 200-216.

Awuchi, C.G. (2019). Medicinal plants: the medical, food and nutritional biochemistry and uses. *International Journal of Advanced Academic Research*. 5(11): 220-241.

Babaiwa, U.F., Eraga, S.O., Ojugo, E.O., Erharuyi, O. and Akerele, J.O. (2020). A preliminary study on the antimicrobial activities and gas chromatography-mass spectrometry (GC-MS) analysis of the ethyl acetate extract of *Dennettia tripetala* seeds. *Bio-Research*. 18(1): 1094-1102.

Bhalodia, N.R. and Shukla, V.J. (2011). Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* L: An ethno-medicinal plant. *Journal of Advanced Pharmaceutical Technology and Research*. 2(2): 104-109.

Bhat, S.G. (2021). Medicinal plants and its pharmacological values. In El-Shemy, H.A. (ed.), *Natural Medicinal Plants*. IntechOpen, London. 10.5772/intechopen.99848.

Bhattacharyya, A., Chattopadhyay, R., Mitra, S. and Crowe, S.E. (2014). Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiological Reviews*. 94: 329-354.

Black, W.D. (2020). A comparison of several media types and basic techniques used to assess outdoor airborne fungi in Melbourne, Australia. *PLOS One*. 15(12): e0238901.

Chauhan, A.K. and Kang, S.C. (2014). Thymol disrupts the membrane integrity of Salmonella ser. Typhimurium *in vitro* and recovers infected macrophages from oxidative stress in an *ex vivo* model. *Research in Microbiology*. 165: 559-565.

Clinical and Laboratory Standards Institute (2015). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Tenth Edition. CLSI document M07-A10. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

Cox-Georgian, D., Ramadoss, N., Dona, C. and Basu, C. (2019). Therapeutic and medicinal uses of terpenes. *Medicinal Plants*. 12: 333-359.

Desbois, A.P. and Smith, V.J. (2010). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*. 85(6): 1629-1642.

Egharevba, H.O., Ibrahim, J.A., Nduaguba, G.A. and Kunle, O.F. (2015). Phytochemical, proximate, pharmacognostic analyses and thin layer chromatography of

Chrysophyllum albidum seed. *Ewemen Journal of Herbal Chemistry and Pharmacology Research*. 1(1): 6-12.

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*. 4: 177.

Evans, W.C. (2002). Trease and Evans Pharmacognosy. Volume III. 15th Edition, Harcourt Health Science, London, pp. 129-146.

Guimaraes, A.C., Meireles, L.M., Lemos, M.F., Guimaraes, M.C.C., Endringer, D.C., et al. (2019). Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules*. 24(13): 2471.

Hochma, E., Yarmolinsky, L., Khalfin, B., Nisnevitch, M., Ben-Shabat, S., et al. (2021). Antimicrobial effect of phytochemicals from edible plants. *Processes*. 9: 2089.

Image, N.O.A. and Urua, E.E. (2013). Chemical and antioxidant evaluation of star apple fruit (*Chrysophyllum albidum*) crude extracts. *Planta Medicinal Congress Abstract*. 79-83.

Kaczmarek, B. (2020). Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials - A mini review. *Materials* (Basel). 13(14): 3224.

Mishra, A., Sharma, A.K, Kumar, S., Saxena, A.K. and Pandey, A.K. (2013). *Bauhinia variegata* leaf extracts exhibit considerable antibacterial, antioxidant and anticancer activities. *BioMed Reseach International*. 2013: 915436.

Moghadamtousi, S.Z., Goh, B.H., Chan, C.K., Shabab, T. and Kadir, H.A. (2013). Biological activities and phytochemicals of *Swietenia macrophylla* King. *Molecules*. 18: 10465-10483.

Murray, P.R., Rosenthal, K.S. and Pfallor, M.A. (2009). Medical Microbiology. Mosby/Elsevier, Philadelphia pp. 234-239.

Oguntoyinbo, O.O., Abdus-Salaam, R.B., Bello, W.A. and Ifesan, B.O.T. (2015). Evaluation of the phytochemical, antioxidant and antimicrobial properties of extracts from *Chrysophyllum albidum* (African star apple) leaf. *Journal of Food Technology Research*. 2(1): 1-10.

Orijajogun, O.J., Olajide, O.O. and Useh, U.M. (2014). Biological analysis and phytochemical studies of the exocarp fruits extracts of African star apple (*Chrysophyllum albidum* G. Don). *Scholars Academic Journal of Pharmacy*. 3(5): 379-382.

Osuntokun, O.T. (2021). Efficacy, properties and therapeutic uses of some major medicinal plants for human health. In: Adejumo, T.O. and Vögele, R.T. (eds). *Biopesticides: Botanicals and Microorganisms for Improving Agriculture and Human Health*. Logos Verlag Berlin GmbH. pp. 179-214.

Oz, M., Lozon, Y., Sultan, A., Yang, K.H.S. and Galadari, S. (2015). Effects of monoterpenes on ion channels of excitable cells. *Pharmacology & Therapeutics*. 152: 83-97.

Pandey, A. and Kumar, S. (2013). Perspective on plant products as antimicrobials agents: A review. *Pharmacologia*. 4: 469-480.

Pandey, G., Zhang, B., Chang, A.N., Myers, C.L., Zhu, J., et al. (2010). An integrative multi-network and multi classifier approach to predict genetic interactions. *PLOS Computational Biology*. 6(9): 33-38.

Tan, B.L., Norhaizan, M.E. and Liew, W.P. (2018). Nutrients and oxidative stress: Friend or foe? *Oxidative Medicine and Cellular Longevity*. 2018: 9719584.

Usman, H., Haruna, A.K., Akpulu, I.N., Ilyas, M., Ahmadu, A.A., et al. (2005). Phytochemical and antimicrobial screenings of the leaf extracts of *Celtis integrifolia* Lam. *Journal of Tropical Biosciences*. 5(2): 72-76.

Verpoorte, R. (2000). Pharmacognosy in the new millennium: Lead finding and biotechnology. Journal of Pharmacy and Pharmacology. 52: 253-262.

Vos, T., Lim, S.S., Afshin, A., Alam, T., Ashbaugh, C., et al. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: A systematic analysis for the global burden of disease study 2019. *Lancet*. 396: 1204-1222.

Welz, A.N., Emberger-Klein, A. and Menrad, K. (2018). Why people use herbal medicine: Insights from a focus-group study in Germany. *BMC Complementary and Alternative Medicine*. 18: 92.

Yoon, B.K., Jackman, J.A., Valle-González, E.R. and Cho, N.J. (2018). Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing and therapeutic applications. *International Journal of Molecular Sciences*. 19(4): 1114.