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Potential of Cashew Gum Exudates as Substrate for Bioethanol Production using *Aspergillus Niger*

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ABSTRACT: Crude cashew gum, a polysaccharide exudates was purified by way of dissolving the crude gum in hot water, centrifuging the solution to remove suspended particles, precipitating the resulting solution with concentrated ethanol, drying and finally grinding the precipitate to fine powder. Physicochemical analysis of the crude and purified gum showed that both solutions were acidic, with the pH ranging from 5.10 to 5.04 and 5.49 to 5.41 in a concentration range of 5 g/10ml to 20 g/ 10 ml respectively in the first week. Both sampled gum solutions showed initial drop in pH in the first day the solutions were made, but the pH stabilized for the remaining days. The pH of the gum solutions was stable throughout the second week, but there was slight drop as the concentration was increased. The purified gum was first adapted to Aspergillus niger and growth was observed only after 72 hours. The zone of clearance formed after Lugol's iodine was applied is indicative of the fact that fermentation of the gum sample to ethanol took place. This result shows that fermentation of cashew gum to ethanol is possible and so the gum could be exploited to produce ethanol for various industrial and domestic uses thus reducing the dependence on grains currently used for the production of ethanol and thus availing humans and animals more grains for food and feed respectively. The practice of the use of cashew gum for ethanol production will increase the economic value of cashew gum globally

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Cashew gums exudates is obtained from the bark of cashew tree (*Anacardium occidentale*) and it is a complex polysaccharide of high molecular mass. The gum is popularly and traditionally used in book binding locally Zhang *et al.,* (2013). Cashew gum is a highly branched polysaccharide of high molecular mass which contains monomeric unit of simple sugars held together in the polymer. The polymer can be broken down into simple sugars through chemical hydrolysis or enzymatic hydrolysis Hughes *et al.,* (2009). Comparative studies among specimens of gums obtained from different geographical areas indicate that there are significant variations in properties associated with climatic conditions, such as specific rotation and composition. Bioethanol can be produced from cellulose and hemicelluloses that originate from various biopolymer sources of biomass.

Bioethanol productions have been sorely dependent on grains and grains related products and place heavy burden on the plant. The burden on grains could lead to food scarcity and its resultant impact on the economy of Nigeria. Cashew gum which is an exudates polymer from the bark of cashew tree has complex polysaccharide that appears usually milky in color but on contact with air changes to light or dark brown Zhang *et al.,* (2013).

Gum production is stimulated by stripping the bark of the gum producing tree (cashew tree). The gum is collected as an air dried droplet. Production of these gums varies from time to time as a result of weather condition, labor strikes and natural disaster. By natural exudation and means of incisions, a gum or resin of a golden brown color soluble in water, and which presents a great potential for industrialization, appear on the trunk and branches of the cashew tree Dickson, (1988). When applied as a varnish, provides remarkable protection, as is unchanged by acids, alkalis, alcohols or heat up to 70 $^{\circ}$C Lima *et al.,* (2002). On hydrolysis, a sample of cashew gum from Brazil gave 70 % galactose, 5 % arabinose, 11 % glucose, 4 % rhamnose, 1 % mannose and 6 % glucoronic acid. But the high content of galactose may decrease the overall ethanol production from a given quantity of the gum; hence the need for

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adaptation of the yeast cells to galactose for efficient conversion of it to ethanol.

Bioethanol can be produced from cellulose and hemicelluloses that originate from various biopolymer sources of biomass. Bioethanol productions have been sorely dependent on grains and grains related products .and place heavy burden on the plant. The burden on grains could lead to food scarcity and its resultant impact on the economy of Nigeria. Cashew gum which is an exudates polymer from the bark of cashew tree has complex polysaccharide that appears usually milky in color but on contact with air changes to light or dark brown Zhang *et al.,* (2013). The phenomenon of yeast adaptation can be defined as a population of cells placed in contact with some substrate in order to acquire, after a lapse of time, the enzymes necessary to metabolize the added substrate (James, 2004). *Aspergillus niger* is one of the most common mould that is adapted for polysaccharide fermentation to ethanol and pyruvic acid as by a product Voca, *et al.,* (2009).

There is much activity in the part of cellulosic ethanol, whereby the cellulose is broken down to sugars and subsequently converted to ethanol as shown in the equations below:

$$C_{12}H_{22}O_{11} + H_2O + \text{Invertase} \longrightarrow 2C_6H_{12}O_6 \quad \text{Eqn. 1}$$

$$2C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2 \quad \text{Eqn. 2}$$

The enzyme will mainly metabolize glucose and galactose to form pyruvic acid through the stages of the reaction pathway (Embden-Meyerhof-Parnas), nevertheless pyruvic acid generated would be decarboxylated to acetaldehyde which then experiences dehydrogenation to ethanol. After fermentation is completed, distillation process can separate ethanol from its mixture with water using the difference in their boiling point. At standard conditions, the boiling point of pure ethanol is 78 ℃ while that of water is 100 ℃. Heating the solution at a temperature range of 78 – 100 ℃ will result in evaporation of most of the ethanol, which will be condensed in the condensation unit as 95 % ethanol by volume Voca *et al.,* (2009). The present research has as objective the sourcing of raw material base for ethanol production from sources that are renewable, cheap, environmentally friendly and non-conventional.

MATERIALS AND METHODS

Crude and purified cashew gums were used for the study. The crude gum was obtained from a cashew plantation at Faculty of Agriculture, Kogi State University, Anyigba, as natural exudates from the stem barks of the tree *Anacardium Occidentale*, family *Anacardiaceae*. The gum was tapped by cutting the bark tissue of the cashew tree and the crude gum was collected and sorted to remove pieces of tree bark. Aspergillus niger culture collection was obtained from the microbiology laboratory of Kogi State University Anyigba, Kogi State.

Reagents: Barium carbonate, Absolute ethanol, Alcohol, distilled water, Fehling solution, Barium hydroxide, Ferric chloride ($FeCl_3$), Concentrated and dilute Sulphuric acid (H_2SO_4), Bial's reagent, Magnesium ribbon, Chloroform, HCl, diethyl-ether, iodine, methylated spirit, Cashew gum (galactose/glucose based moiety), PDA (potato dextrose agar), S.D.A (Sauborraw Dextrose agar).

Apparatus and Equipment: pH meter (PHS-25), Electric weighing machine (PB 303-N), Water bath, Magnetic hotplate stirrer (Corning PC-420 D), Uniscope laboratory centrifuge (Model SM 112), Mortar and pestle, Electric laboratory oven (Gallenkamp 300 Plus Series, Apeldoorn Zuid, Netherlands), Buckner funnel, Auto-clave, Incubator, wire loops, inoculating needles, cotton wool, liquid soap, Whatman filter paper, funnel, Vacuum pump, Retort stand, Spatula, Retsch Laboratory sieve, Binatone Electric blender, Beakers and Conical flasks, Aluminum foil, Petri dishes (glass and plastic), Measuring cylinders, Test-tubes, string rod, matches and rutter among others were the equipment and apparatus used for this project.

Collection and processing of cashew gum exudates: The gum exudates were collected and sorted to remove pieces of tree bark and other foreign matter and dried in a laboratory electric oven at 30 ˚C for one week. The dried and clean samples were pounded using mortar and pestle, milled to finer particles with an electric blender and sieved using sieve number 38 after which Some of this was used as crude cashew gum for subsequent tests and analyses.

Purification of Crude Cashew Gum: The grated sample was purified using the procedure of Ofori-Kwakye, *et al.,* (2010) in which 20 g (W1) of the sample was weighed into 100 ml of distilled water and stirred. The solution was centrifuged at 800 r/ min for 20 min. using Uniscope laboratory centrifuge (Model SM 112) followed by decantation. The residue was further filtered using suction through a fine muslin cloth to remove any extraneous matter and the entire filtrates were precipitated with 100 ml absolute ethanol and washed with diethyl ether. The

precipitate was dried in an electric oven at 50 °C for 3 hrs. The dried purified gum was then ground with a mortar and pestle and screened through sieve number 38 and weighed (W2). The gum was then stored in an air-tight container and used for subsequent tests. The same procedure was repeated using cold distilled water and the percentage yield was calculated using the relationship:

$$\% \text{ Yield} = \frac{\text{Weight of purified gum (W2)}}{\text{Weight of crude gum (W1)}} \times 10 \ldots\ldots (1)$$

pH of cashew gum sample: Crude and Purified cashew gum samples were prepared with distilled water at concentrations of 5, 10, 15 and 20 % w/v. The pH of the samples was determined at daily intervals for three days at 25 °C, using a standardized pH meter (Eutech Instruments, The Netherlands).

Enzymatic hydrolysis: Saccharification of the raw material was conducted according to Guo *et al.,* 2008; Brodeur *et al.,* 2011; Chaturvedi and Verma (2013) protocol with some modifications. *Aspergillus niger* culture collection was obtained from the Microbiology Laboratory of Kogi State University Anyigba, Kogi State. Then the prepared enzyme was added to the diluted substrates (purified cashew gum exudates) and incubated for 72 hrs. For process optimization, sterile distilled water was used as solvent.

Isolation and selection of mould strains: Mould samples were isolated in sterilized condition at approximately 36 °C using the technique called enrichment, according to Consuelo *et al.,* (2010) at pH 3.9 and at adjusted pH of 4.7. The pH adjustment was made with Ammonium sulphate (0.05 %) and (volume by volume) ethanol (4 %). Cultures of pure mould were isolated and kept on agar slants (One percent yeast extract, two percent peptone, two percent galactose/glucose and two percent agar) and stored at 10 °C.

Utilization of galactose and glucose by Aspergillus niger: Potato Dextrose Agar (PDA) of 150 ml was prepared by weighing 6 g of PDA agar and dissolving it in 100 ml of sterile water. The above specification was that of the manufacturer, of Oxoid India and as reported by Chesborough, (2008). The resulting mixture was stirred to avoid lump and it was eventually digested in a heating mantle at 150 °C for 15 min with constant stirring using a sterile stirring rod. The resulting solution of digested PDA in a conical flask was covered with a cotton wool wrapped with aluminum foil to prevent evaporation of media constituent during sterilization and the covered media was sterilized in an autoclave at 129°C and a pressure of 15 MPa for 15 min. The media was allowed to cool to 40-45 °C before the incorporation of the galactose/glucose based compound. The galactose/glucose based compound was prepared by weighing 5 g of purified cashew gum exudates and dissolving it in 50 ml of sterilized water in a ratio of 1:10. The galactose/glucose compound (50 ml) was added to the sterilized media i.e. 100 ml prepared PDA agar and cooled to 40-45°C, to give 150ml of modified PDA of incorporated galactose/glucose based compound. The media was plated out in to 10 Petri plates by introducing approximately 15 ml of the media into each Petri plates under aseptic condition. The ten plates were allowed to set (gel) under a burning flame to reduce every possibility of contamination. After the media have successfully gelled, a tightly twisted wire loop was sterilized by passing it through a burning flame till red hot before inoculation. Inoculation of the prepared media was done by carefully scooping a loop full of *Aspergillus niger* into the solidified modified PDA media using a spread plate platting method as described by Chesbrough, (2008). The spread plate technique was repeated for the rest of the 9 Petri plates and incubated at room temperature for 72 hours. After 72 hours, one of the resulting cultures was flooded with Lugol's iodine to see if zone of clearance formed as a way to determine if fermentation took place.

RESULTS AND DISCUSSION

The crude and purified cashew gum exudates were acidic as shown in (Tables 1, 2,); confirming the findings of previous studies of the gum by Ofori-Kwakwe *et al.*, (2010). Variation of pH with duration was observed in the first two days and as the concentration increases across the table. The acidity of the gums could be attributed to the acidic functional group (uronic acid) of sugar units in the gum. High concentrations of the gum mucilage were slightly more acidic than low concentrated ones (Table 1). The trend in the pH drop across the table is more pronounced in Table 1 than in Table 2, representing crude gum and purified gum respectively.

Table 1: pH of crude gum solution in the first week

Concentration (g/10mL)	5	10	15	20
Days				
1	5.10	5.08	5.06	5.04
2	5.06	5.04	5.02	5.00
3	5.05	5.03	5.01	4.90
4	5.05	5.03	5.01	4.90
5	5.05	5.03	5.01	4.90

No marked change in pH of the samples was observed after storage at room temperature over two weeks period. The slight reduction in pH observed in the first week of storage of the samples could be attributed to fermentation of the sugar units and the gradual hydrolysis of uronic acid units making the solutions more acidic (Table 3 and 4).

Table 2: pH of purified gum solution in the first week

Concentration (g/10mL)	5	10	15	20
Days				
1	5.49	5.46	5.43	5.41
2	5.43	5.40	5.37	5.35
3	5.40	5.37	5.34	5.32
4	5.40	5.37	5.34	5.32
5	5.40	5.37	5.34	5.32

Table 3: pH of crude gum solution in the second Week

Concentration (g/10mL)	5	10	15	20
Days				
1	5.05	5.03	5.01	4.90
2	5.05	5.03	5.01	4.90
3	5.05	5.03	5.01	4.90
4	5.05	5.03	5.01	4.90
5	5.05	5.03	5.01	4.90

Table 4: pH of purified gum solution in the second Week

Concentration (g/10mills)	5	10	15	20
Days				
1	5.40	5.37	5.34	5.32
2	5.40	5.37	5.34	5.32
3	5.40	5.37	5.34	5.32
4	5.40	5.37	5.34	5.32
5	5.40	5.37	5.34	5.32

An average yield of 85.25 % was obtained when batches of Cashew gum exudates were purified using absolute ethanol as solvent for purification. This result was much higher than the 55 % yield for purification of cashew gum using acetone as reported by Kumar *et al.,* (2009) but slightly above 78.5 % yield reported for extraction using ethanol by Ofori-Kwakwe *et al.*, 2010).

The variations in extraction yield results of gums in general may be due to quality of the crude gum, purity of the extraction solvent and thoroughness of the extraction procedure.

Plate 1 illustrates the plating of media for fermentation in which the enzyme (*Aspergillus niger*) was able to utilize galactose and glucose growing entirely over the surface of the medium within a period of 72 hrs. Plate 2 illustrates the media when flooded with Lugol's iodine to show zones of clearance.

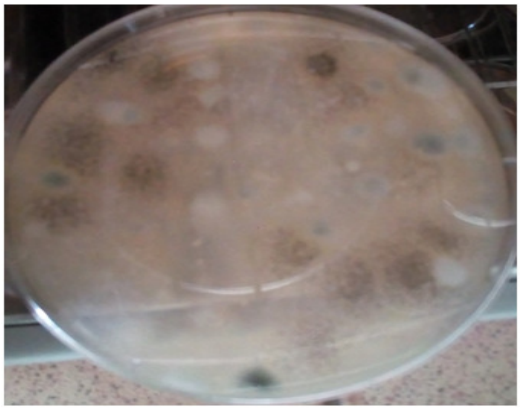

Plate 1: Media showing *Aspergillus niger growing* on the plate

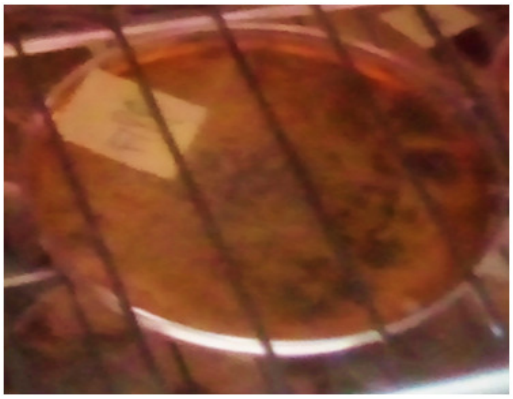

Plate 2: Clearer stains on media flooded with Lugol's Iodine

Conclusion: Cashew gum have previously been shown to have potential applications in pharmacy as tablet binders, suspending agents, filling agents and also in the food industry, cosmetics, in paper and textile industries. Our finding has shown that the gum can also serve as raw material in the production of bioethanol. We therefore recommend that the gum be used in the production of ethanol as an alternative/supplementary raw material to grains which are used for food and feed for human and animals respectively. Interestingly, cashew tree grow over wide areas in Nigeria.

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