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Evaluating the Potential of *Chrysophyllum albidum* (African Star Apple) as an Alternative Culture Media

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ABSTRACT

The feasibility of developing an alternative media different from the conventional culture media namely Potato Dextrose agar and Nutrient Agar were assessed using locally available cheap materials *Chrysophyllum albidum* (African star apple). In this study *Chrysophyllum albidum* pulp and *Chrysophyllum albidum* pulp with skin which were collected, air dried and blended to give a fine texture and was used to grow bacteria and fungi. The test microorganisms used were *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli* and *Aspergillus niger, Mucor mucedo, Aspergillus flavus, Trichoderma viridae* respectively for bacteria and fungi. The bacteria count, fungi growth were determined, proximate and mineral composition of the locally prepared media were also determined using standard methods. All the test bacteria grew on the *Chrysophyllum albidum* media except for *Escherichia coli*. The bacteria count ranged from TNT to 1.0 x 10^{-4} at 24hrs to 72hrs of incubation and no growth were observed for *Escherichia coli* while *Staphylococcus aureus* recorded the highest bacteria growth. All test fungi grew well on the *Chrysophyllum albidum* medium. The proximate composition of *Chrysophyllum albidum* pulp with skin and *Chrysophyllum albidum* pulp only has high carbohydrate content respectively (31.92%) and (35.1%) and the mineral composition of *Chrysophyllum albidum* pulp with skin and *Chrysophyllum albidum* pulp only has high potassium content respectively (274.3mg/g) and (193mg/g) which might have contributed to the growth of bacteria and fungi. The present study clearly showed the possibility of using cheap locally available materials such as African star apple as an alternative nutrient media for bacteriological and mycological studies.

KEYWORDS: *Chrysophylum albidum*, Culture media, Bacteria and fungi.

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I. INTRODUCTION

The African star apple (*Chrysophylum albidum* Linn.) is an angiosperm belonging to the order Ebernales, family Sapotaceae[1]. The plant has been reported to grow up to a height of 36.5m and is known to occur in diverse ecological zones in Nigeria, Uganda, Niger Republic, Cameron and Cote d'Ivoire[2]. Its fully ripe fruit becomes available from January through March in the Southwestern part of Nigeria. The pink-colored pulp and the whitish cover of the brown-colored seeds of the fruit are consumed, while the empty pale yellow pericarp is discarded. Cherry (*Agbalumo* in Yoruba, *Udara* in Igbo) is a native of many parts of tropical Africa. The tree grows as a wild plant which has up to 800 species and make up almost half of the order[1]. Its rich sources of natural antioxidants have been established to promote health by acting against oxidative stress related diseases such as diabetics, cancer and coronary heart diseases[3]. The fruit-pulp has been reported to contain significant amount of ascorbic acid[4], vitamins, iron and food flavors[5] fat, carbohydrate and mineral elements[6]. The fruit-peel has been shown to be a rich source of fiber and mineral[7] while the seed shell pericarp has been reported to be a good source of carbohydrate and minerals[8]. The fruits are not only consumed fresh but also used to produce stewed fruit, marmalade, syrup and several types of soft drinks[9].

Figure 1: Chrysophyllum Albidum fruits, tree and seed

Microbiological study depends on the ability to cultivate and maintain microorganisms under laboratory conditions by providing suitable culture media that offer good environmental condition[11]. A nutrient material prepared for the growth of microorganisms in a laboratory is called culture media. Culture media used in the laboratory for the cultivation of microorganism supply the nutrients required for the growth and maintenance. A medium is solid or a liquid preparation containing materials for the culture (growth) of microorganisms, animal cells or plant tissue cultures[12]. To culture organisms in laboratory, it requires the preparation of substances which can be used as food[13]. Most often, a culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors. Besides these, the pH of the medium must be set accordingly. Some of the ingredients of culture media include water, agar, peptone, casein hydrolysate, meat extract, yeast extract and malt extract[14]. Culture media is a term used to describe a complex or synthetic substance (chemically defined) found in one of two states of matter: either the liquid (broth) or solid (such as agar in a Petri dish) [15]. The two major types of culture media are those used for cell culture, which use specific type of cell types derived from plants or animals, and microbiological culture, which are used for growing microorganisms, such as bacteria or fungi. The most common culture media for microorganisms are nutrient broths and agar plates[16]. This experiment is to use *Chrysophyllum albidum* for the production of local media, to determine the microbial population on *Chrysophyllum albidum* media and to observe the growth of some microorganisms on the locally produced media by comparing them with the conventional media.

II. METHODS

Sources of materials

African star apples (Chrysophyllum albidum) were purchased from Akoda, Ede, Osun State, Nigeria. They were bought in sterile polythene bags and transferred into the laboratory for processing. The conventional media used for the research work was purchased from Fumac pharmacy Ibadan Nigeria. The bacterial cultures used were *Esherichia coli, Klebsiella pneumoniae, Staphlococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis,* and were obtained from Microbiology laboratory, University Teaching Hospital (UCH), Ibadan, Oyo State, Nigeria. The fungi culture used were *Aspergillus niger, Mucor mucedo, Aspergillus flavus, Trichoderma viridae,* and were obtained from Microbiology laboratory Federal University of Technology and Agriculture, Akure, Ondo state, Nigeria.

Preparation of chrysophyllum albidum media

African star apple (*Chrysophyllum albidum*) pulp and pulp with the skin were peeled separately and blended in a warring blender (Variable Speed Laboratory Blender) with occasional addition of water. The pulp and pulp with skin were sieved separately using a muslin cloth and the filtrate of the pulp only and pulp with skin filtrate were dissolved in distilled water, agarose at low concentration was added to aid solidification of the media. The *Chrysophyllum albidum* media were prepared in ratio 2: 1 and 3:1 of *Chrysophyllum albidum* to agarose, where 15.75g of *Chrysophyllum albidum* to 5.25 of agarose and 10.50 to .25 of agarose.

Preparation of test isolates

Eighteen hours old culture of *Esherichia coli, Klebsiella pneumoniae, Staphlococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis* and seven two hours old culture of *Aspergillus niger, Mucor mucedo, Aspergillus flavus, Trichoderma viridae* were used.

Inoculation of media

Already identified bacteria (*Esherichia coli, Klebsiella pneumoniae, Staphlococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis*) were inoculated on the formulated media and was incubated at 37^0C for 24 hours as described by[17]. This same identified bacteria were also inoculated on a conventional media; Nutrient agar which was used as control. Identified fungal organisms (*Aspergillus niger, Mucor mucedo, Aspergillus flavus, Trichoderma viridae*) were also inoculated on the formulated media and incubated for 2 to 3days as described by[17]. This same identified fungal organisms was inoculated on a conventional media; Potatoe Dextrose Agar which was also used as control.

Serial dilution of the selected bacteria

Already identified bacteria (*Esherichia coli, Klebsiella pneumonia, Staphlococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis*) were taken. 1ml of each bacteria isolate in a 24hr old broth was serial dilute and 0.1ml of the bacterial suspension was. It was inoculated in to a petri dish used using pour plating method. Then all the plates were incubated at 37^0C for 72 hours. After the incubation all the plates were observed for bacterial growth and the number of colonies was counted.

III. RESULT/ DISCUSSION

Tables 1 and 2 are showing the bacteria count of *Chrysophyllum albidum* Pulp with Skin Media and *Chrysophyllum albidum* Pulp only media at 24hrs to 72hrs. For *Pseudomonas aeruginosa* no growth was observed at 24hrs of inoculation for both *Chrysophyllum albidum* Pulp with Skin Media and *Chrysophyllum albidum* Pulp only media, the bacteria population ranged from 1.3 x 10^{-4} to 2.8 x 10^{-4} and 1.4 x 10^{-4} to 2.3 x 10^{-4} respectively for *Chrysophyllum albidum* Pulp with Skin Media and *Chrysophyllum albidum* Pulp only media at 24hrs to 72hrs. For *Klebsiella pneumonia* its growth ranged from 1.5 x 10^{-4} to 5.3 x 10^{-4} and 1 .4 x 10^{-4} to 5.8 x 10^{-4} respectively for *Chrysophyllum albidum* Pulp with Skin Media and *Chrysophyllum albidum* Pulp only media at 24hrs to 72hrs. No growth was observed for *Esherichia coli* both at Pulp with Skin and Pulp only media. For *Proteus mirabilis*, no growth were observed at 24hrs for Pulp with Skin and Pulp only media, at 48hrs to 72hrs growth ranged from 1.0 x 10^{-4} to 1.8 x 10^{-4} and 1.2 x 10^{-4} to 2.2 x 10^{-4} respectively for Pulp with Skin and Pulp only media. For *Staphylococcus aureus* growth ranged from 3.8 x 10^{-4} to TNT and 2.3 x 10^{-4} to TNT respectively for Pulp with Skin and Pulp only media at 24hrs to 72hrs.

Klebsiella pneumonia, Esherichia coli, Proteus mirabilis and *Staphylococcus aureus* Percentage proximate composition and mineral Composition of *Chrysophyllum albidum* pulp with skin and *Chrysophyllum albidum* pulp are shown on figure 2 and 3. It reflects moisture content as (10.6%), raw lipid content as (10.9%), lipid, raw ash content has (5.8%), crude fibre content has (26.8%), protein content has (14.9%) and raw carbohydrate content has (31.9%) for *Chrysophyllum albidum* pulp with skin and moisture content as (6.6%), raw lipid content as (10.9%), lipid content as (12.1%), raw ash content has (5.8%), crude fibre content has (24.2%), protein content has (16.2%) and raw carbohydrate content has (35.1%) for *Chrysophyllum albidum* pulp only (figure 2). The Percentage Mineral Composition of *Chrysophyllum albidum* pulp with skin and *Chrysophyllum albidum* pulp as shown on (figure 3) it reflects ranges in differences in values of different mineral such as phosphorus, potassium, calcium, zinc, sodium. Raw phosphorus has (11.9mg/g) value for *Chrysophyllum albidum* pulp with skin and (22.03mg/g) for *Chrysophyllum albidum* pulp only, raw potassium has (274.3mg/g) for value *Chrysophyllum albidum* pulp with skin and (193mg/g) for *Chrysophyllum albidum* pulp only, raw calcium has (16.03mg/g) value for *Chrysophyllum albidum* pulp with skin and (18.02mg/g) for *Chrysophyllum albidum* pulp only, raw zinc has (4.55mg/g) value for *Chrysophyllum albidum* pulp with skin and (3.8mg/g) for *Chrysophyllum albidum* pulp only, raw sodium has (7.5mg/g) value for *Chrysophyllum albidum* pulp with skin and (3.4mg/g) for *Chrysophyllum albidum* pulp only.

Table 1 Bacteria Count on Chrysophyllum albidum Pulp with Skin Media

Time (hours)/organisms	*Pseudomonas aeruginosa* (cfu/ml)	*Klebsiella pnenoniae* (cfu/ml)	*Escherichia coli* (cfu/ml)	*Proteus mirabilis* (cfu/ml)	*Staphylococcus Aureus* (cfu/ml)
24hours	No growth	1.5×10^{-4}	No growth	No growth	3.8×10^{-4}
48hours	1.3×10^{-4}	2.6×10^{-4}	No growth	1.0×10^{-4}	8.2×10^{-4}
72hours	2.8×10^{-4}	5.3×10^{-4}	No growth	1.8×10^{-4}	TNT

Table 2 Bacteria Count on Chrysophyllum albidum Pulp Only Media

Time (hours)/organisms	*Pseudomonas aeruginosa* (cfu/ml)	*Klebsiella pneumonia* (cfu/ml)	*Escherichia coli* (cfu/ml)	*Proteus mirabilis* (cfu/ml)	*Staphylococcus Aureus* (cfu/ml)
24hours	No growth	1.4×10^{-4}	No growth	No growth	2.3×10^{-4}
48hours	1.1×10^{-4}	2.2×10^{-4}	No growth	1.2×10^{-4}	7.2×10^{-4}
72hours	2.3×10^{-4}	5.8×10^{-4}	No growth	2.2×10^{-4}	TNT

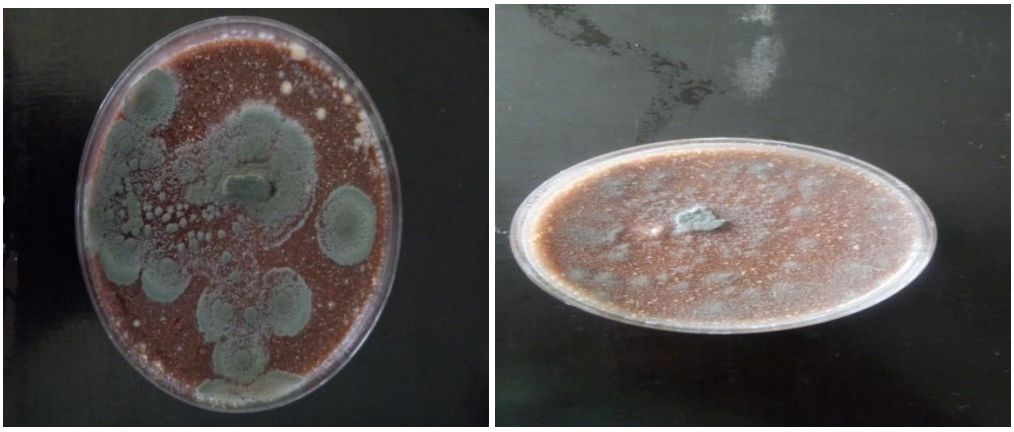
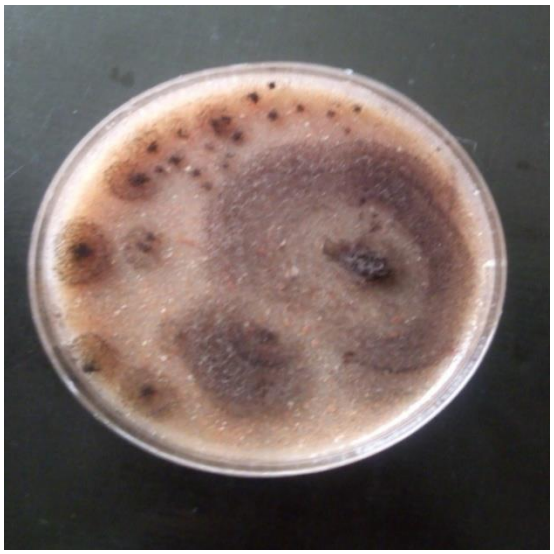


Plate 1 **Plate 2**

Plate 1: Growth of *Trichoderma virdae* on *Chrysophyllum albidum* pulp with skin agar plate
Plate 2: Growth of *Trichoderma virdae* on *Chrysophyllum albidum* pulp Only agar plate

Plate 3 **Plate 4**

Plate 3: Growth of *Mucor mucedo* on *Chrysophyllum albidum* pulp with skin agar plate
Plate 4: Growth of *Mucor mucedo* on *Chrysophyllum albidum* pulp Only agar plate



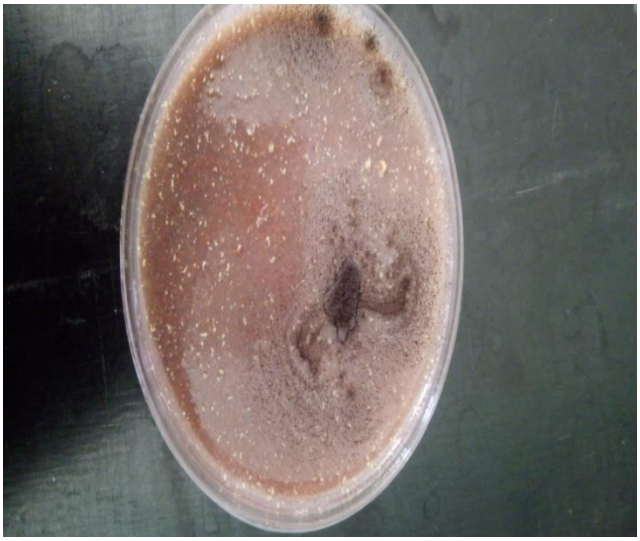
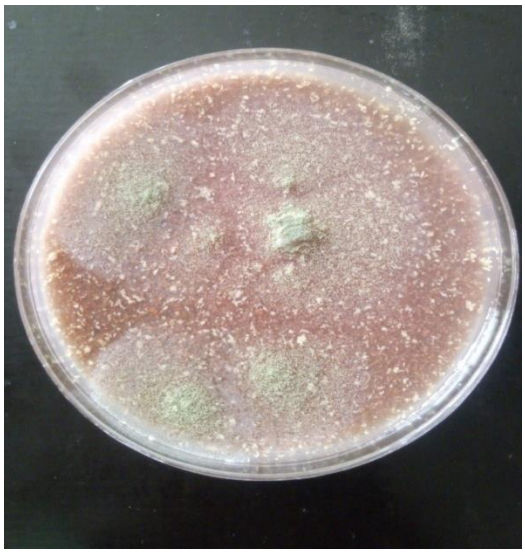


Plate 5 **Plate 6**

Plate 5: Growth of *Aspergillus niger* on *Chrysophyllum albidum* pulp with skin agar plate
Plate 6: Growth of *Aspergillus niger* on *Chrysophyllum albidum* pulp Only agar plate



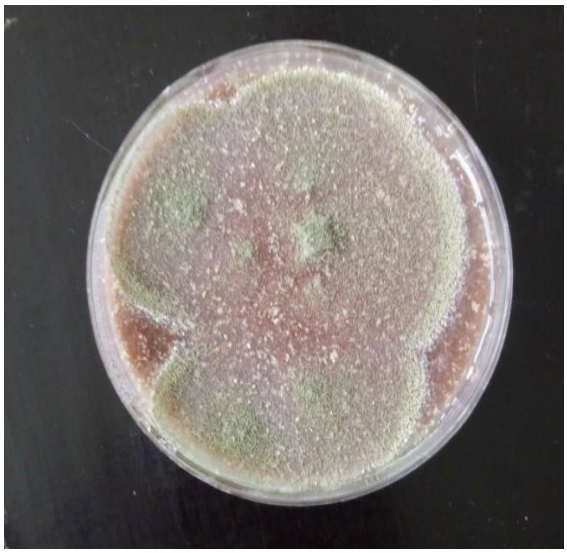
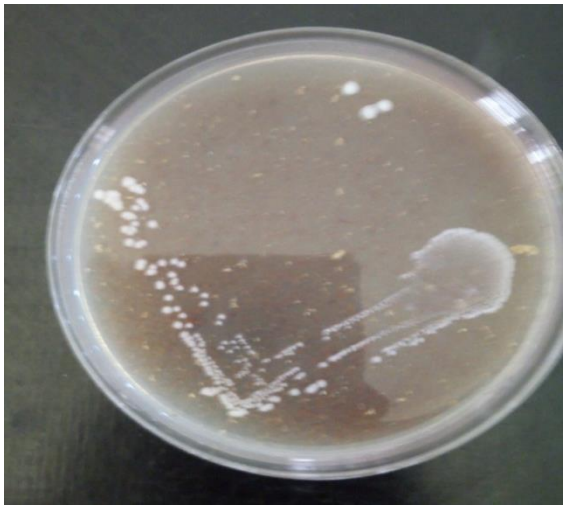


Plate 7 **Plate 8**

Plate 7: Growth of *Aspergillus niger* on *Chrysophyllum albidum* pulp with skin agar plate
Plate 8: Growth of *Aspergillus niger* on *Chrysophyllum albidum* pulp Only agar plate



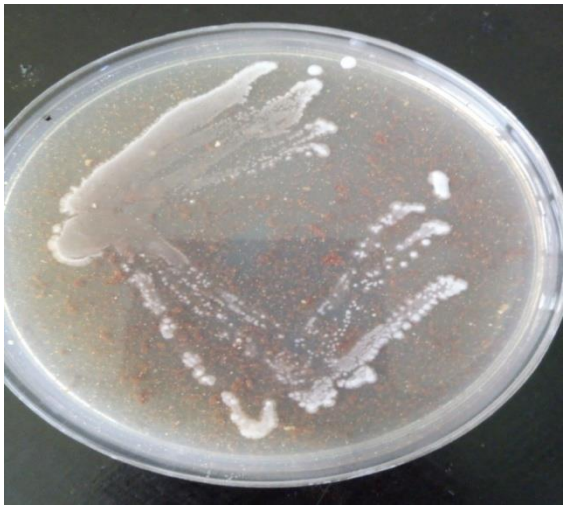


Plate 9 **Plate 10**

Plate 9: Growth of *Staphylococcus aureus* on *Chrysophyllum albidum* pulp with skin agar plate
Plate 10: Growth of *Staphylococcus aureus* on *Chrysophyllum albidum* pulp Only agar plate

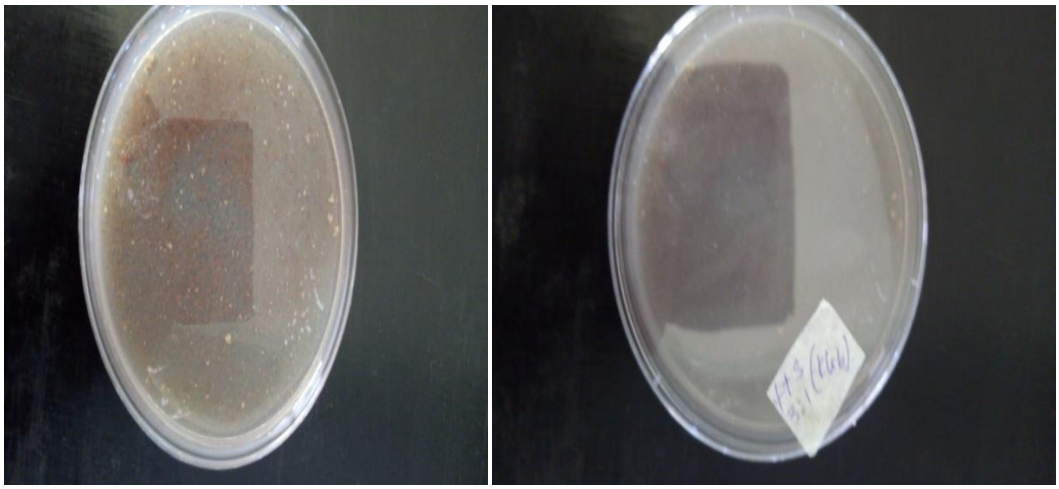


Plate 9: Growth of on *Klebsiella pneumoniae Chrysophyllum albidum* pulp with skin agar plate
Plate 10: Growth of *Klebsiella pneumoniae* on *Chrysophyllum albidum* pulp Only agar plate

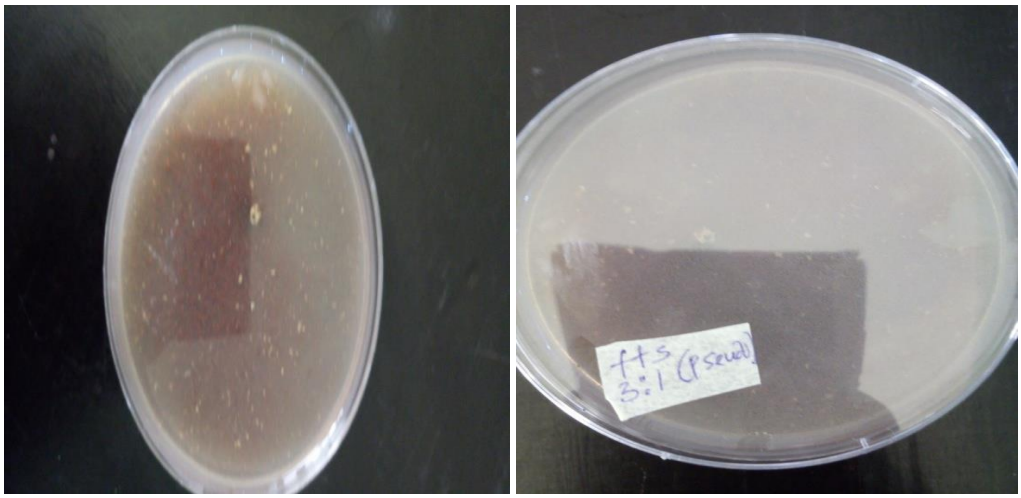


Plate 11: Growth of on *Pseudomonas aeruginosa Chrysophyllum albidum* pulp with skin agar plate
Plate 12: Growth of *Pseudomonas aeruginosa* on *Chrysophyllum albidum* pulp Only agar plate

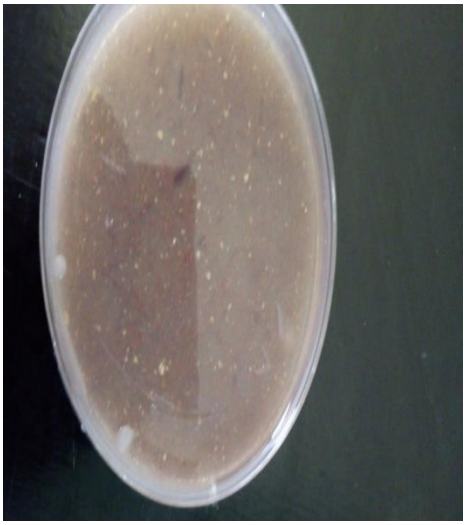
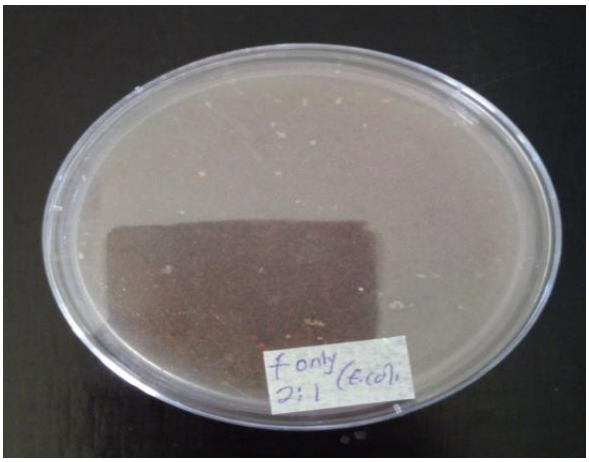



Plate 11: Growth of on *Proteus mirabilis Chrysophyllum albidum* pulp with skin agar plate
Plate 12: Growth of *Proteus mirabilis* on *Chrysophyllum albidum* pulp only agar plate



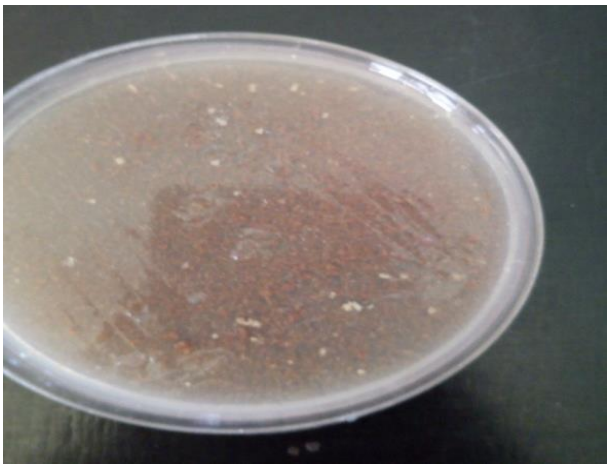


Plate 13: Growth of on *Proteus mirabilis* Escherichia coli pulp with skin agar plate indicating no growth
Plate 14: Growth of *Proteus mirabilis* on Escherichia coli pulp only agar plate indicating no growth

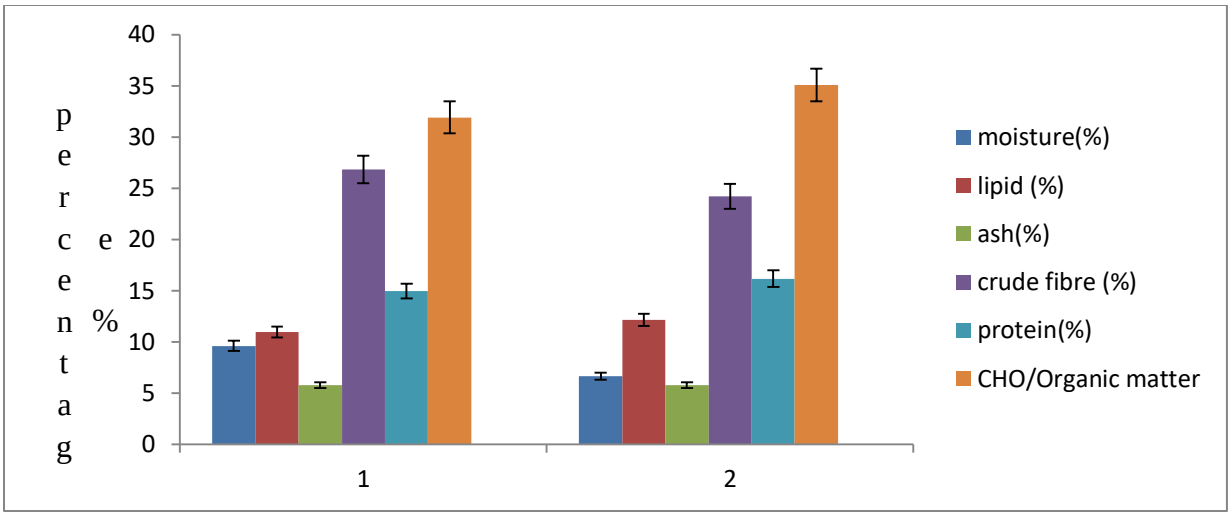


Figure 2: Proximate analyses of *chrysophyllum albidum* pulp with skin and *Chrysophyllum albidum* pulp only
Key 1 – *Chrysophyllum albidum* pulp with skin 2 – *Chrysophyllum albidum* pulp only

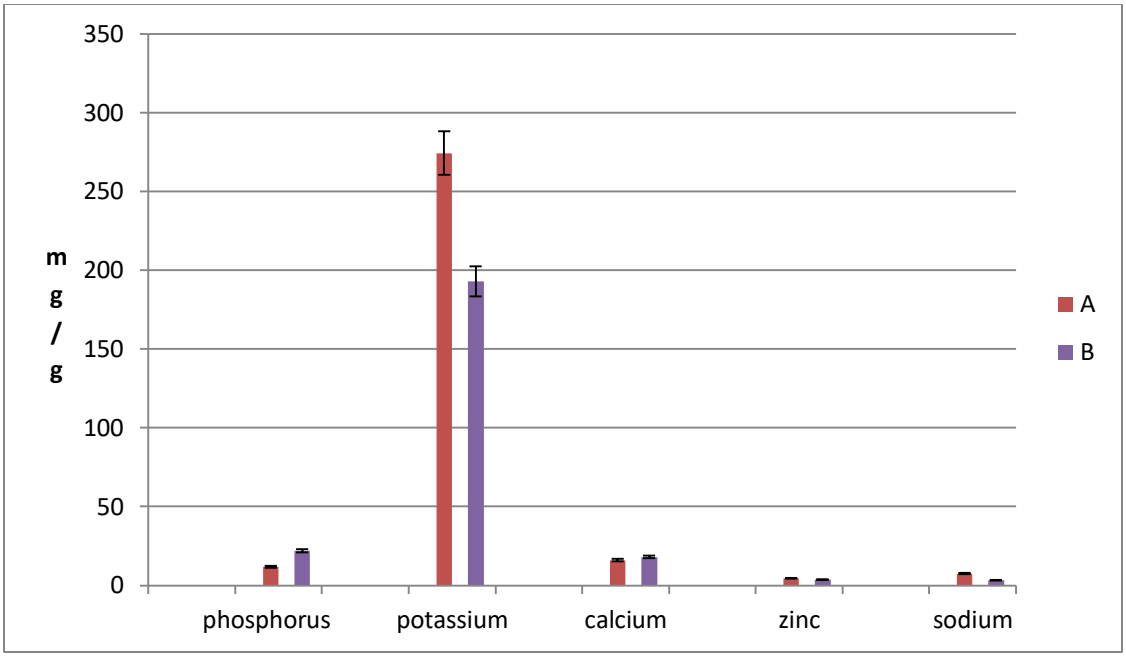


Figure 3: Mineral analyses of *Chrysphyllum albidum* pulp with skin and *Chrysphyllum albidum* pulp only
Keys: A – *Chrysophyllum albidum* pulp with skin B - *Chrysophyllum albidum* pulp only

There was a higher microbial count in *Chrysophyllum albidum* pulp with skin when compared with *Chrysophyllum albidum* pulp only. This might be as a result of higher nutritional content and mineral content when compared with *Chrysophyllum albidum* pulp only.

Among the five bacteria inocula *Staphylococcus aureus* has showed significantly high growth rate on *Chrysophyllum albidum* pulp with skin media and *chrysphyllum albidum* pulp only media (Tables 1 and

2) which might be due to minerals present in the *Chrysophyllum albidum* medium are favourable for the growth of *Staphylococcus aureus*. *Klebsiella pneumoniae* also showed significantly high growth in *Chrysophyllum albidum* pulp with skin media and showed less growth rate in *Chrysophyllum albidum* pulp only media which might be as a result of less nutrient present. *Proteus mirabilis* and *Pseudomonas aeruginosa* showed less growth rate in *Chrysophyllum albidum* pulp only media than *Chrysophyllum albidum* pulp with skin media which might have accounted to the *Chrysophyllum albidum* pulp with skin richer in nutrients (Figures 2 and 3). *Escherichia coli* showed no growth in the two formulate media this is due lack of enough minerals and carbohydrate that is enough to support the growth of *Escherichia coli*. In this study the test fungi such as *Trichoderma virid*ae., *Aspergillus flavus Aspergillus niger*, *Mucor mucedo* showed significantly higher growth in the *Chrysophyllum albidum* media formulation which shows that the media could supply them with the needed nutrients for growth. The result gotten in this studies correlate with the study conducted by[19], he screened for alternative culture media to replace PDA using Hommali brown rice flour (HMBRF) to investigate the growth of *Aspergillus niger* using the extract as culture media. Protein and carbohydrate rich raw materials like Soya, Potato, dates, Groundnut, Cereals, Cassava, Yam, Pigeon pea, Maize and Beans have been successfully used in formulation of cheap alternative bacteriological media[19,21].

CONCLUSION

Based on this study it is concluded that *Staphylococcus aureus* and all the fungi isolates used showed high growth rate with maximum growth in *Crysophyllum albidum* flesh with skin media and *Crysophyllum albidum* only.

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