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Evaluation of Bioethanol Potential of *Solenostemon rotundifolius* (Spreng) Pulp and Peel

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***Abstract*—** *This research is design to determine the proximate composition and feasibility of Solenostemon rotundifolius pulp and peel as a raw material for bioethanol production as a biofuel. The results obtained for proximate analysis showed the following: moisture (78.17 ± 0.63% 75.50 ± 0.82%) Ash (1.33 ± 0.24% and 5.17 ± 0.24%), Crude protein (2.22 ± 1.12% and 2.77 ± 0.08%), Crude lipids (0.50 ± 0.00% and 2.67 ± 0.24%), Crude fibre (0.50 ± 0.00% and 1.83 ± 0.24%), Carbohydrate (95.45 ± 0.33 and 87.56 ± 0.56) and Organic matter (20.50 ± 19 33) for pulp and peel respectively. The results demonstrated that both pulp and peel contains sufficient amount of starch and carbohydrate to guarantee use of plant for bioethanol production. The qualitative test on the distillate with various chemicals revealed the characteristics reaction of alcohol, while the HPLC analysis of the produced bioethanol has shown similar peaks compared with standard ethanol. On the FTIR, the spectrum has shown a sharp peaks at 3300cm^{-1} for alcohol, 2900-1450cm^{-1} for alkane and 1200cm^{-1} for carbonyl which is an identification of ethyl alcohol. The observed results indicate that the Solenostemon rotundifolius pulp and peel could be a potential feedstock for bioethanol production.*

***Keywords*—** *Bioethanol, Enzymatic hydrolysis, fermentation and S. Rotundifolius.*

I. INTRODUCTION

The rising demand for energy across the world has necessitated the search for alternative source for fuels from renewable resources and waste materials. Globally, about 80 million barrels of petroleum are consumed on a daily basis and among 195 countries in the world only 40 countries generate petroleum (Gautam, 2013 and Delilah, 2011). As a result of an increasing world population and rapid industrialization, energy demand is constantly increasing; therefore consumption of fossil fuels such as oil, coal and natural gas has increased and caused significant environmental pollution due to the release of environmental pollution gasses such as CO, CO_2, NO_2, SO_2 and CH_4 that cause greenhouse effect and global warming. Consequently, the search for alternatives energy sources has gained great importance because the uses of fossil fuels is harmful to the environment and the supply is limited (McKendry, 2002)

Nigeria joined the league of biofuel users with the aim of generating wealth (Aisien *et al.*, 2010). The plan had generally been production from sugarcane and cassava characterized cassava alongside with sweet potato and yam as main starches that serve as staple foods for people through the world's hot and humid regions. These plants are so accomplished at supplying essential calories that they are considered the vital subsistent crops. On the other hand, the success of these starch crops as staple foods confines their potential development and general economic growth, for example, cassava which has become an important biofuel crop (Amadi *et al.*, 2015).

Solenostemon rotundifolius is an erect, semi-succulent annual herb. It is bushy from the base up to 30cm tall, has a succulent stem and thick leaves. The flowers are blue pinkish white or pale violet in a distal inflorescence. Plant is highly tolerant of more drought and rainfall (Priya and Anbuselvi, 2013). It grows well in loose or sandy soil and direct sunlight. The tubers are harvested about four to five months after planting, flowering and aerial parts of plant have died. Tubers of *S. rotundifolius* can be used as edible potatoes in Tamil Nadu. This tuber is oval shaped and smaller than commercial potatoes. They are usually cooked by baking and frying. The taste of potato is fairly bland than sweet potato (Manikandan *et al.*, 2016)

Some local names include: Hausa potato (Ghana), Innala (Srialanka); Kembili (Mali.); Ketang (Indonesia.); Koorka (India.); Madagascar potato (France.); Ratala (Srialanka.); Saluga (Nigeria.); Sudan potato, Tumuku (Niger.); Vatke (Ethophia.). Frafra potato (FP) is a small, herbaceous having an aromatic smell resembling that of mint. Flowers are small, pale violet in colour, produced on an elongated terminal raceme. The tubers are small dark-brown and produced in clusters at the base of the stem (Sugri *et al.*, 2013).

Hausa potato (*Solenostemon rotundifolius*) is amongst the lesser known tropical root crops in Nigeria, and some other Sub-Saharan Africa (SSA) countries. Though many communities in northern Nigeria consume these starchy tubers (mostly as boiled or fried pieces), there is dearth of information (in scientific literature) on their processing and culinary characteristics (Ukpabi *et al.*, 2011)

Fig. 1. *Solenostemon rotundifolius* plant

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Fig. 2. *Solenostemon rotundifolius* tubers

Moreover, *S. rotundifolius* tuber peel and pulp are potential sources for local bioethanol system and can be processed for second generation biothanol by implementing a sustainable bioenergy convertion technology (Antizar-Ladislao and Turrion-Gomenez, 2008) *S. rotundifolius* has the potential to compete with other available biomass feed stocks for biorefineries based on yam, cassava, sweet potatoes and cocoyam for the integrated production of bioethanol ((Akpabio *et al.*, 2012, Ojewumi *et al.*, 2018, Lewu *et al.*, 2010)

The aim of the present work is to determine the bioethanol potential of *Solenostemon rotundifolius* by means of enzymatic hydrolysis. The results obtained in the present investigation may have an important contribution in value addition of the inedible and low cost bio resource. The scientific knowledge obtained from the present study can be useful in bioethanol preparation from *S. rotundifolius* tuber.

II. Materials and Method

A. Sampling

Matured tubers of *S. rotundifolius* were ontained from various locations in Zuru Local Government area of Kebbi State in Nigeria. The fresh tuber samples were authenticated at the Herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria voucher No.UDUSH/ANS/0235.

B. Sample Treatment

The sample tubers were washed with distilled water to avoid surface contamination (Ahmed and Birnin Yauri, 2008). The pulp and the peel were separated manually using mechanical filler. The sample was dried at room temperature for three (3) weeks, crushed into fine powder mechanically using a blender, sieved through 20mm–mesh and stored in room condition in an air tight plastic container for analysis.

C. Determination of Starch Content

Proximate factors were determined using Association of Official Analytical Chemists, [AOAC] (2005) method from which available carbohydrate and crude protein was calculated using relationship reported by James (1995).

$$\% \, Starch = 0.9 \; x \; available \; CHO$$

D. Enzymatic Hydrolysis of S. Rotundifolius

Thirty grams (30g) of pre-treated sample was put into 500 cm^3 conical flask, 300 cm^3 of distilled water was added to each conical flask and then allowed to absorbed water in order to obtain homogeneous mixture. The conical flasks were covered with cotton wool, wrapped with aluminium foil and sterilized at 121^oC for 15 minutes using autoclave. The sterilized conical flask was allowed to cool and inoculated with *Aspergillusniger* and incubated for 5 days for hydrolysis to take place (Huprey and Cavitus, 2007).

E. Fermentation of S. Rotundifolius

In fermentation process, *Saccharomyces cerevisiea* (baker yeast) was used to ferment the simple sugar to ethanol and carbon dioxides. To determine the effects of temperature, inoculums concentration and pH on ethanol yields. The fermentation process continued for 48 hours.

F. Distillation of Ethanol

This was carried out according to the method described by Oyeleke and Jibrin (2009). The fermented broths were dispensed into round bottom flask fixed to distillation column enclosed on running tap water. Conical flasks were fixed to the other end of the distillation column to collect the distillate. A heating mantle with temperature adjusted to 78^oC (boiling point of bioethanol) was used to heat the round bottom flask containing the fermented sample until all the sample has distilled. This was repeated for the sample of each run. The distillate was a mixture of water and ethanol also known as "azeotrope" which was further purified by dehydrating with calcium hydroxide that help break or alter ethanol- water azeotrope. The calcium hydroxide formed was separated by filtration and the filtrate redistilled to obtained pure ethanol. The bioethanol produced from distillation were assessed for it biofuel properties such as, density, viscosity, flash point, pour point, cloud point, specific gravity, octate number, sulphate, ash and corrosive properties.

G. Qualitative Test on the Distillate

The samples distillates were distinctly tested with litmus papers, acidified $KMnO_4$ potassium heptaoxochromate (VI) and piece of sodium metal to make certain of their reactivity. A confirmatory test was performed on the distillate using Iodine solution and NaOH solution as described by Sokoto *et al.*, (2016).

H. Fourier Transform Infrared Spectroscopy

FTIR was used to check the chemical content of the samples as described by Ifran *et al*, (2011). Mixture of sample and KBr (5% sample and 95%KBr) were passed into a disk for FTIR measurement. The spectrum was recorded with 32 scans in the frequency range of 4000-400cm^{-1} with a resolution of 4cm^{-1}

I. High-performance Liquid Chromatography Spectroscopy

Bioethanol produced was analysed by high performance liquid chromatography (HPLC). 20 µL of the sample was injected into HPLC system to determine the bioethanol yield.

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The HPLC analysis parameters were determine using the following condition: column C18 RP (53 x7mm); injector temperature was 30°C, 20 µL of the sample was injected into the HPLC system. The mobile phase was phosphoric acid and the flow rate was 1.5mL/min; and detection was set at a wavelength of 210 nm.

J. Statistical Analysis

Data generated in triplicates were expressed as mean ± standard deviation.

III. Results and Discussion

The results of proximate of proximate composition of the peel and pulp were presented in Table 1 which indicate that the pulp contains higher amount of moisture content, available carbohydrate, calorific energy value, potassium, organic matter and C/N ratio while peel was found to contain higher ash content, crude protein, crude lipids and phosphorus.

TABLE 1: Proximate compositions of *S. rotundifolius* tuber (g/100g) dry weight.

Parameter	Pulp	Peel
Moisture (% wet weight)	78.17 ± 0.63	75.50 ± 0.82
Ash	1.33 ± 0.24	5.17 ± 0.24
Crude Protein	2.22 ± 0.12	2.77 ± 0.08
Crude Lipid	0.50 ± 0.00	2.67 ± 0.24
Crude Fibre	0.50 ± 0.00	1.83 ± 0.24
Available Carbohydrate	95.45 ± 0.33	87.56 ± 0.56
Calorific Value (kcal/100g)	395.18±2.03	385.35±1.01
Organic Matter	20.50	19.33

Carbohydrate obtain by difference

The data are mean ± standard deviation of the three replicates determinations

Proximate Compositions of Pulp and Peel

The proximate analysis is the quickest and simplest way of investigating the fuel quality of solid materials. From the results, moisture content of the sample was 78.17± 0.63% and 75.50±0.82% for pulp and peel respectively. This was higher than 61.29% for sweet potato as reported by Ojewumi *et al.*, (2018) and 66.62±1.02% for cocoyam but lower than 81.53±1.39% for potato (Lewu *et al.*, 2010). The results obtained revealed that the pulp contains higher moisture compared to peel. The moisture contents of materials subjects it to increased microbial spoilage and deterioration (Adepoju and Onasanya 2008). The ash content was found to be 1.33±0.24% and 5.17±0.24% for pulp and peel respectively. The values obtained are lower than 6.41% (61.4gkg^{-1}) and 7.82% (78.2gkg^{-1}) for potato flesh and peel (Vaitkeviciene, 2019). The result of pulp obtained is also lower than 4.09±0.07% and 4.58±0.02% for cocoyam and potato tubers respectively but lower than the obtained value for peel while the peel is comparable with 5.6% for sweet potato peel as reported by (Ojewumi *et al*., 2018). It was recorded that the ash content in *S. rotundifolius* peel was much high than that of the pulp. The lower ash contents of the subtrates qualify it as good resources for bioenergy production. This indicate that the peel has more mineral elements present compared to the pulp which may have lower minerals contents especially macro minerals. A crude fibre measures the cellulose, hemicellulose and lignin contents of the substrate, and their processed products. Lignin constitutes of polymers of phenolic acid and hemicellulose is made of heteropolymer of polysaccharides (Zakpaa *et al.*, 2010). The results obtained are 0.50±0.00% and 1.83.±0.24% for pulp and peel respectively which is lower than 2.42 %(24.2g/kg) and 6.64% (66.4.g/kg) for potato flesh and peel reported by vaitkeviciene, (2019

Crude lipid content of the pulp and peel was 0.50±0.00 and 2.67±0.24 respectively. The result of pulp is lower than 1.63±0.24% for cassava bagasse as reported by Adedu and Enesi 2002. The value obtained for pulp is found to be higher than 0.24±0.03 for potato but lower than 0.78±0.07 for cocoyam as reported by Lewu *et al*, (2010). This crude lipid determines the free fatty acid of a product. This property can be used as the basis in determining processing temperatures as well as auto-oxidation which can lead to acidity. The higher the crude lipid presents in a biomass the less biodegradability of cellulose for biofuels production (Demirbas and Demirbas 2007). This indicates that *S. rotundifolius* pulp and peel could be potential raw materials in ethanol and biogas production.

Crude protein content of the substrates was found to be 2.22±0.12% and 2.77±0.08% for pulp and peel respectively. The results obtained are very much lower than 10.33% (103.3g/kg) and 14.72% (147.2g/kg) for potato flesh and peel respectively

The available carbohydrate content of the pulp and peel was 95.45±0.33% and 87.56±0.56 respectively which is higher while compared with 86.58±1.69% and 83.21±0.46% for cocoyam and potato tubers respectively. This values obtained is much higher than 72.72±0.06% for cassava bagasse (Enenebeaku *et al.*, 2016). The values may have been so because *S. rotundifolius* tuber is a carbohydrate rich food. The high carbohydrate content of this sample explains why it yielded a good quantity of reducing sugar and bioethanol. The calorific energy value of the pulp and peel was 315.18± 2.03kcal/100g and 385.35±1.01kcal/100g. The value obtained from the pulp is much lower than 378.93±10.39k/cal and 376.80k/cal for cocoyam and potato respectively but slightly lower than peel which is an indication of energy potentials of the substrate.

The pulp and peel were found to contains 20.50% and 19.33% organic matter which is lower 94./4% for sweet potato reported by (Ojewumi *et al.*, 2018). The organic matter content in the substrate indicate the carbon element present in the samples due to carbohydrates, fats, sugar and starch in the pulp and peel. The percentage carbon contents in the sample was 10.25% and 9.67% for pulp and peel which shows that the pulp has slightly high carbon content both substrates displayed to have potential in bioethanol production. The values obtained ware found to be very much lower than 43.8% for potato peel as reported by Liang and MCDonald (2015). Therefore the obtained result of this study shows that the C/N ratio is within the range of methane production and as such suitable for bioethanol production.

Generally, the pulp contain high amount of moisture, available carbohydrate, calorific energy value and organic matter while the peel was found to be higher in the ash content, protein, lipid and fibres contents. These results have

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shown similar trends as reported for potato peel and flesh by vaitkeviciene (2019).

Qualitative Analysis of Bioethanol produced

TABLE 2: Qualitative test of the distillates (Pulp and Peel)

Test	Observation	Inference
Distillates + Acidified $KMnO_4$	Decolourized the purple colour immediately	OH present
Distillates + Sodium metal	Vigorous effervescence colourless gas evolved	OH present
Gas evolved + Moist litmus paper	No effect on both litmus papers	Neutral to litmus paper
Distillates + CH_3COOH + few drops of conc. H_2SO_4	Fruity smell observed	OH present
Distillates + Iodine solution	Yellow precipitate observed	OH confirmed
Distillates + NaOH solution	Antiseptic smell	OH confirmed

The distillate was qualitatively tested with conventional laboratory reagents. Testing the distillates with litmus paper separately no visible reaction was observed, which indicate the neutrality of the distillate. Likewise acidified $KMnO_4$ were also decolourized by the distillates. Vigorous effervescence was observed by addition of sodium metal to the distillate which is colourless and has no effect on the litmus paper. Further test was carried out on the distillates with acetic acid and in the presence of few drops of concentrated H_2SO_4 in which a fruity smell was observed. Beside, a confirmatory test was performed on the distillate using Iodine solution and NaOH solution. Appearance of yellow precipitate and an antiseptic smell indicated the presence of ethanol. Therefore, the bioethanol produced showed similar characteristics with that of commercially prepared ethanol.

TABLE 3: FTIR Analysis of the Distillate (Bioethanol) for both pulp and peel.

Frequency of literature	Frequency of sample	Band	Functional group
3500 – 3200	3300	O – H	Alcohol (H – bonded)
3000 – 2800	2900	CH_2 and C – H	Alkane (stretching)
1670 – 1640	1650	C = O	
1450 and 1375	1450	CH_3	Alkane
1300 – 10000	1200	C – O	Alcohol

Table 3 showed the FTIR spectroscopic analysis of bioethanol produced from *S. rotundifolius* pulp and peel. The broad absorption band was observed at a range of 3500 – 3000 cm^{-1} frequency which comprises a band related to the crystalline structure of cellulose (Colon *et. al*., 2003). The region is of great importance and is related to the sum of the valence vibration of H – bonded for OH intermolecular and intermolecular hydrogen bonds. Another small peaks were recorded between 3000-2850 cm^{-1} which to CH_2 and CH symmetric and asymmetric stretching respectively. Absorption around 1450 cm^{-1} and 1375 cm^{-1} refers to CH_3 for alkane. A frequency range between 1300-1000cm^{-1} has a large concentration of hemicellulose and cellulose with maximum at 1200 cm^{-1} due to C – O stretching mode. The strong absorption at 1650 cm^{-1} is attributed to C = O unconjugated stretching of hemicellulose but also with the contribution of lignin which indicates chemical changes in hemicellulose or lignin as shown in appendix.

HPLC

To test HPLC methodology commercial Industrial Fuel Ethanol Standard was quantified (3.758*mAU*) and the result compared with the one obtained from *S. rotundifolius* pulp and peel (3.596 *mAU* and 3.819 *mAU*). The results are all within an acceptable +/- 10%.

IV. Conclusion

The results presented in this research work shows that both pulp and peel contained reasonable amount of starch and carbohydrate which can be hydrolyzed for fermentation to produced bioethanol. The determined properties of the bioethanol produced agrees with those repeated for bioethanol standard. The underutilized *Solenostemon rotundifolius* tuber which is considered as non-valuable could be effectively utilized for bioethanol production.

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