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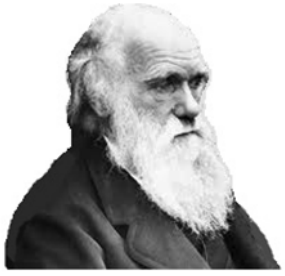

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Chemical composition and diluted acid hydrolysis pretreatment of *Acacia mellifera* sawdust as a raw material for bioethanol production

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ABSTRACT

Biofuels are alternatives to fossil fuels for ensuring energy security and for mitigating climate change. Currently, most biofuels are in the form of a bioethanol that is generated from starch or sugar. Increasing energy demand, food insecurity, and ecological concerns lead to evaluating agricultural, forestry, and urban lignocellulosic wastes as being very important for energy production. This is because all celluloses and hemicelluloses of lignocellulosic wastes can be converted into bioethanol reducing sugars. Hence, the current study was initiated to determine the chemical composition and best diluted acid hydrolysis pretreatment of *Acacia mellifera* sawdust for bioethanol production. Our study indicates that the chemical composition of *Acacia mellifera* sawdust exhibits different composition in extractives such as alcohol, cellulose, lignin, and ash. Accordingly, the extractive content of the sawdust was 6.3% soluble alcohol toluene, 52.9% cellulose, 23.9% lignin, 4.2% ash, and 6.92% moisture content, respectively. In our experiment, the biomass at a solid loading rate of 0.66% was pretreated at 121 °C with different sulfuric acid concentrations (0.5, 0.75, and 1%, w/w) and residence times (10, and 20 min). Total reducing sugars in the hydrolyzed sample with acid and time were then analyzed. The reducing sugars obtained at 0.50%, 0.75%, and 1% dilute sulfuric acid concentration with time residence of 10 min and 20 min were: 7.39±0.24 and 8.4±0.9, 8.03±0.64 and 9.18±0.43, 9.68±1.30 and 10.23±0.80, respectively. With the increasing acid concentration and residence time, the amount of glucose in the filtrates increased. Therefore, the total reducing sugar concentration in the hydrolysate of *Acacia mellifera* saw dust was significantly influenced by the sulfuric acid concentration and residence time. From this study, it can be concluded that total reducing sugars in lignocellulosic wastes are widely available and easily obtainable, they can be considered as potential feedstocks for bioethanol production.

***Keywords*:** Pretreatment, lignocellulose, reduced sugar, *Acacia mellifera*, bioethanol production

1. INTRODUCTION

Global warming and energy crisis are of great concern to government and people around the world. Greenhouse gases in the earth's atmosphere, such as carbon dioxide, cause the biosphere to warm up. Traditional fossil fuels will be used up in a few decades. Therefore, biofuel is an option for resolving both, the energy crisis and global warming. Bio-fuels converted from biomass are renewable energy and mitigate global warming by recycling carbon dioxide from the atmosphere. Plants absorb carbon dioxide and release oxygen back in to the atmosphere. Carbon becomes stored in a plants cellulose, hemicellulose, starches, and sugars and oils.

Then, plants are processed into Biofuels used in transportation. Bio-fuels are combusted back to carbon dioxide when people drive their cars, trucks and other modes of transportation [1]. Biofuel has been a source of energy that human beings have used since ancient times. Increasing the use of biofuels for energy generation purposes is of particular interest nowadays because they allow alleviation of greenhouse gases, provide means of energy independence and may even offer new employment possibilities.

Bio-fuels are being investigated as potential substitutes for current high pollutant fuels obtained from conventional sources. In fact, this biofuel has gained worldwide acceptance, specially to overcome severe energy crisis, fossil fuels depletion and environmental pollution. Hence, development of biofuel production from readily available feedstock from agro-based industries is urgently needed.

Lignocellulosic materials are renewable, largely unused, and abundantly available sources of raw materials for the production of ethanol. It's a potential source or future low-cost ethanol production which is to utilize lignocellulosic materials such as sawdust, crop residues, grasses and trunks [2]. It is one of the promising raw material for future energy alternatives contribute to the reduction of negative environmental impacts generated by the use of fossil fuels [3].

Bioethanol has been produced from a variety of lignocellulosic raw materials containing fermentable sugars such *Acacia mellifera* sawdust. *Acacia mellifera* is one of the commercially important tree in Ethiopia and Nigeria. It provides raw material for papermaking and is widely used in the construction industries, although large amounts of wood residue, such as bark, leaves, cork residue, cross-cut ends, edgings, grinding dust and saw have not been efficiently utilized. Considering the high cellulose content and the fact that waste generated during wood processing has no human and animal food values, the plant could serve to provide the much-needed feedstock for bioethanol production. Ethiopians have diverse climate conditions and different tree species with multi-uses.

Acacia mellifera (gerar) is indigenous hardwood species, wide-spread in Africa, occurring in Egypt, Sudan, Somalia, Angola, Kenya, Uganda and Tanzania, Sahel East of Niger River to Southern Arabian Peninsula, Eastern and Southern Africa. It is a re-sprouting multi-stemmed shrub with an average height of 75 cm [4, 5].

Therefore, the current study was initiated to characterize the chemical composition and diluted acid hydrolysis pretreatment of *Acacia mellifera* saw dusts for bio-ethanol production, in order to utilize these locally available and cheap lignocellulosic materials for the production of bio-ethanol as new source of energy to meet the rising energy demand and to reduce environmental pollution.

2. MATERIALS AND METHODS

2. 1. Biomass

Acacia mellifera was chosen as a raw material in this work, because it is a widely grown tree in Ethiopia and much of the sawdust is going to waste. The *Acacia mellifera* samples were harvested, dried and milled to a particle size of 1-3 mm and dried to a moisture content of less than 10% to determine the extractive, cellulose, lignin Ash, and moisture contents of samples were determined in the laboratory of biochemical and bioenergy of wood technology research center (**Table 1**). Standard methods of Association of Official Analytical Chemists (AOAC 973.18) and UV spectroscopy) were used in the analysis.

2. 2. Moisture content determination

About 2 g of sample was taken in a weighing bottle (A) and dried in an oven for 2 h at 105 °C. Then Stopper was put on the bottle and cooled in a desiccator containing silica gel to room temperature and weight. The process of heating and cooling in desiccators was repeated until the difference in two successive weighing was less than 1 mg.

The lowest weight (B) was record [6].

$$\text{Moisture content (\%)} = \frac{A - B}{A} \times 100$$

where: A = Initial Weight
B = Final Weight

2. 3. Alcohol-Toluene Extraction Solubility (ASTMD 1107-56)

The extraction apparatus consisted of a Soxhlet extraction tube which was connected with a reflux condenser on the top and joined at the bottom to a boiling flask. 2 g (O.D.) samples were placed into extraction thimbles. The thimbles were placed in a Soxhlet extraction tubes. The boiling flasks contained a 2:1 solution of 95% of ethyl alcohol and toluene in to the top of the condenser when the solvent mixture surrounding the sample exceeds a certain level, it overflows back down into the boiling flask 3 times and is placed on heating mantles. The flask containing the extractive solution was transferred into a round bottom flask. The extractive solution in the flask was then evaporated using distillation.

The remaining extractives in the flask were then placed in oven at 105 °C for an average of 1h, cooled in desiccators, and weighed until a constant weight was obtained. The following formula was used to obtain the alcohol - toluene solubility content of *Acacia mellifera*.

$$\text{Alcohol} - \text{toluenesolubles (\%)} = \frac{W_2}{W_1} \times 100$$

where:

W_1 = weight of oven-dry test specimen, g
W_2 = weight of oven-dry extraction residue, g.

2. 4. Cellulose Content Determination

Cellulose content was determined by treatment of 1 g of extractive-free wood meal with 20 ml of 3% nitric acid and boiling for 30 min. The solution was filtered and the residue was treated by 25 ml of sodium hydroxide (3%) and boiling for 30 min. The residue was filtered, washed with worm water, dried and weighed [7]:

$$Cellulose\ (\%) = \frac{W_2}{W_1} \times 100$$

where:

W_1 = the amount of extract free samples taken for analysis, g
W_2 = the residual mass of cellulose, g.

2. 5. Lignin Content Determination

Klason lignin content was determined by treatment of the extractive-free wood meal (0.5 g) with a mixture of 85% phosphoric acid and 75% sulfuric acid (in a ratio of 1:6) at 35 °C. After one hour, the samples were boiled for half an hour with 200 ml distilled water, filtrated, dried, and weighed [8], (ASTM D 1106-84, 1989):

$$\text{Lignin}\ (\%) = \frac{W_2 - W_1}{\text{O. D. Weight of Sample}} \times 100$$

W_2 – stands for weight of crucible + sample
W_1 – stands for weight of empty crucible.

2. 6. Dilute Acid Hydrolysis

2. 6. 1. Acid Impregnation

The acid pretreatment experiments were carried out in a 250-ml volumetric flask. The wood was impregnated with dilute acid before being subjected to high-temperature hydrolysis to ensure that uniform treatment was obtained and diffusion of acid into the chips was not a rate-limiting factor. Acid impregnation was carried out by soaking the chips in dilute sulfuric acid (0.5–1%) solution at 121 °C for 10 min and 20 min in autoclave. After completion of the acid pretreatment, the solids and liquids were separated through Whatman No. 1 filter paper. Hydrolysates (liquid fractions) were stored for sugar analysis. Then the total reducing sugar concentration was determined by phenol-sulfuric acid method.

2. 6. 2. Phenol-sulfuric acid method

The total sugar concentration was determined by using UV-visible spectrophotometer (NV203 Spectrophotometer) at 540 nm wavelength of glucose absorbance and the quantification was made from calibration curve using glucose as standard (**Figure 1**), and calculation was performed by equation of the linear regression obtained from calibration curve. A 2-ml aliquot of a sample solution was mixed with 0.4 ml of 5% aqueous solution of phenol in a test tube. Subsequently, 2 ml of concentrated sulfuric acid was added rapidly to the mixture. The test tubes were allowed to keep for 10 min at room temperature, and placed in a water bath

for 20 min for color development. Then, light absorption at 540 nm was recorded on a spectrophotometer.

Blank solutions were prepared in the same way as above, except that the 2-ml aliquot of a sample solution was replaced by distilled water [9].

2. 6. 3. Experimental Design

The study was conducted using completely randomized design (CRD) with 2 factors. Factor 1 is the concentration of sulfuric acid consists of three variations, namely 0.5%, 0.75%, and 1% H_2SO_4. Factor 2 is the time of the hydrolysis process that consists of two variations, namely 10 and 20 min. Of the two factors obtained, 6 treatment combinations and the parameters that were analyzed were for reduction sugar levels. The data obtained were statistically analyzed using SPSS software with real different test (*t* test).

3. RESULTS AND DISCUSSION

The results of the *Acacia mellifera* chemistry testing are listed in Table 1. The saw dust consisted of 52.9% cellulose, 23.9% lignin, 6.92% moisture content, 4.2% ash, and 6.3% alcohol toluene solubility. The lignin-to-cellulose ratio was 2.1. The results of lignin and cellulose contents in *Acacia mellifera* were 23.9 % and 52.9%, respectively, which place wood at the high end of the normal range of 20-25% and 40-50% reported for hardwoods [10], similarly, [11] reported 23.3% and 51.5% lignin and cellulose, respectively. Additionally, the result of ash (4.2%) and Alcohol-Toluene Solubility (6.3%) content was determined [11-16]. It has been reported 1.9% ash and 3.0% extractive contents, and summarized in (Table 1).

Table 1. Chemical composition of *Acacia mellifera*

Country	Properties	Ash	Moisture	Cellulose	Lignin	Alcohol-Toluene Solubility	Cellulose/ lignin ratio
Ethiopia	%	4.2	7.17	52.9	23.9	6.3	2.21
Sudan*	%	1.9	n. a	51.5	23.3	3.0	2.2

n. a not available and *Source: [11]

In this study, the total reduced sugar content through hydrolysis process was investigated. The saw dust of *Acacia mellifera*, hydrolyzed at different acid concentration, hydrolysis time, and hydrolysis temperature on the amount of sugar produced, was determined by phenol-sulfuric acid.

The glucose equivalents (GE) were calculated from the calibration curve of glucose standards. The concentrations of unknown sugar samples were determined from a standard curve of glucose ($y = 13.395x + 0.073$; $R^2 = 0.9939$) (Figure 1).

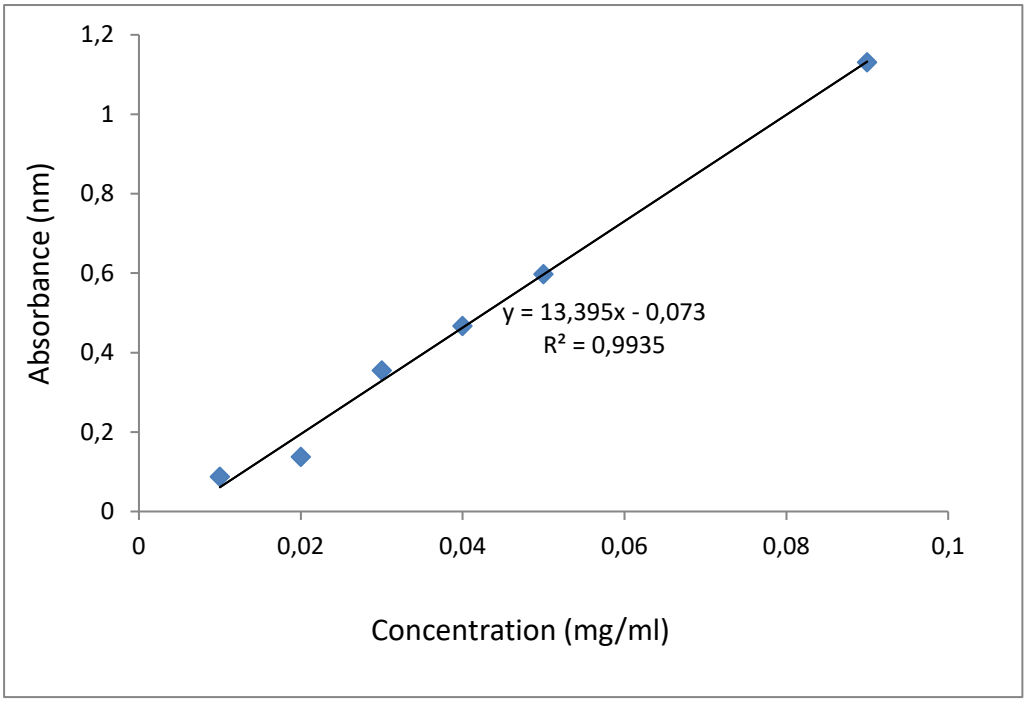

Figure 1. Calibration curve of glucose standard for determination of total reducing sugar content

After pretreatment at different sulfuric acid concentrations and times, the hydrolyzed was collected. The amount of total reducing sugars released from *Acacia mellifera*is shown in **Table 3**. Pretreatment time and sulfuric acid concentration significantly influence the release of sugar from the biomass ($P < 0.05$), as shown in **Table 2**.

Table 2. Analysis of effect of different concentration of sulfuric acid and treatment time on release of glucose.

Source	Df	Mean Square
Acid	2	6.338*
Time	1	3.915*
Acid * Time	2	0.168 ns
Error	12	0.637
$R^2 = 0.689$		

*indicate significant difference between treatments' (Tukey's test; *$p < 0.05$), ns indicate not significant difference between treatments', $p > 0.05$

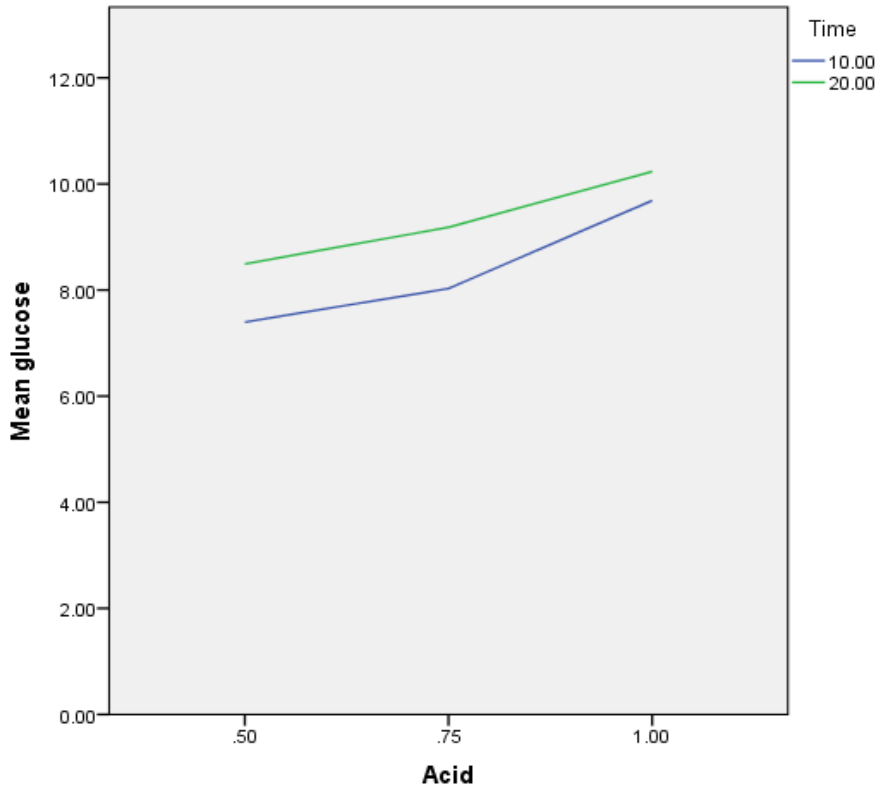

Figure 2. Analysis of the effect of different concentrations of sulfuric acid and treatment time on release of glucose during hydrolysis.

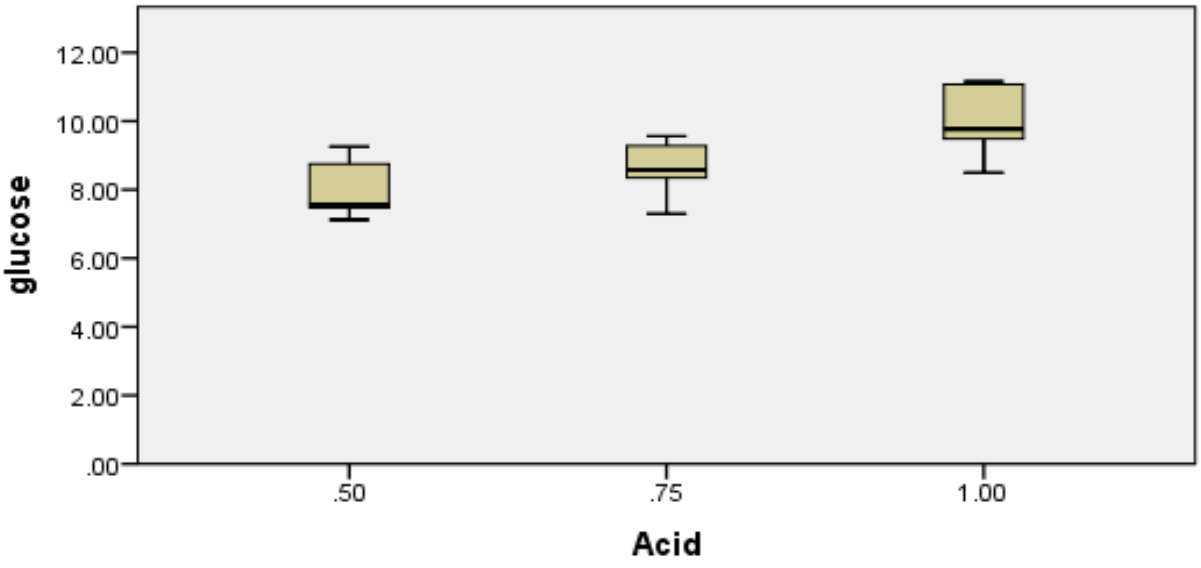

Figure 3. Impact of various sulfuric acid concentrations on the release of sugars from *Acacia mellifera*

Studying the output of the ANOVA (**Figure 2**) we saw that there was no evidence of a significance of interaction effect ($p > 0.772$). Therefore, it concluded that there is no interaction between concentration of the acid and the treatment time for releasing of glucose. The effect of sulfuric acid concentration was not changing depending on the pretreatment time.

The test for the main effect of treatment acid concentration ($p < 0.05$) shows a significant interaction of acid concentration on the release of glucose level such that acid whose concentration had 1% released higher glucose than 0.75% and 0.5% H_2SO_4. **Figure 3** shows that there appears to be a different in release of glucose amount for different sulfuric acid concentration. The best glucose yield in the liquid fraction was 10.23 ±0.80 when pretreated in 1% sulfuric acid concentration.

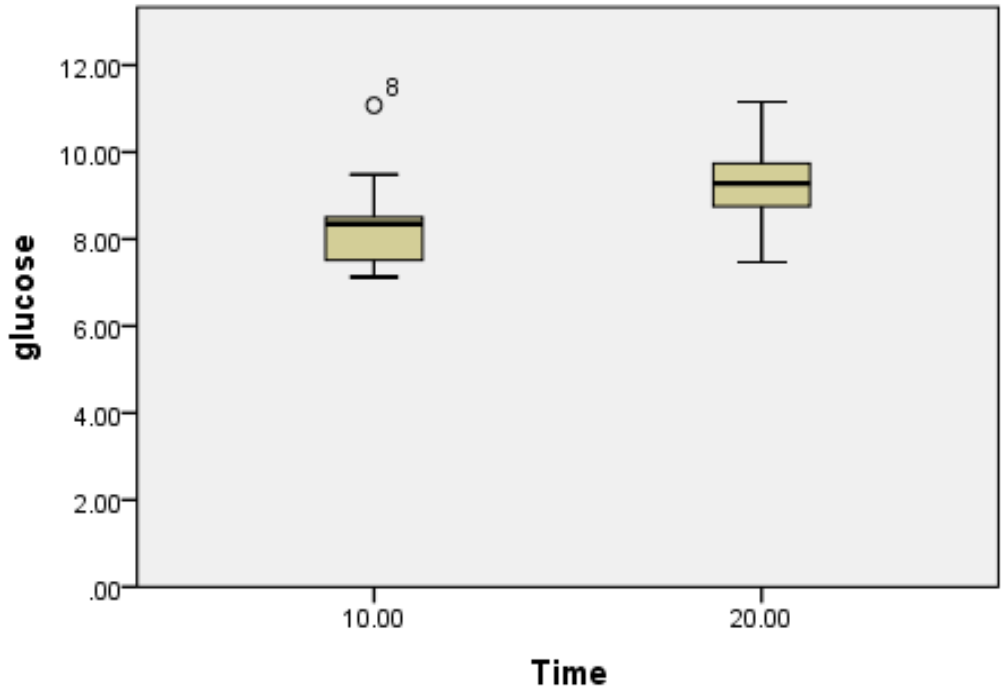

Figure 4. The impact of different pretreatment time on the release of sugars from *Acacia mellifera* during hydrolysis

Figure 4 shows that the difference in glucose is also pronounced between the two pretreatment times. As the treatment time increased, the glucose yield increased, the test for the main effect of time ($p < 0.05$) tells us there is enough evidence to conclude that there is a significant time effect on the release of glucose such that when pretreatment time was 20 min, it had released higher glucose amount 9.18 ±0.433 than at 10 min when glucose had 8.02±0.63.

The result indicated that the residence time of 10 min was not enough for the solubilization cellulose in the biomass.

Table 3. The yields of glucose in the hydrolysis at different hydrolysis temperature and retention time.

Acid (%)	Time	Mean± Std Deviation of glucose
0.50	10.00	7.34±0.24
	20.00	8.49±0.92
0.75	10.00	8.02±0.63
	20.00	9.18±0.43
1.00	10.00	9.68±1.30
	20.00	10.23±0.80

The results of chemical analysis are shown in Table 3, for 10 min autoclave treatment. It appeared that using a 1% sulfuric acid concentration released of 9.68±1.30 total reducing sugar of *Acacia mellifera* compared to the 0.5% and 0.75% acid concentration which released only 7.34±0.24 and 8.02±0.63 for 10 min, respectively. For 20 min autoclave treatment, it appeared that using a 1% sulfuric acid concentration released 10.23±0.80 of *Acacia mellifera* compared to the 0.5% and 0.75% acid concentration which released only 8.49±0.92 and 9.18±0.43 of total reducing sugar released, respectively. In 1% sulfuric acid, the treatment was the best and statistically different (Tukey, $\alpha = 0.05$) from the others. This suggests that high acid treatments with high exposure time and temperature yield higher reducing sugar concentrations.

Table 4. Correlation coefficient of *Acacia mellifera* sawdust hydrolysis by acid.

Correlations				
Factor		Acid	Time	Glucose
Acid	Pearson Correlation	1	.000	.705**
	Sig. (2-tailed)		1.000	.001
	N	18	18	18
**. Correlation is significant at the 0.01 level (2-tailed).				

According to the above Table 4, the Pearson correlation between acid and glucose is about 0.705**, which indicates that there is a strong positive relationship between the variables.

Sulfuric acid concentration had a significant correlation with reducing glucose. This indicated that there is a strong and positively relationship, between acid concentration and reducing sugars.

4. CONCLUSION

Determinations of chemical composition and total reduced sugar of *Acacia mellifera* saw dust were investigated by using different factors. The chemical composition of *Acacia mellifera* saw dusts exhibited different chemical composition in extractives; cellulose, lignin and ash contents were studied. Total reducing sugars in the pretreatment and hydrolyzed sample with acid and time were analyzed. The 0.50%, 0.75%, and 1% dilute sulfuric acid concentrations, with time residence of 10 min and 20 min, were studied. With the increasing acid concentration and residence time, the amount of glucose in the filtrates increased. Therefore, the total reducing sugar concentration in the hydrolysate of *Acacia mellifera* saw dust was significantly influenced by the sulfuric acid concentration and residence time. As a result, *Acacia mellifera* has the potential for fuel ethanol production.

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