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Assessment of Antimicrobial, Phytochemical Screening and Gas Chromatography-Mass Spectrophotometric Profile of Crude Chrysophyllum Albidum Essential Oil

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Abstract The main purpose of this research work is to investigate and compare the antimicrobial properties of *Chrysophyllum albidum* seed and stem bark essential oils, against some selected pathogenic isolates, to compare the phytochemical composition of the oil of *Chrysophyllum albidum* seeds and stem bark, to analyze the chemical compounds responsible for activities of the essential oil using Gas Chromatography – Mass Spectrometer (GC-MS) method in order to provide scientific validation for their use and as potential source of drug development. Phytochemical profile of air-dried *Chrysophyllum albidum* seed and stem bark essential oils shows that it contains an array of biologically active substances that include alkaloids, steroids, tannin, phenol, reducing sugar and flavonoid. However, *Chrysophyllum albidum* seed contains cardiac glycosides and saponin which are absent in the stem bark. Antimicrobial activity was determined using agar well diffusion method. The seed part reveals comparatively great antimicrobial activity against the test organisms (bacteria and fungi) used in the study. *Salmonella typhi* was the most susceptible bacterial isolate with 29mm zone of inhibition at 100mg/ml, 18mm at 50mg/ml and 13mm at 25mg/ml, while *Trichophyton rubrum* was the most susceptible fungi isolate with 18mm and 13mm at 100mg/ml and 50mg/ml respectively. Further study on the essential oil of the *Chrysophyllum albidum* seed using the Gas Chromatography and Mass Spectrometer reveals fifteen bio actives chemical compounds which invariably are the most volatile of the thousands of compounds that might be present in the essential oil. The compounds are said to possess antimicrobial activity, their various heights (%) includes; hexadecanoic acid, methyl ester (2.02), Pentadecanoic acid (11.77), Cycloheptan(a)indole (1.13) , Methyl 10-trans,12-cis-octadecenoate (4.99),9-Octadecanoic acid (z)-,methyl ester (7.07), 6-Octadecanoic acid,(z)- (47.81), octadecanoic acid (14.94), 2,2,3-Trimethyl-2-3-methyl-buta 1,3-dienyl (0.91), Squalene (3.35), Chondrillasterol (0.45), 7,22- Ergostadienone (1.51), 17-(1,5- Dimethyl-3-phenylthiohex-4-enyl)-4 (0.54), Beta.-Amyrin (0.49), Lup-20(29)-en-3-ol, acetate, (3-beta) (0.77) and Phthalic acid, di(2-propylpentyl) ester (2.26). The results of this research justify the use of *Chrysophyllum albidum* for traditional medicine and further research on this plant parts is encouraged.

Keywords Antimicrobial, Phytochemical, *Chrysophyllum albidum*, Gas Chromatography/Mass Spectrometer (GC-MS), Essential oil

1. Introduction

Since ancient times, essential oils are recognized for their medicinal value and they are very interesting and powerful natural plant products. They continue to be of paramount importance until the present day. Essential oils

have been used as perfumes, flavors for foods and beverages, or to heal both body and mind for thousands of years [1-2]. Record findings in Mesopotamia, China, India, Persia and ancient Egypt show their uses for many treatments in various forms. For example, in the ancient Egypt, the population extracted oils by infusion. Later; Greeks and Romans used distillation and thus gave aromatic plants an additional value. With the advent of Islamic civilization, extraction techniques have been further refined. In the era of the Renaissance, Europeans have taken over the task and with the development of science; the composition and the nature of essential oils have been well established and studied [3-6]. Nowadays, peppermint, lavender, geranium, eucalyptus, rose, bergamot, sandalwood and chamomile essential oils are the most frequently traded ones.

Essential oils (also called volatile or ethereal oils, because they evaporate when exposed to heat in contrast to fixed oils) are odorous and volatile compounds found only in 10% of the plant kingdom and are stored in plants in special brittle secretory structures, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts [7-12].

They are hydrophobic, soluble in alcohol, non polar or weakly polar solvents, waxes and oils, but only slightly soluble in water and most are colourless or pale yellow, with exception of the blue essential oil of chamomile (*Matricaria chamomilla*) and most are liquid and of lower density than water (sassafras, vetiver, cinnamon and clove essential oils being exceptions) [13-16].

Because of the variability of amounts and profiles of the components of essential oils, it is likely that their antimicrobial activity is not due to a single mechanism, but to several sites of action at the cellular level. Then, different modes of action are involved in the antimicrobial activity of essential oils. One of the possibilities for action is the generation of irreversible damage to the membrane of bacterial cells, that induce material losses (cytoplasmic), leakage of ions, loss of energysubstrate (glucose, ATP), leading directly to the lysis of bacteria (cytolysis) and therefore to its death. Another possibility of action is inhibition of production of amylase and protease which stop the toxin production, electron flow and result in coagulation of the cell content [3, 17-19].

Antifungal actions are quite similar to those described for bacteria. However, two additional phenomena inhibiting the action of yeast are worth mentioning: the establishment of a pH gradient across the cytoplasmic membrane and the blocking of energy production of yeasts which involve the disruption of the bacterial membrane [20]. Essential oils and their components are widely used in medicine as constituents of different medical products, in the food industry as flavouring additives and also in cosmetics as fragrances and pharmaceutical industries [21].

Chrysophyllum albidum (African Star Apple) is one fruit of great economic value in tropical Africa due to its diverse industrial, medicinal and food uses. The tree grows as a wild plant and belongs to the family of Sapotaceae which has up to 800 species and make up almost half of the order Ebernales [22]. *Chrysophyllum albidum*, commonly called African star apple is a lowland rain forest tree species that grows up to 25 to 37m in height at maturity with a girth varying from 1.5 to 2 m [23]. The Scottish botanist George Don described it as a forest fruit tree [24]. It is primarily a forest tree species, its natural occurrences have been reported in diverse ecozones in Nigeria, Uganda, Niger Republic, Cameroon, and Cote d'Ivoire [25].

The plant has become a crop of commercial value in Nigeria [22, 26].When it is ripe, the fruit is ovoid to sub-globose, pointed at the apex, and up to 6 cm long and 5 cm in diameter. The skin or peel, is orange to golden yellow when ripe and the pulp within the peel may be orange, pinkish, or light yellow. Within the pulp are three to five seeds which are not usually eaten. The seed-coats are hard, bony, shiny, and dark brown, and when broken reveals white-coloured cotyledons. The fruit (Fig. 1) is seasonal (usually from the months of December to March). The seeds are also used for local games or discarded. The fleshy fruit pulp is suitable for jams and is eaten especially as snack by many locals.

Chrysophyllum albidum is a plant which has been used in traditional/alternative medicine in Nigeria to treat health problem, various parts of this herb have been proved to have a wide range of therapeutic effects. Generally, the roots, barks and leaves of C. *albidum* is/are widely used as an application to sprains, bruises and wounds in southern Nigeria [27].

In folklore medicine, *Chrysophyllum albidum* bark is employed for the treatment of yellow fever and malaria. In the Southern Nigeria, the roots and stem barks are employed in urinary related infections [28]. The leaf is used as an emollient and for the treatment of skin eruption, stomachache and diarrhea [29-30]. The decocted leaves are

administered as a cancer remedy and as pectoral in Cuba [31]. In Venezuela, the bitter pulverized seed is taken as a tonic, diuretic, febrifuge and in the treatment of diarrhea. The seeds and roots extracts of C. *albidium* is used to arrest bleeding from fresh wounds, and to inhibit microbial growth of known wound contaminants and also enhance wound healing process [31]. In addition, its seeds are a source of oil, which is used for diverse purposes.

Figure 1: Chrysophyllum albidum plant

2. Materials and Methodology

2.1. Collection of Plant Materials

The stem bark of *Chrysophyllum albidum* was collected from a local farm at Owo (710'59.998N and 534'59.988E), Ondo State, Nigeria at WAT UTC+1 time zone on 26th and 27th of February, 2016. Fresh and healthy fruits of the plant were also collected during its fruiting season, between January and April 2016, from the same geographical locations.

2.2. Raw Material Preparation

2.2.1. Seed Preparation

The *Chrysophyllum albidum*, seeds undergo various processing in the course of its preparation for extraction. The seeds were separated from its fleshly pulp and then air dried in room temperature. The seeds were later broken up to yield cotyledon and seed coat. The seed coat was separated from the cotyledon. The needed part which is the cotyledon is grinded using mortar and pestle. The cotyledon were crushed to obtain a size of 1.18 mm sieve size, in order to weaken or rupture the cell walls to release castor fat for extraction [32-33].

2.2.2. Stem Bark preparation

The plant sample was air-dried until a constant weight was obtained. The dried stem bark was ground into powder using a mill. The powdered material was stored in an air-tight container for further use.

2.3. Extraction Method: Soxhlet Extraction of the seed and stem bark oil content

About 250 ml of n-Hexane was poured into a round bottom flask. 10 g of the powdered seed sample was placed in the thimble and was inserted in the centre of the soxhlet extractor. The extractor was then heated to and held constant at 65 °C. As the solvent begins boiling; the vapor rose through the vertical tube of the extractor into the condenser at the top of extractor. The liquid condensate then dripped into the filter paper thimble in the centre which contained the solid sample from which oil is extracted. The extract seeped through the pores of the thimble and filled the siphon tube, where it flowed back down into the round bottom flask. This was allowed to continue for 5 hours. It was then removed from the tube, dried in the oven, cooled in the desiccators and weighed again to determine the amount of oil extracted. Further extraction was carried out at 30 min intervals until the sample weight

at further extraction and the previous weight became equal. At the end of the extraction, the resulting mixture containing the *Chrysophyllium albidum* oil was heated to recover solvent from the oil [34].

2.4. Standardization of Plant extracts

The extracts was reactivated at aseptic condition by adding 1g of each extracts to 2.5ml of DMSO (Dimethylsulphoxide) and 7.5ml of sterile distilled water making it 100mg/ml. for each extracts, 5ml of distilled water is measured into four sterile bijoux bottles making it 50mg/ml. the serial concentration was prepared to get concentrations of 50mg/ml, 25mg/ml and 12.5mg/ml [35].

2.5. Test organisms

Standard strains of pathogenic organisms; *Staphylococcus aureus* (ATCC 55620), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC23922), *Salmonella typhi* and *Klebsiella pneumonia* (ATCC 15380) obtained from the stock culture of organisms at the college of medicine, University of Lagos and pure cultures of clinical fungal isolates; *Trichophyton rubrum*, *Aspergillus flavus* and *Aspergillus fumigatus* sourced from the Microbiology laboratory, Department of microbiology, Adekunle Ajasin University, Ondo State, Nigeria were used. The bacterial isolates were maintained on agar slant of Mueller hinton agar in bijoux bottles at 4 °C and transported to the Laboratory of Microbiology Department, Adekunle Ajasin University, Akungba Akoko and then incubated in an incubator for reactivation of the bacteria. These strains were sub - cultured on a fresh appropriate agar plate 24 hrs and 3days prior to any antimicrobial test, bacterial and fungal respectively [36].

2.6. Standardization of Test Organisms

Bacterial and fungal isolates were sub-cultured onto freshly prepared Mueller Hinton agar and Potato Dextrose agar plates and incubated for 24 h at 37°C and 3 to 5 days at room temperature respectively. A portion of the streaked bacterial colonies were transferred into McCartney bottles containing 1 ml of sterile distilled water likewise a small inoculum from the lawn of fungal growth was transferred into McCartney bottles containing 1 ml of sterile distilled water. Vortexing was carried out and the turbidity was adjusted to match 0.5 Mc Farland standards (106 cfu/ml and 106 spores /ml) [36].

2.7. Determination of Antimicrobial Activity of the Extract

All the test bacteria, were sub-cultured onto sterile Mueller Hinton agar plates, and incubated at 37 ºC for 18-24 hours. Five distinct colonies for each organism were inoculated onto sterile Mueller Hinton broth and incubated for 3-4 hours. All inocula were standardized accordingly to match the 0.5 McFarland standards, and this standard was used for all susceptibility tests. All the extracts were reconstituted accordingly into the following concentrations; 100, 50, 25, 12.5, 6.25mg/ml, using Dimethylsulphoxide (DMSO). The susceptibility testing was investigated by the agar well diffusion method. A 0.1ml of 1: 10,000 dilutions (equivalent to 10^6 cfu/ml) of fresh overnight culture of the clinical isolates grown in Mueller Hinton agar and Sabouraud dextrose agar was seeded into 40ml of Mueller 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 6mm diameter, equidistant wells were made in the agar. Drops of the re suspended, (2ml per well) extracts with concentrations between 100mg/ml to 6.25mg/ml were introduced into the wells till it was filled. Levofloxacin 50mg/ml was used as the control experiment for bacteria, while fluconazole 50mg/ml was used as the positive control for fungi. 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 for the bacterial isolates and 24 ºC for 48 hours for the fungal isolates. The zones of inhibition were measured to the nearest millimetre (mm) using a standard transparent metre rule. All experiments were performed in duplicates [37].

2.8. Phytochemical Analysis of the Plant Extract

2.8.1. Qualitative Phytochemical Analysis of *Chrysophyllum albidum*

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.

Test for Alkaloid

TLC method

The powdered test samples were wet with a half diluted NH_4OH and lixiviated with EtOAc for 24hr at room temperature. The organic phase was separated from the acidified filtrate and basify with NH_4OH (pH 11-12). It was then extract with chloroform (3X), condense by evaporation and use for chromatography. The alkaloid spots were separated using the solvent mixture chloroform and methanol (15:1). And the spots were sprayed with Dragendorff's reagent. Orange spot shows it's a positive result [38-39].

Test for Anthraquinone

Borntrager's test: Heat about 50mg of extract with 1ml 10% ferric chloride solution and 1ml of concentrated hydrochloric acid. Cool the extract and filter. Shake the filtrate with equal amount of diethyl ether. Further extract the ether extract with strong ammonia. Pink or deep red coloration of aqueous layer [40-41].

Test for Cardiac glycosides

TLC method - The powdered test samples were extracted with 70% EtOH on rotary shaker (180 thaws/min) for 10hr. 70% lead acetate was added to the filtrate and centrifuge at 5000rpm/10 min. The supernatant is then further centrifuged by adding 6.3% Na_2CO_3 at 10000 rpm/10min. The retained supernatant was dried and redissolved in chloroform and use for chromatography. The glycosides were separated using EtOAc-MeOH-H_2O (80:10:10) solvent mixture. The color and hRf values of these spots can be recorded under ultraviolet (UV254 nm) light [38].

Test for Flavonoid

TLC method - 1g powdered test samples was extracted with 10ml methanol on water bath (60°C/ 5min). The filtrate was condensed by evaporation, a mixture of water and EtOAc (10:1 mL) was added and mixed thoroughly. The EtOAc phase was retained and use for chromatography. The flavonoid spots were separated using chloroform and methanol (19:1) solvent mixture. And then the color and hRf values of these spots were recorded under ultraviolet (UV254nm) light [38, 40].

Test for Phenol

Phenol test: The extract was spotted on a filter paper. A drop of phoshomolybdic acid reagent was added and expose to ammonia vapors. Blue coloration of the spot shows it's a positive result [40].

Test for Saponin

TLC method: two grams of powdered test samples were extracted with 10 ml 70% EtOH by refluxing for 10 min. The filtrate was condensed, enriched with saturated n-BuOH, and mix thoroughly. The butanol was retained, condensed and use for chromatography. the saponins were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The chromatogram was exposed to the iodine vapors. The colour (yellow) and hRf values of these spots were recorded by exposing chromatogram to the iodine vapours [38, 42].

Test for Steroid

TLC method: Two grams of powdered test samples were extracted with 10ml methanol in water bath (80°C/15 min). The condensed filtrate was used for chromatography. The sterols was separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The color and hRf values of these spots were recorded under visible light after spraying the plates with anisaldehyde- sulphuric acid reagent and heating (100°C/6 min). The color (Greenish black to Pinkish black) and hRf values of these spots were recorded under visible light [38].

Test for Tannin

Braemer's test: 10% alcoholic ferric chloride was added to 2-3ml of methanolic extract (1:1). Dark blue or greenish grey coloration of the solution shows it's a positive result [40, 42].

2.8.2. Quantitative Phytochemical Analysis of *Chrysophyllum albidum*

1. Test for 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 was heated using a hot water bath. At about $55^{O}C$, 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 40ml over a water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20rnl of diethyl ether were added and then shaken

vigorously. The aqueous layers were recovered while the ether layer was discarded. The purification process was repeated three times. 60rnl of *n*-butanol were added. The combined *n*-butanol extracts were washed twice with 10 m1 of 5% aqueous sodium chloride. The remaining solutions 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

2. Test for Flavonoids: About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solutions 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 was weighed to a constant weigh.

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

4. Test for Alkaloids: 5 grams of the plant samples 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 was 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.

2.9. Analysis of the essential oil

Capillary gas chromatography was performed using Hewlett–Packard 5890 gas chromatograph equipped with a flame ionization detector and fused silica capillary column HP-5 (5 % diphenyl and 95 % dimethylpolysyloxane, 30 m × 0.25 mm, 0.25 μm film thickness); injector and detector temperatures were 270 ^{o}C and 300 ^{o}C, respectively. The components of the essential oil were separated by the GC and identified by mass spectrometry (GC–MS) using Agilent 6890N gas chromatography coupled to Agilent5973N mass selective detector. GC settings were as follows: the initial oven temperature was held at 60^{o}C for 1 min and ramped at 10^{o}C min−1 to 180^{o}C where it was held for 1 min, and then ramped at 20C min−1 to 280 C and held there for 15 min. The sample (1 μl, diluted 1:100 in acetone) was injected, with a split ratio of 1:10. The carrier gas was helium at a flow rate of 1.0ml min−1. Spectra were obtained over the scan range 20 to 550 m/z at 2 scans s-1. Most constituents were identified by gas chromatography by comparison of their retention indices with those published in the literature or with those of authentic compounds available in the laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8–C24) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [43]. Relative percentages of the oil components were calculated based on GC peak areas without using correction factors.

3. Results

3.1. Antimicrobial Activity of Plant Extracts

Results presented in Table 1 and 2 shows the zones of inhibition of the of the bacterial growth against the essential oil extracted from *Chrysophyllum albidum* stem bark and seed cotyledon respectively at different concentration. The samples of the plants extracts were prepared at different concentration of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml.

Table 1: This shows the zones of inhibition of bacteria and fungi isolates against *Chrysophyllum albidum* seed oil. *Samonella typhi* is the most sensitive organism to the seed cotyledon extract at 100 mg/ml concentration having zone of inhibition of 29mm. similarly, *Klebsiella pnemoniae* has a high zone of inhibition of 27mm at same concentration. Also, *Escherichiacoli* has its highest zone of inhibition of 6mm at same concentration of 100mg/ml and lowest of 5mm at 50mg/ml. All test isolates (bacterial and fungal) show resistance at 12.5mg/ml. The fungal isolates, *Trichophyton rubrum* exhibits zones of inhibition of 18mm and 13mm at 100mg/ml and 50mg/ml

respectively. *Aspergillus fumigatus* shows zone of inhibition of 10mm at the highest concentration of 100mg/ml and resistance at other concentrations. *Aspergillus flavus* shows resistance at all concentration against the plant extract. *Samonella typhi* was more susceptible to the essential oil of *Chrysophyllum albidum* seed than to the synthetic antibiotics, ofloxacin.

Table 1: Antimicrobial Activity of the Essential Oil of *Chrysophyllum albidum* Seed against Selected Organisms

Organisms Control	**Zones of Inhibition (mm)**					
	100	**50**	**25**	**12.5**	**Ofloxacin/ Fluconazole (50mg/ml)**	**DMSO (30%)**
Staphylococcus aureus	11.0	6.0	0.0	0.0	28.0	0.0
Pseudomonas aeruginosa	25.0	18.0	11.0	0.0	0.0	0.0
Klebsiella pneumonia	27.0	19.0	10.0	0.0	19.0	0.0
Escherichia coli	6.0	5.0	0.0	0.0	24.0	0.0
Salmonella typhi	29.0	18.0	13.0	0.0	27.0	0.0
Trichophyton rubrum	18.0	13.0	0.0	0.0	14.0	0.0
Aspergillus fumigatus	10.0	0.0	0.0	0.0	19.0	0.0
Aspergillus flavus	0.0	0.0	0.0	0.0	0.0	0.0

Positive control for bacteria= Ofloxacin; Positive control for fungi= Fluconazole

Table 2: Shows the antimicrobial activity of essential oil of *Chrysophyllum albidum* stem bark against all the test organisms. *Samonella typhi* was observed to have the highest susceptibility to the stem bark extract with 19mm in diameter zone of inhibition at 100mg/ml 14mm in diameter zone of inhibition at 50mg/ml and 8mm at 25mg/ml. *S. aureus* showed susceptibility to this extract having 11mm zone of inhibition at 100mg/ml, and 6mm zone of inhibition at 50mg/ml.

From this study, *Pseudomonas aeruginosa* was also observed to have the lowest susceptibility to the stem bark extract of the plant, with 6mm at 100mg/ml and resistant in other concentrations. The antifungal activities essential oil of *Chrysophyllum albidum* of stem bark against the test fungal isolates was also recorded in this table. The fungal isolates which includes *Trichophyton rubrum, Aspergillus fumigatus and* A. *flavus* showed resistance against the plant extract at all concentrations. The bacterial and fungal isolates were more susceptible to Ofloxacin than the essential oil of *Chrysophyllum albidum* stem bark part.

Table 2: Antimicrobial Activity of the Essential Oil of *Chrysophyllum albidum* Stem bark against Selected Organisms

Organisms Control	**Zones of Inhibition (mm)**					
	100	**50**	**25**	**12.5**	**Ofloxacin/ Fluconazole (50mg/ml)**	**DMSO (30%)**
Staphylococcus aureus	11.0	6.0	0.0	0.0	28.0	0.0
Pseudomonas aeruginosa	6.0	0.0	0.0	0.0	0.0	0.0
Klebsiella pneumonia	10.0	9.0	0.0	0.0	19.0	0.0
Escherichia coli	10.0	5.0	0.0	0.0	24.0	0.0
Salmonella typhi	19.0	14.0	8.0	0.0	27.0	0.0
Trichophyton rubrum	9.0	5.0	3.0	0.0	14.0	0.0
Aspergillus fumigatus	0.0	0.0	0.0	0.0	0.0	0.0
Aspergillus flavus	0.0	0.0	0.0	0.0	19.0	0.0

Positive control for bacteria= Ofloxacin; Positive control for fungi= Fluconazole

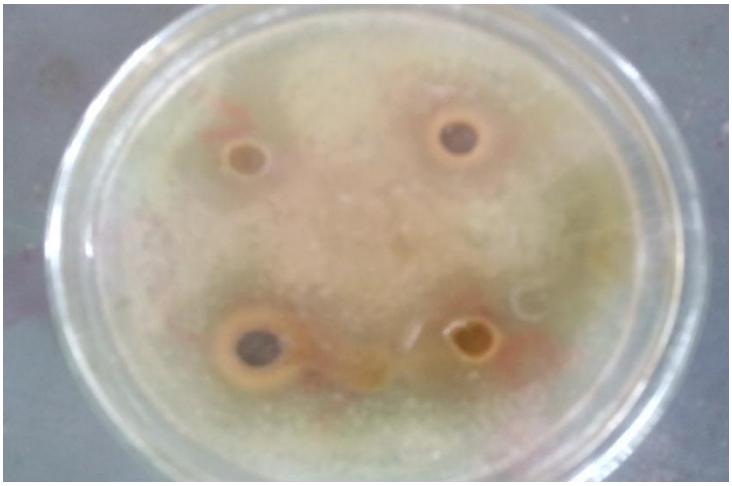


Plate 1: Antimicrobial activity of Chrysophyllum albidum seed oil against Staphylococcus aureus

3.2. Qualitative Phytochemical Analysis of *Chrysophyllum albidum*

Table 3: This shows the Phytochemical screening of *Chrysophyllum albidum* seed and stem bark oil using methanol. The presence of alkaloid, tannin, anthraquinone, phenol, cardiac glycosides and reducing sugar and was devoid of steroids, and `flavonoids were seen in the seed part. The plant stem bark part also shows the presence alkaloid, cardiac glycosides, tannins and flavonoids and absence of steroids, phenol, saponin and reducing sugar as presented in Table 3.

Table 4: Represents phytochemical screening of *Chrysophyllum albidum* seed using Ethyl acetate reveals the presence of alkaloid, cardiac glycosides, steroids, anthraquinone , phenol, tannins, saponins, flvonoids and reducing sugar for both samples aside anthraquinone and reducing sugar which were absent in the stem bark part. And anthraquinone was not detected in the seed sample (Table 4)

Table 5: The solvent dichloromethane as represented in table 5 recovers alkaloid, anthraquionone, tannins, flavonoids and reducing sugar in the seed cotyledon part except steroids and phenol. Stem bark in same solvent also showed the presence of cardiac glycoside, steroids, phenol, saponin and flavonoids while alkaloid, anthraquinone, tannins and reducing sugar were absent in the sample.

Table 6: Phytochemical analysis of C. *albidum* stem bark and seed cotyledon using n-Hexane reveal the presence of Alkaloid, Cardiac glycoside, Steroids, phenol, tannins, saponin, flavonoids and reducing sugar. Cardiac glycoside and saponin were absent in the stem bark sample and Anthraquinone was not detected in the sample.

Table 3: Qualitative phytochemical analysis of *Chrysophyllum albidum* (Methanol)

Test	Seed	Bark
Alkaloid	+	+
Cardiac Glycoside	+	+
Steroids	-	-
Anthraquinone	+	ND
Phenol	+	-
Tannins	+	+
Saponin	+	-
Flavonoids	-	+
Reducing sugar	+	-

ND: Not Detected; +: Positive; -: Negative

Table 4: Qualitative phytochemical analysis of *Chrysophyllum albidum* (Ethyl acetate)

Test	Seed	Bark
Alkaloid	+	-
Cardiac Glycoside	+	+

Steroids	+	+
Anthraquinone	ND	-
Phenol	+	-
Tannins	+	+
Saponin	+	+
Flavonoids	+	+
Reducing sugar	+	-

ND: Not Detected; +: Positive; -: Negative

Table 5: Qualitative phytochemical analysis of *Chrysophyllum albidum* (Dichloro methane)

Test	Seed	Bark
Alkaloid	+	-
Cardiac Glycoside	+	+
Steroids	-	+
Anthraquinone	+	-
Phenol	-	+
Tannins	+	-
Saponin	+	+
Flavonoids	+	+
Reducing sugar	+	-

+: Positive; -: Negative

Table 6: Qualitative phytochemical analysis of *Chrysophyllum albidum* (*n*- hexane)

Test	Seed	Bark
Alkaloid	+	+
Cardiac Glycoside	+	-
Steroids	+	+
Anthraquinone	ND	ND
Phenol	+	+
Tannins	+	+
Saponin	+	-
Flavonoids	+	+
Reducing sugar	+	+

ND: Not Detected; +: Positive; -: Negative

3.3. Quantitative Analysis of Phytochemical Constituents

Table 7 to 9 present the quantitative analysis of the phytochemical screening of *Chrysophyllum albidum* seed and stem bark using the solvents methanol and ethyl acetate.

Table 7: The quantitative analysis of the various constituents; alkaloid, oxalate, phytate, phenol, tannins, saponin and flavonoids in C. *albidum* seed cotyledon sample shows larger amounts compared to stem bark using methanol as solvents.

Table 8: The quantitative analysis of the phytochemical screening of C. *albidum* seed cotyledon and stem bark using the solvent ethyl acetate as presented in Table 6. The result shows that saponin (21.14) is the most present in the seed cotyledon and flavonoids (1.20) the least present. The stem bark also reveals the least in flavonoids (2.56) and most present in oxalate (24.98).

Table 9: The quantitative analysis of phytochemical components using N-hexane reveals large amounts of the constituents in the seed cotyledon and stem bark ranging from Tannins (23.32) to Alkaloid (14.62) and flavonoids a bit lower of 11.20 and 12.56 in the seed cotyledon and stem bark samples respectively.

Table 7: Quantitative phytochemical analysis of *Chrysophyllum albidum* (Ethyl acetate) (%)

Test	Seed	Bark
Alkaloid	14.62	19.00
Oxalate	15.24	24.98
Phytate	19.78	23.12
Phenol	20.10	20.34
Tannins	20.32	16.89
Saponin	21.14	22.12
Flavonoids	1.20	2.56

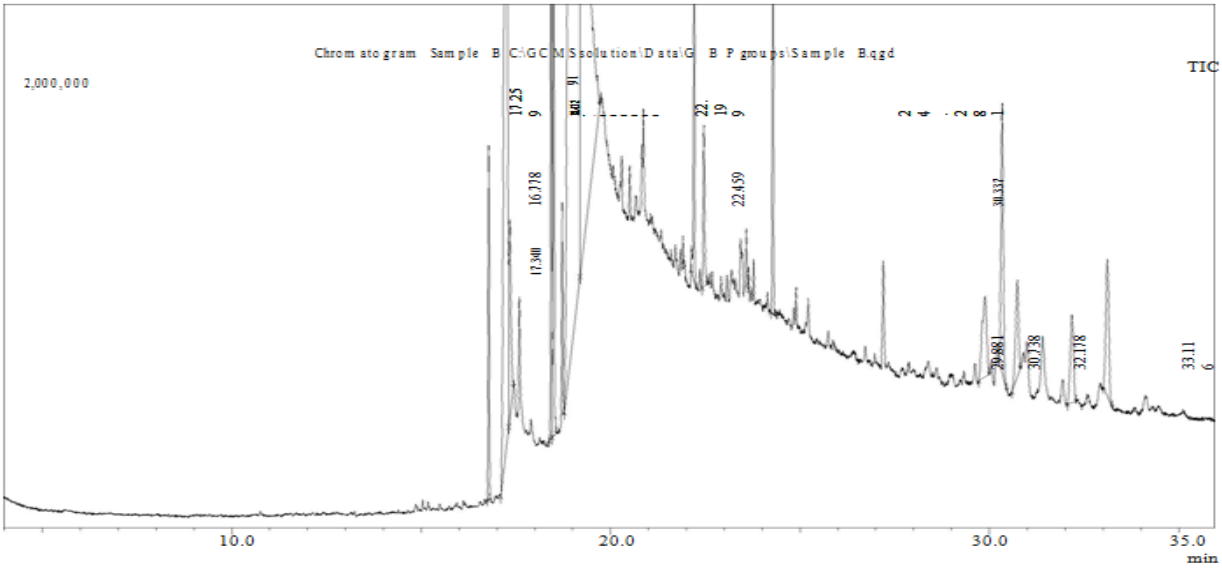
Table 8: Quantitative phytochemical analysis of *Chrysophyllum albidum* (n-Hexane) (%)

Test	Seed	Bark
Alkaloid	14.62	
Oxalate	15.24	
Phytate		
Phenol		
Tannins		
Saponin		
Flavonoids		

Table 9: Quantitative phytochemical analysis of *Chrysophyllum albidum* (n-Hexane) (%)

Test	Seed	Bark
Alkaloid	14.62	19.1
Oxalate	15.24	24.98
Phytate	19.78	23.12
Phenol	22.10	20.34
Tannins	23.32	16.89
Saponin	21.14	22.12
Flavonoids	11.20	12.56

3.4. Gas Chomatography-Mass Spectrometer

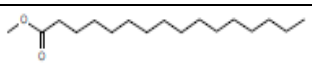
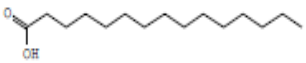
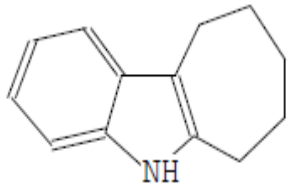
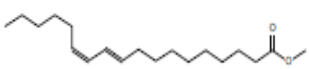
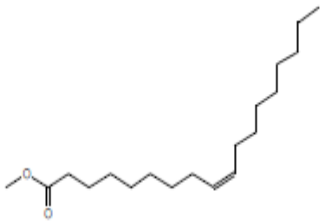
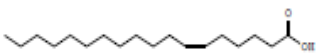
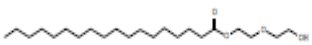


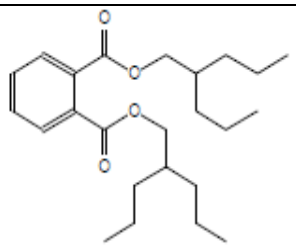
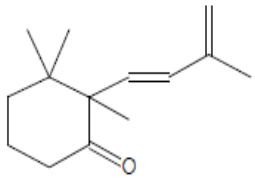
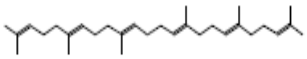
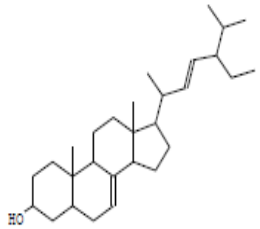
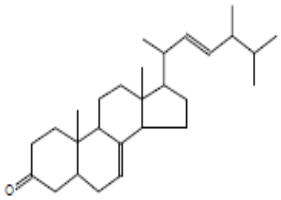
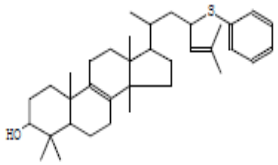
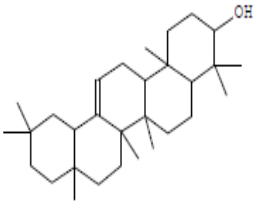
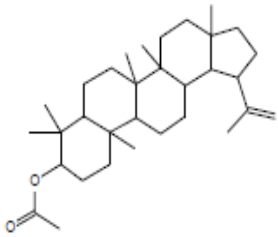
Spectra of Gas Chromatography/Mass Spectrophotometry Analysis of Chrysophyllum albidum

Using Gas Chromatography Mass Spectrometer (GC-MS), 15 fifteen components were detected in *Chrysophyllum albidum* seed essential oil, with Cycloheptan(a)indole, 9-octadecenoic acid(z)-, Methyl ester and Octadecanoic acid being identified as marker compounds. *Chrysophyllum albidum* essential oil extract showed the presence of large 5 to 6 peaks including other minor peaks.

The main peak of about 19130 secs match the library mass spectra to 6-octadecenoic acid, (z)-. The second main peak about 19246 secs matches to the library mass spectra toOctadecanoic acid. The Gas Chromatography Mass Spectra also reveal the presence of hexadecanoic acid, methyl ester, Pentadecanoic acid, Methyl 10-trans, 12-cis-octadecenoate,2,2,3- Trimethyl-2-3-methyl-buta 1,3-dienyl, Squalene, Chondrillasterol, 7,22- Ergostadienone, 17-(1,5- Dimethyl-3-phenylthiohex-4-enyl)-4, Beta.-Amyrin, Lup-20(29)-en-3-ol, acetate, (3-beta)and Phthalic acid, di(2-propylpentyl) ester. Further identification information, including retention time, molecular similarity and probability values, greatly increases the reliability of this analysis and is reported in the table below (Table 10).

Table 10: Compounds identified in the essential oil of *Chrysophyllum albidum* seed Gas Chromatography Mass Spectrometry (GC-MS)

S/N	RT	BP	Height%	Molecular weight	Compound Name	Compound Formula	Compound Structure
1	16778	74.00	2.02	270	Hexadeconoic acid, Methyl ester	$C_{17}H_{34}O_2$	
2	17259	73.00	11.77	242	Pentadecanoic acid	$C_{15}H_{30}O_2$	
3	17340	185.10	1.13	185	Cycloheptan(a)indole	$C_{13}H_{15}N$	
4	18432	67.05	4.99	294	Methyl 10-trans, 12-cis-octadecadienoate	$C_{19}H_{34}O_2$	
5	18491	55.05	7.07	296	9-octadecenoic acid(z)-, methyl ester	$C_{19}H_{36}O_2$	
6	19128	65.05	47.81	282	6-octadecenoic acid, (z)-	$C_{18}H_{34}O_2$	
7	19246	73.00	14.94	284	Octadecanoic acid	$C_{18}H_{36}O_2$	

8	22199	149.05	2.26	390	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	
9	22459	123.10	0.91	206	2,2,3-Trimethyl-2-3-methyl-buta 1,3-dienyl	$C_{14}H_{22}O$	
10	24281	69.05	3.35	410	Squalene	$C_{30}H_50$	
11	29881	271.20	0.45	412	Chondrillasterol	$C_{29}H_{48}O$	
12	30337	269.20	1.51	382	7,22- Ergostadienone	$C_{28}H_{44}O$	
13	30738	69.05	0.54	426	17-(1,5-Dimethyl-3-phenylthiohex-4-enyl)-4	$C_{36}H_{54}OS$	
14	32178	105.05	0.49	424	Beta. -Amyrin	$C_{30}H_{50}O$	
15	33116	189.15	0.77	468	Lup-20(29)-en-3-ol, acetate, (3-beta)	$C_{32}H_{52}O_2$	
	Total		**100**				

Key: Retention time=RT, Base Peak= BP

Table 13: Medicinal value of the major peaks of GC-MS analyzed chemical constituents of *Chrysophyllum albidum* seed essential oil

Compound Name	Compound Nature	Activity
Pentadecanoic acid	Fatty acid	Antimicrobial
n-Hexadecanoic acid	Fatty acid	Antioxidant, Hypocholesterolemic
6-Octadecenoic acid	Petroselinic acid	Antioxidant, Hypocholesterolemic
Octadecanoic acid	Fatty acid	Antifungal, antitumor
Octadecanoic acid, methyl ester	Stearic acid methyl ester	Antioxidant, Hypocholestero-lemic
Hexadecanoic acid methyl ester	Palmitic acid methyl ester	Antioxidant, Hypocholestero-lemic
9-Octadecenoic acid, methyl ester	Oleic acid methyl ester	Antioxidant, Hypocholesterolemic
Phthalic acid, di(2-propylpentyl) ester	Bicyclic Aromatic Hydrocarbon	Anti-inflammatory, Antimicrobial
Squalene	Triterpenoid	Antiradioant and Antioxidant

4. Discussion

Essential oils are recognized for their medicinal value and they are very interesting and powerful natural plant products. They are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides [1]. The screening of eesential oil from *Chrysophyllum albidum* seeds and stem bark for antimicrobial activity has shown that this plant represent a potential source of novel antibiotics.

The main goal of this work was to determine and compare the antimicrobial properties of *Chrysophyllum albidum* seed and stem bark essential oil by testing it against some selected clinical isolates, to determine and compare the phytochemical composition of the oil of the *Chrysophyllum albidum* seeds and stem bark, to analyze the chemical compounds of the essential oil from the seed, the biological properties of the compounds in order to provide scientific validation for their use and as potential source of drug development.

Chrysophyllum albidum seed oil exhibited broad spectrum and antifungal activity. It inhibited the growth of seven (7) pathogenic organisms; *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Salmonella typhi, Trichophyton rubrum and Aspergillus fumigatus.* The *Chrysophyllum albidum* stem bark oil also exhibited antibacterial activity against all the five (5) Gram-negative and Gram-positive bacteria tested. The fungi *Trichophyton rubrum, Aspergillus flavus and Aspergillus fumigatus* were resistant to the plant extract [44].

The zones of inhibition for the essential oil of *Chrysophyllum albidum* seed range from 29mm to 6mm at a concentration of 100mg/ml, and 13mm to 10mm at 25mg/ml. *Salmonella typhi* was discovered from this study to be the most susceptible organism to the *Chrysophyllum albidum* seed oil, Escherichia coli shows low susceptible to this oil extract while *Aspergillus flavus* was seen to be resistant to the seed oil extract. Other test organisms showed very good susceptibility to the seed oil extract. The essential oil of *Chrysophyllum albidum* seed as observed from this study, can be a source of a novel antimicrobial agent, especially with good activities against organisms like *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Samonella typhi*. It can also be a source of antimicrobial agent against the dematophytes *Trichophyton rubrum* which was resistant to the antibiotic Ofloxacin and was seen to be susceptible to this extract. *Chrysophyllum albidum* was also very active against *Aspergillus fumigatus* used in this study, having 10mm zone of inhibition at 100mg/ml, where in *Trichophyton* has the highest zones of inhibition of 18mm and 13mm at 100mg/ml and 50mg/ml respectively. The most glaring aspect is the antibacterial activity of *Chrysophyllum albidum* having relatively good activity against Pseudomonas aeruginosa at a concentration of 25mg/ml, a bacterial isolate that was resistant to 50mg/ml Ofloxacin.

On the other hand, *Chrysophyllum albidum* stem bark oil exhibited comparatively low antimicrobial activity against the selected organisms to the essential oil from *Chrysophyllum albidum* seed. The zones of inhibition for the essential oil of *Chrysophyllum albidum* stem bark range from 19mm to 6mm at a concentration of 100mg/ml, and 8mm at 25mg/ml. *Salmonella typhi* was discovered from this study to be the most susceptible organism to the *Chrysophyllum albidum* stem bark oil, Pseudomonas aeruginosa shows low susceptible to this oil extract while the

fungal isolates were seen to be resistant to the seed oil extract. Escherichia coli was also resistant to the stem bark of *Chrysophyllum albidum*. Ofloxacin thus has relatively good activity against all of the selected bacterial and fungal isolates.

The health promoting properties of plant-based foods have largely been attributed to their wide range of phytochemicals [46]. *Chrysophyllum albidum* seed oil phytochemical analysis reveals the presence of metabolites that are of great importance in phytomedicines development. Aside anthraquinone that was not detected, flavonoids, alkaloids, cardiac glycosides, steroids, phenol, tannins, saponins and reducing sugar were present in the seed samples and flavonoids, alkaloids, steroids, phenol, tannins and reducing sugar in the stem bark part using n-hexane. This supports the use of different parts of *Chrysophyllum albidum* plants in the treatment of many diseases as claimed by herbalists. In that, Flavonoids and Tannins are important sources of natural antioxidants preferred over synthetic ones as they are less toxic [46] and are present in the seed and stem bark extract. Flavonoids scavenge free radicals produced by Reactive Oxygen Species (ROS) thereby preventing diseases caused by oxidative stress. Alkaloids have anti-inflammatory property [47].

Li *et al*. [48] reviewed the biological activities of tannins and observed that tannins have remarkable activity in cancer prevention and anticancer, thus suggesting that *Chrysophyllum albidum* could be a possible source of important bioactive molecules for the treatment and prevention of cancer. Alkaloid is also one of the phytochemical compounds observed in both parts of *Chrysophyllum albidum.* Alkaloids have been associated with medicinal uses for centuries. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. It is also a known active compound of most anti-malarial drugs.

Saponins which have been ascertained to be responsible for numerous pharmacological properties [49] were also present in *Chrysophyllum albidum* seed and stem bark. Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects in medicinal plants [50]. Saponins present in the seed part and absent in stem bark exhibits hypocholesterolemic property through the formation of insoluble complexes with cholesterol and consequently slowing down its absorption [51]. It is not unlikely that these bioactive compounds found in *Chrysophyllum albidum* seed and absent in *Chrysophyllum albidum* stem bark are the reason for the wide antimicrobial activity exhibited by the essential oil from *Chrysophyllum albidum* seed when compare to stem bark part.

With the development of botanical drugs, including traditional herbal medicines, analysis of their bioactive components is becoming more popular. Many botanical drugs have bioactive components in their essential oils, so characterization of plant essential oils is an important and meaningful task. Gas chromatography (GC) or gas chromatography-mass spectroscopy (GC-MS) are used almost exclusively for the qualitative analysis of the volatiles [52].

The Gas chromatography/mass spectrometer analysis identified fifteen (15) constituents from the essential oil extract of *Chrysophyllum albidum* seed. They are bioactive compounds mostly saturated and monounsaturated fatty acids, lipid and compounds of Methyl esters. These compounds are responsible for the antibacterial, antifungal and antioxidant activity of the essential oil. 9-octadecenoic acid (z)-, methyl ester, 6-octadecenoic acid, (z) - are known to exhibit hypocholesterolemic activity. Also the compounds, Hexadeconoic acid, Methyl ester Pentadecanoic, acid Octadecanoic acid, Phthalic acid, di(2-propylpentyl) ester possess antimicrobial activity.

Squalane is also reported by cancer researchers to possess anti-carcinogenic agent with the observed correlation between a high amount of squalene in shark fatty tissues and the absence of cancer in this species [53] as well as possibly playing a significant role in lowering cancer rates found with the Mediterranean Diet [54]. According to Smith and Theresa [55], Squalene can act as a free radical scavenger and seems to enhance the anti-carcinogenic effect of co-ingested drug treatments and shows synergism against cancer with oleic acid, another constituent found in the *Chrysophyllum albidum* seed oil.

The major peaks of the mass spectra matches compounds; The Phtalates, octadecenoic acid, 6-Octadecenoic acid, Hexadecanoic acid methyl ester, 9-Octadecenoic acid, methyl ester, Squalene and Methyl 10-trans, 12-cis-octadecadienoate are majorly antioxidant and antimicrobial. And thus makes this study correlates with the proposition drawn by some personalities [56-57] from the anti - microbial activity of different solvent extracts of C.

albidium seed studied that *C. albidium* seed oil and ethanolic extract of *C. albidium* seed were broad spectrum antibiotics and anti - fungal drugs.

4.1. Conclusion

The presence of phytoconstituents is responsible for the antibacterial activity and antifungal potential of *Chrysophyllum albidum* seed essential oil and stem bark. And that n-hexane is a better solvent on the plant compare to methanol, ethyl acetate and dichloromethane. The presence of some compounds in the seed and absent in the stem bark is an indication of the difference in the antimicrobial activity of the seed oil stem bark oil. In that the seed oil of *Chrysophyllum albidum* exhibited relatively higher antimicrobial activities when compared with stem bark of the plant. However, it was observed that the selected organisms were all resistant to *Chrysophyllum albidum* seed and stem bark at lower concentration of 25mg/ml which is an indication that this plant parts may be effective against diseases only at higher quantities.

It can also be seen in the quantitative analysis that the solvents N-hexane reveals larger quantity of the metabolites present than methanol. This reveals that n-hexane is a good solvent in extraction of the *Chrysophyllum albidum* essential oil. Identification of chemical compounds in the form of lipid such as squalene and saturated fatty acid such as the Hexadeconoic acid, Methyl ester, Pentadecanoic acid, Octadecanoic acid (Stearic acid), and monounsaturated fatty acid such as 6-octadecenoic acid, (z)-(Oleic acid), in *Chrysophyllum albidum* seed oil by GC-MS confirmed the plant part to be a potential source for bioactive substances that supports several pharmaceutical uses and therapeutic value.

4.2. Recommendation

Most often the seed are thrown away after the consumption of its juicy pulp. I recommend that the seed be exploited because of the wide spectrum of pharmacological activities associated with the biologically active chemicals present in this plant components.

As GC-MS is the first step towards understanding the nature of active principles, further investigation in this plant parts is suggested for the development of novel drugs. I recommend that more work should be done on the structural elucidation of *C. albidium* Seeds extract, using Fourier transform infrared spectroscopy (FTIR), UV-visible spectroscopy, GCMS and NMR equipment. This will help to isolate some medicinally active compounds that might be present in it and effort would be made in formulating them into drugs and help to improve healthcare delivery system.

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