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Preliminary Assessment of African Star Apple Seeds (*Chrysophyllum albidum*) as Potential Feedstock for Production of Bioethanol

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Abstract: Production of Bioethanol from renewable feedstocks has gained considerable scientific attention since they are able to supply energy and they serve as alternative fuels. This study presents a preliminary assessment of the production of Bioethanol from African star apple seeds. The mildly pre-treated seeds were fermented for bioethanol production by wild microbes. These organisms responsible for the fermentation were then isolated and characterized using microbiological techniques. Proximate analysis was carried out on the fermented milled seed during the fermentation period. High percentage carbohydrate, fat, and protein were obtained. Bioethanol produced was comparable to conventional ethanol physically and chemically. Specifically noticeable, flash point (12.5 °C), refractive index (1.364), and relative density (0.762 g/cm^3). The different wild microorganisms isolated during the fermentation period were *Bacillus brevis, Bacillus cereus, Aspergillus niger, Penicillium verruculosum, Lactobacillus fermentum, Lactobacillus rossiae, Serratia marcescens, Absidia Spinosa, Rhizopus stolonifer, Pediococcus damnosus, Klebsiella planticola Rhizopus stolonifer* and *Fusarium culmorum*. Although the mild pretreatment was ineffective for releasing fermentable sugar needed for bioethanol production, these findings demonstrate that *Chrysophyllum albidum* seeds which are usually wild can be an excellent renewable feedstock of fermentable sugars for the production of biofuels such as Bioethanol with suitable pretreatment techniques.

KEYWORDS: *Chrysophylum albidum* seeds, Bioethanol, fermentation and, proximate analysis

I. INTRODUCTION

In the 21st century, the demand for energy for transportation, heating, and industrial processing increases day by day. The heavy reliance on fossil fuels has led to its rapid depletion. Furthermore, environmental issues are a point of concern with the use of conventional fossil fuels. Consequently, research is presently focused on developing alternative energy sources such as biofuels (Bioethanol, biohydrogen, biogas, biodiesel). Biofuels are renewable energy sources that are renewable, cost-effective, and environment-friendly. Bioethanol is one of the bioenergy sources with high efficiency and low negative environmental impact [1]. In the last decades, there was a vast interest in producing and using liquid biofuels, most especially biodiesel or Bioethanol, as promising alternatives to fossil fuels. This feedstock would reduce fossil fuel consumption and consequently the negative impact on the environment [2]. The use of organic biomass for biofuels production lines with the 2030 Agenda for Sustainable Development of the U.N. They are affordable Clean Energy at the E.U. level and the production of biofuels from bio-waste is reflected in the Renewable Energy Directive 2009/28/E.C [3].

Moreover, other attractive features of Bioethanol include its renewable nature, higher combustible oxygen content, and higher-octane rating [4]. Ethanol is a high octane fuel and could replace lead as an octane enhancer in petrol. By blending ethanol with gasoline, we can also oxygenate the fuel mixture to burn more completely and reduce pollution emissions. To the above merits of Bioethanol is the ease of storage; bioethanol storage, transportation, and utilization are compatible with existing infrastructure for fossil fuel products [5]. Production of Bioethanol is from lignocellulosic materials in a process that entails pretreatment, enzymatic hydrolysis, fermentation, and ethanol recovery. The pretreatment stage of the production is necessary to modify the structural characteristics in the raw material facilitating the enzymes' access and maximizing sugar

monomers production. The enzymatic hydrolysis phase targets the structural carbohydrates starch, cellulose, and hemicellulose. At this phase, pentoses and hexoses that can be further used in the fermentation step are liberated. In the subsequent fermentation step, microorganisms metabolize those readily available sugars, producing ethanol, which is subsequently recovered through the distillation process [6]. Bioethanol is a principal fuel that can be used as a petrol substitute for the vehicle. Although ethanol can be produced by the chemical process of reacting ethylene with steam, Bioethanol can be produced mainly by the sugar fermentation process [7]. The main source of sugar required to produce Bioethanol comes from fuel or energy crops [8]. These crops include maize, cassava, wheat crops, and sugarcane. The use of this crop is a threat to food security. Hence, agricultural waste such as waste straw, guinea corn husk, millet husk, sawdust, and sorghum leaves are presently being used. Despite technical and economic complications, renewable lignocellulosic raw materials represent low-cost feedstock that does not compete with the food and feed chain, thereby motivating sustainability. Bioethanol has been produced in batch fermentation with fungi strain [9].

African Star Apple (*Chrysophyllum albidum*) is an edible tropical fruit that is classified as a wild fruit belonging to the family *Sapotaceae* [10] and it is a seasonal fruit. Therefore, it could be a suitable feedstock for the production of Bioethanol. The fruit has been shown to have tremendous economic value [11]. [11] reported that jams comparable to raspberry jams and jellies could be made from it. Its high pectin content [12] is also suggestive of its vast medicinal benefits. In Nigeria is generally known to dispose *Chrysophyllum albidum* seeds to the immediate environment after its fruit has been consumed serving as an environmental nuisance. Introducing as a potential biomass for bioethanol production is a form of ecological cleanup to such an environment.

Despite the merits of microbial bioethanol production processes from agriculture waste and wild fruits, several challenges plague its application. Significant bioethanol limitations production may include; (1) high cost and energy requirement, (2) lack of a suitable substrate, and (3) low product yield on the substrate [13]. Hence, current research on bioethanol production is gear towards the search for suitable substrates, low-cost pretreatment strategy, process optimization, strain selection, and strain engineering [14, [13]. The substrate's suitability depends on the richness of the carbohydrate content and the ease of susceptibility to low energy and harsh pretreatment regime to recover the fermentable sugar [13].

Therefore, the objectives of this research are; (i) to assess the suitability of *Chrysophyllum albidum* seeds as a substrate for bioethanol production, (ii) to isolate and identify microorganisms responsible for the fermentation of *Chrysophyllum albidum* seeds, and (iii) to characterize the Bioethanol from *Chrysophyllum albidum* seeds and compare with conventional ethanol.

2. MATERIALS AND METHODS

Preparation of African Star Apple *(Chrysophyllum albidum)* seed

Healthy fruits of *C. albidum* of the same variety were purchased from two local markets in Oyo and Ondo state, Nigeria. *C. albidum* seeds were obtained by carefully slicing open the fruit pulp to remove the seeds, and then sun drying the obtained seeds.

Pretreatment of African Star Apple seed

They were dehulled (seed coat removed), oven-dried at 90°C until there was no significant change in weight. After cooling, the seeds were milled to powder using a traditional grain mill REF 121 and sieved with 40 meshes, packed in an air-tight bag, and stored in the refrigerator until further usage.

Chrysophyllum albidum seeds→ Sundry → Cut seed open → washed with distilled water→Dry in an oven at 90^{o}C→ ground into powder in a mill→ Sieve →Stored in airtight cellophane bag →store in a refrigerator.

Figure 1: Flow chart for preparation of *Chrysophyllum albidum* seed powder.

Preparation of sample

Ten grams of the substrate (*Chrysophyllum albidum* seed powder) was introduced into a fermentor, and distilled water was added. The pH was monitored and sampling at regular intervals for the isolation of fermenting bacteria and fungi.

Bioethanol production

100g of the powdered seed was added to 250ml of distilled water in a 500ml fermenter, supplemented with an inoculum of *Saccharomyces cerevisiae* culture. This was followed by fermentation for 72hrs at 30^{o}C. Samples were taken at regular intervals for analysis.

3. ANALYTICAL METHODS

Determination of the viable microbial count of samples

Serial dilution of *C. albidum* powder was made before and after fermentation, pour plating technique was employed. Nutrient agar (N.A.) which is a general-purpose media, was used for the isolation and enumeration of bacteria, Man Rogosa and Sharpe (MRS) media was used for isolation and cultivation of *Lactobacilli* and Potato dextrose agar (PDA) was used for isolation and cultivation of fungi. Pure culture of each of the isolated bacteria and fungi was obtained and identified according to the methods of [15].

Proximate analysis

Compositional (moisture content, ash content, Lipid content, crude fiber, crude protein, and carbohydrate) proximal analysis was analyzed using previous standard conventional protocols [16].

Bioethanol parameter

Distilled bioethanol parameters were analyzed using standard convention techniques [17].

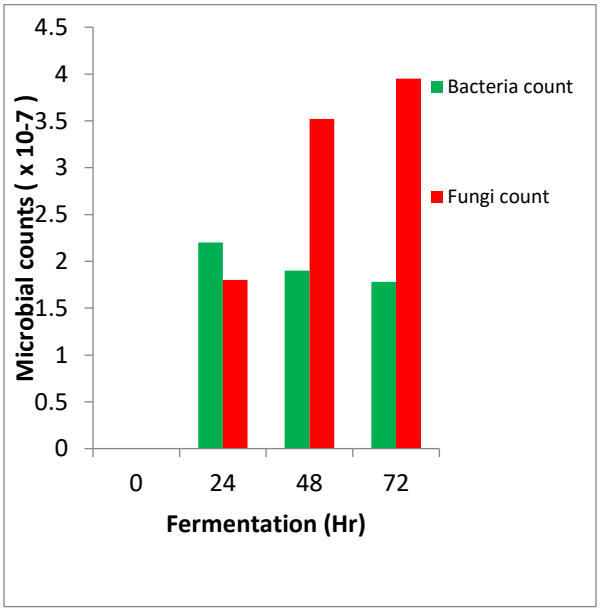

Figure 1: Bacteria and fungi count isolated from seeds during fermentation

Table 1: Microorganisms isolated at different hours during fermentation of African star apple seed

Fermentation (hour)	Bacteria	Fungi
24	*Bacillus brevis*, *Bacillus cereus*	*Aspergillus niger* and *Penicillium verruculosum*
48	*Lactobacillus fermentum*, *Lactobacillus rossiae* and *Serratia marcescens*	*Absidia* Spinosa and *Rhizopus stolonifer*
72	*Pediococcus damnosus* and *Klebsiella planticola*	*Fusarium culmorum*

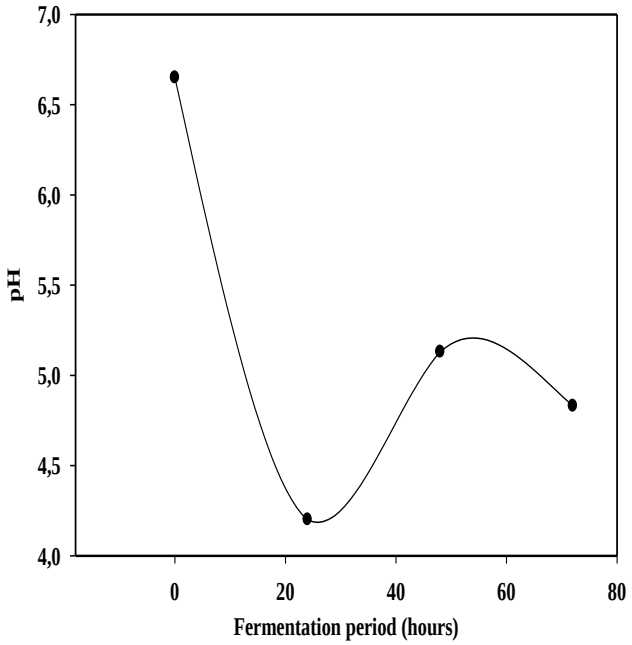

Figure 2: pH of the seeds during fermentation

Table 2: Proximate composition of the fermented and unfermented sample

Parameters	**0hrs**		**24hrs**		**48hrs**		**72hrs**	
(%) Moisture	F 6.35	U 5.24	F 5.23	U 5.05	F 5.05	U 5.05	F	
Ash	2.85	2.84	1.50	1.50	1.45	1.46	F 5.05	U 5.40
Fat	10.50	10.68	12.40	12.40	12.47	12.46	0.70	0.70
Protein	10.97	10.98	11.97	12.07	12.82	12.83	13.30	13.32
Crude Fibre	2.10	2.11	3.08	3.07	4.75	4.76	12.80	12.82
Carbohydrate	67.23	67.14	65.79	65.73	63.46	63.46	4.14	4.15

KEY:
F = Fermented
U = Unfermented

Table 3: Comparative properties of African star apple seed bioethanol and convectional ethanol Parameters

Parameters	**Bioethanol**	**Ethanol**
Appearance	Colourless	Colorless
Relative density	0.762g/cm^3	0.789g/cm^3
Melting point	-111°C at 15°C	-114^{0}C at 15°C
Boiling point	77.8°C	78°C
Burning characteristics	Burning with blue flame	Burning with blue flame
Refractive index	1.364	1.362
Flashpoint	12.5°C	13-14°C

4. RESULTS AND DISCUSSION

Table 2, shows the nutrient composition Chrysophyllum *albidum* seed. The presence of high carbohydrate content reveals *Chrysophyllum albidum* seed could be a suitable substrate for bioethanol production. To increase the recovery of the fermentable sugar locked up in the carbohydrate structure, a more convenient and efficient pretreatment regime will be required [18]. The microbial population of the fermented African star apple seed shows fluctuation in the microbial count. There was a reduction in the bacterial count and a significant increase in the fungal count (Fig. 1). Fungi such as *Saccharomyces cerevisiae* have been reported to ferment sugars for the production of ethanol. This could account for the significant increase in the fungal count. The pH pattern shows the uncontrolled condition under which the process took place (Fig. 2). Enzyme activities during microbial fermentation have been known to be pH-dependent; a buffering effect will keep them in a physiological state for optimum activities [19]. Hence, a buffered or a control pH system would be required to increase the process productivity [19]. An initial decrease in pH is probable due to acidic metabolic released by microorganisms present. The presence of different microbial species such as *Aspergillus niger*, *Bacillus cereus*, *Lactobacillus Plantarum*, and *Lactobacillus rossiae* (Table 1) reveal possible microbial activities during the fermentation period. This consortium of microorganisms could lower the efficiency of ethanol production. Most fermentation process monoculture system, which has been considered efficient. The comparative properties of Bioethanol obtained in the present study and convectional ethanol shows close similarity in the parameters tested (Table 3). This agrees with previous reports on bioethanol production from organic biomasses. And the potential to replace ethanol production with a biological process of production [5].

5. CONCLUSION

This study report on the potential of *Chrysophyllum albidum* seed for bioethanol production. The properties of the obtained Bioethanol are comparable to the convention ethanol. The constant search for alternative biofuel sources could be focused on Chrysophyllum *albidum* seed and other biomass, contributing to a safer natural environment. Further pretreatment process is required to promote bioethanol production from *Chrysophyllum albidum* seed.

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