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Assessment of Bioenergy Potential of Lemon Grass

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Authors' contributions

This work was carried out in collaboration between all authors. All authors participated in the research activities. All authors read and approved the final manuscript.

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ABSTRACT

Grasses can serve as an alternative biomass for the production of bioenergy which plays a vital role in solving some of the challenges faced in the production of renewable energy and also help in tackling some of the environmental challenges faced all over the world. In this research, the phytochemical and biofuel content of *Cymbopogon citratus* was assessed. The phytochemical screening and proximate analysis were carried out according to standard qualitative and quantitative methods. Alkaloids, balsam, flavonoids, glycosides, saponin, carbohydrates, protein, volatile oil, minerals are analyzed. Bioenergy was produced using enzymatic hydrolysis after pretreatment under different pH viz: pH 5, pH 7 and pH 8 all under an ambient temperature of 36˚C. Each of the pretreated samples of varying pH was then fermented using *Saccharomyces cerevisiae*. Each of the pre-treatment under three different pH was made in triplicate. The absorbance was determined for reducing sugar and at the end of the production, the pretreated samples were subjected to Gas

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chromatography and mass spectroscopy to analyze the end product of bioenergy produced. The result for phytochemicals shows the presence of flavonoids and alkaloids (1.60%) followed by volatile oil (7%), saponin (3.20%) and tannins (0.6%). The result for proximate analysis shows that lemon grass has the highest percentage of carbohydrates (87.63%). This was followed by ash (11.67%), protein (11.14%) and fibres (2.83%). However, the mineral analysis shows the presence of Calcium (1.88 mg/ml), Magnesium (0.13 mg/ml), Phosphorous (5.87 mg/ml), Potassium (2866.67 mg/ml) and Sodium (89.17 mg/ml). The absorbance in *C. citratus* is higher at pH 5 (0.0651). The GC-MS analysis of the bioenergy produced shows the presence of Ethyl alcohol (bioethanol) in all the samples at different pH. Hydrazine carboxamide was present in all the treatments under different pH. In addition glycidol, acetyldehyde, acetic acid, 1,2-propanediamine were found present, having fuel potential and are good source of gasoline. These are produced as a result of fermentation and enzymatic activities of the organic compound present in the biomass sample used.

Keywords: Bioenergy, Cymbopogon citratus, pH and phytochemical screening.

1. INTRODUCTION

A wide range of agricultural and forestry waste, as well as other energy crops, are being considered as feed stock for bioenergy production [1-4] most commonly demonstrated biomass are raw materials from corn Stover, sugar cane baggase, wheat straw, switch grass and rice straw are currently in operation. This biomass has different chemical compositions which further depend on factors such as seasonal variation, weather and climatic factor, crop maturity and storage conditions [2,3]. Bioethanol is the largest volume product produce in most biotechnology industries [3]. Its production is currently based on fermentation of cane sugar or hydrolyzed corn starch with the yeast *Saccharomyces cerevesea* [4] over the past decades, a large international effort involving researcher (academics, research institutes and industries) have being searching for an alternative and abundantly available agricultural and forestry residue, as well as growing biomass as a substitute for biofuel production [5].

The search and debate on an alternative source of energy is a worldwide challenge faced, though developed countries like U.S.A and Brazil have been producing and utilizing biofuel produced from different biomass. The most common used biomass for bioenergy productions are food crops mainly sugar cane (*Sarcharium officinarum*), corn (*Zea mays*) and rice husk (*Oryza sativa*), which all belong to poaeceae family (C4-grasses). They are highly efficient at converting solar energy during photosynthesis. Due to this the need to replace this food crops for security purposes warrants the search for an alternative source of energy [5,6].

Lemon grass is compacted tufted perennial crop grown under irrigation or non- irrigation condition having a characteristic scent of lemon [7,8,9,10]. According to Valtcho et al. [9], lemon grass is a high- biomass crop that may have applications to biofuel production due to the high content of essential oil. However, the cost of production of biomass for biofuel may be low because the biomass is a byproduct of essential oil production. Lemon grass proves to be a new high value specialty crop and good source for biofuel in the temperate area with high temperature.

Lemon grass being a C4 perenial grass having a characteristic smell of lemon grass, growing this grass plants requires little input to reduce cost effectiveness, water use efficiency, fertilizer arable land [7]. The phytochemical screening and proximate analysis of lemon grass show the presence of some organic constituent which is good source of biofuel [8], and also seeing the quench and search for environmental friendly fuel this motivates the interest of this research on bioenergy production using a biological sources for enzymatic hydrolysis and fermentation activities.

There are a lot of limitations influencing the type of biomass to use for energy production that will be sustainable [7]. The main limitations are the amount of land to be used for the production of biomass, cost input, water and fertilizer. However, the use and choose of which biomass on the geographic location, environmental condition and time for the availability of the biomass has a great impact.

Moreover, most of the biomass used for the production of biofuel are switch grass, rice straw, wheat straw coconut husk and other none agricultural waste. So far less research was explored on bioenergy or biofuel potential of lemon grass, which is an abundant and less cost effective species. The aim of this research is to assess the phytochemicals and Bioenergy contents of *Cymbopogon citratus*. The objectives were to: Determine the phytochemical and nutritive content in the grass; to evaluate the effect of pretreatment and enzymatic hydrolysis in the production of bioenergy under three different pH.

2. METHODOLOGY

Lemon grass sample collected was air-dried under shade. The sample was pulverized and sieved into a fine powder using mortar and pestle for phytochemical analysis. Another sample of the grass was crushed with hammer into small sizes in order to obtain 20 g of the substrate for bioenergy production [11]. In this analysis moisture content, ash, crude protein, carbohydrates, lipids and mineral content of the grass species was determined using standardized methods as described by Amina et al. [8].

The crushed sample was then diluted with 300 cm^3 distilled water. The suspension prepared was then autoclaved at 121˚C for 15 minutes to sterilized and prevent the growth of other microorganisms as well as to degrade the complex carbohydrate (ligno-hemicellulose material) present in the plant species prior to the release of sugar [11]. Three different set of pH was set for the production of bioenergy viz: Acidic (pH5), neutral (pH7) and alkali (pH8). The pH of the grass samples for the first sets which were acidic was retained (pH5). For the remaining sets the pH was adjusted with 1 Mol. of Sodium Hydroxide (NaOH) to Neutral (pH7) and Alkaline (pH 8.0) using a pH Meter Lenway Model No 3015. Hydrolytic organism (*Aspergilus niger*) was inoculated for enzymatic hydrolysis as explained below after culturing, sub-culturing and identification of the species.

Enzymatic Hydrolysis - Saccharification of enzyme pre-treated biomass was done enzymatically to get fermentable sugars [10,11]. Twenty (20) grams of *Aspergilus niger* was used for enzymatic hydrolysis; these was transferred into 500 cm^3 volumetric flask. Thereafter, the volumetric flask fitted with cotton and aluminium foil was placed in an incubator for period of seven days at room temperature (36˚C) which is the normal room temperature of the study area. It was allowed to stay for enzymatic activity to occur in order to break the hemi-cellulose present in the plant samples. At the end of hydrolytic analysis, the sample produced (glucose) was collected and subjected for test for reducing sugar using the method of [12]. The reducing sugar content was determined by making reference with a standard curve of known concentration for Glucose.

Fermentation of the substrates to simple sugars (Glucose) was carried out using activated baker yeast (*Saccaromycese cerevisea*) as described by Nasrullah and Touseef, [13]. The bioenergy produced was subjected to fractional distillation in order to remove water and other impurities. Bioenergy was then tested qualitative and quantitatively using Fourier transformer and infrared spectroscopy (FT-IR) analysis. The absorbance was measured at 588nm using UV-visible-1650pc, Schimadzu Spectrophotometer. Spectrophotometric graph of absorbance was plotted against concentration which is used as the standard curve. After the hydrolysis and fermentation process, the sample produced was subjected to GC-MS analysis to determine the end product. The analysis was performed using GC-MS-QP2010 plus (Shimadzu, Japan) equipped with flame ionization detector (FID). The injection was conducted in split less mode at 250˚C for 3 minutes using an inlet of 0.75 mm ionization detector to minimize peak broadening.

Statistical analysis - The data obtained was subjected to analysis of variance and means that were significant were separated using t-test at $p< 0.05$. Statistical Package for Social Sciences (SPSS) version 20 was used for this analysis.

3. RESULTS

The results obtained for phytochemical screening of the leaf extract of *Cymbopogon citratus* was shown in Table 1. The percentage of flavonoid presents is higher (13%) than the remaining constituents that's alkaloids, glycosides, saponin and tannins. However, the results for proximate analysis presented in Fig. 1 indicated that carbohydrate content is much higher (87.81%) followed by crude proteins (8.69%), moisture content (7.17%), ash (1.17%), fibres (2.8%) and lipid (3.67%). The quantity of inorganic minerals present in the grass samples (Table 3) reveals high amount of potassium (2866.67 mg/ml) followed by Sodium (89.17 mg/ml). Where

Calcium (1.88 mg/ml) and Magnesium (0.13 mg/ml).

Table 1. Quantitative test for Phytochemical present in *C. citratus* leaf powder

Compounds	Quantitative value (%)
	***C. citratus* (lemon grass)**
Alkaloids	1.60
Flavonoids	1.60
Glycosides	0.84
Saponins	3.20
Tannins	0.62
Volatile oil	7.01

The effect of pH on concentration of *C. citratus* (lemon grass) was shown in Fig. 2. It indicates that the highest bioenergy concentration was observed at pH5 (0.058 mg/ml) followed by pH7 (0.047 mg/ml) and pH8 (0.041 mg/ml) having the least concentration. The interaction between reducing sugar, absorbance and volume of the grass samples tested was shown in table 3. There was no significant difference between the tested samples in terms of absorbance, reducing sugar and volume. pH has effect on the absorbance and concentration of bioenergy produced under different pH. The difference between pH shows that the absorbance for spectrometric analysis is less at pH8 (0.051) than pH 5 (0.058) and pH 7 (0.056). However, the significant difference for the absorbance of reducing sugar too is higher in pH7 and 8 than pH of 5. There was no significant difference at 0.05% level of significance between volumes of the samples at different pH level.

Table 2. Concentration of Mineral Constituents in *C. citrates*

Parameters	Quantity (mg/ml)
Calcium	1.88
Magnesium	0.13
Phosphorus	5.87
Potassium	2866.67
Sodium	89.17

Table 4 shows the results for GC-MS for the determination of the bioenergy produced using lemon grass sample. Under pH Hydrazine carboxamide with a peak area of 54.23% was detected at 1.60 minutes, Ethyl alcohol peak area of 23.20% at 1.70 minutes and Glycidol at a peak area of 22.57% at 2.24 minutes Were present. At pH7 the following compounds were detected: Hydrazine carboxamide at a peak

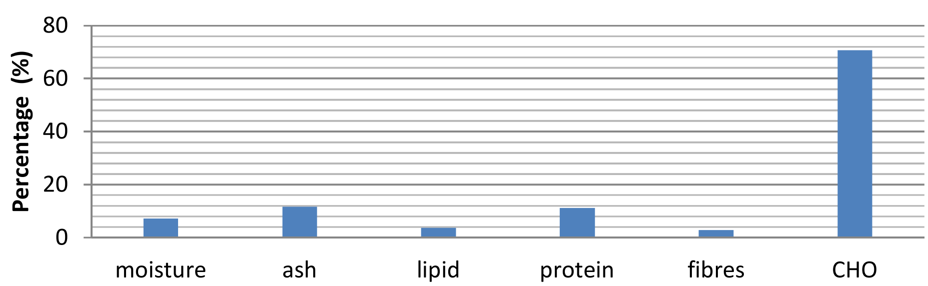

Fig. 1. Percentage ofnutritive content of lemon grass

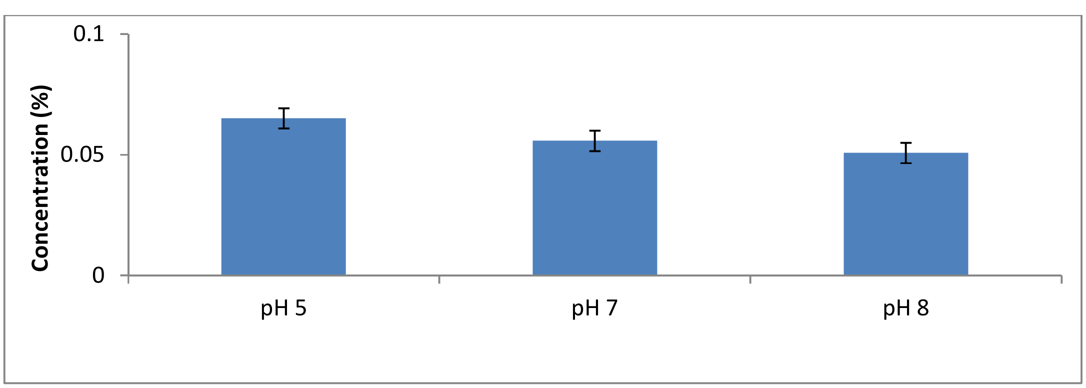

Fig. 2. Effect of pH Bioenergy concentration of *C. citratus* (Lemon grass)

Table 3. Effect of pH on absorbance and concentration of bioenergy produced from *C. citratus*

Samples	Ph	Absorbance (nm)	Concentration (mg/ml)	Concentration (g/g)
C. citratus	5	0.0651^{a}	0.0576^{a}	0.058
	7	0.0558^{a}	0.0466^{a}	0.047
	8	0.0508^{ab}	0.0407^{ab}	0.041
Significance		**	**	**
Standard error		0.0099	0.0116	0.012

Table 4. Gc-Ms analysis of the bioenergy produced from lemon grass under different pH

pH	Retention time (min)	Compound	Concentration (%)
pH5	1.604	Hydrazine carboxamide	54.23
	1.701	Ethyl alcohol	23.20
	2.238	Glycidol	22.57
Total			**100**
pH7	1.595	Hydrazine carboxamide	37.35
	1.701	Ethyl alcohol	19.71
	2.237	Acetyldehyde	42.94
Total			**100**
pH8	1.596	Acetic acid	23.95
	1.701	Ethyl alcohol	12.27
	2.225	1,2-propanediamine	63.78
Total			**100**

area of 37.35% at 1.60 minutes of the analysis, Ethyl alcohol was detected at 1.70 minutes at a peak area of 19.71% and Acetyldehyde at 2.24 minutes at a peak area of 42.94%. However, at pH 8 as shown in Table 4 the following compounds were detected from the GC-MS analysis viz: Acetic acid, Ethyl alcohol and 1,2-propanediamine. Acetic acid with a peak area of 23.95% at a retention time of 1.60 minutes, while Ethyl alcohol and 1,2-propane diamine having a peak area of 12.27% and 63.78% at a retention time of 1.70 minutes and 2.23 minutes.

4. DISCUSSION

Quantitative investigation revealed that *C. citratus* contained several phytochemicals such as saponins, tannins, alkaloids, glycosides, steroids and flavonoids (Table1). These components are naturally occurring in most plant materials and known to possess interesting biological activity such as antioxidant, anti-carcinogenic, antiviral, antibacterial, antidiabetic, anti-inflammatory [12-16] that inhibit and effect the process of fermentation activities in bioethanol and bioenergy production.

The presence of minerals and nutrient in the plant samples used in the analysis favours the means of the fermentative yeast, because as a living constituent for its activity to take place there is need for some basis such as protein, carbohydrates lipids, moisture and minerals (Potassium, Magnesium, Calcium and Sodium) for the life processes and physiological activities of the yeast to take place [17,18]. This also helps in the fermentation processes. For example, in transportation or cellular activities happening in and out of the cells, the presence of Potasium, Sodium and Calcium serves as a transportation channel [19]. From the analysis, it was observed that pH has influence on the absorbance of the reducing sugar. The absorbance is higher at different pH levels. At neutral level (pH7) the absorbance increases, toward Alkalinity (pH8) the absorbance decreases slightly when compared to acidic level (pH5).

Grass samples used in this analysis contain high level of carbohydrates this is in line with Parameswara et al. [20] finding on rice straw which belong to the same family compose of complex carbohydrates. The content of carbohydrates in all the samples was high with no significant differences between the samples at $p \geq 0.05$.The high content of carbohydrates shows that the grass is good source of sugar suitable for the production of biofuels. The amount of carbohydrates in the grass sample makes it a good source of bioethanol production [21]. This is in line with Alessia et al. [21] on carbohydrates rich materials dispose in the pineapple canneries makes this waste an interesting source of bioethanol production. However, the presence of different polymeric substances (hemicellulose) on the cell wall justifies the need for pre-treatment on waste. According to Sebayang et al. [11] and Alessia et al. [21], enzymatic activities are not affected by fermentation parameters used in his analysis the presences of Nitrogen act as a supplements which might reduce the lag phase and increase the yeast biomass [20].

The water content is less in lemon grass when compared to moisture content of wheat (12.3%), corn (15%) and Barley (11.1%) in a finding and review by Sebayang et al. [11]. Ash content depend on the plant type, water uptake, inorganic sources from the environment or fertilizer used particularly with regard to mineral element (Potassium, Chlorine, Phosphorus), soil conditions, or part of the plants where leaves have the highest ash content [19]. Lemon grass has fuel potential grass and the inorganic content is much too due to the presence of ash. When compared to Sorghum, wheat and sugar cane, the ash content of the grass species in this research is higher than the content in Sorghum (0.4%), wheat (1.7%), corn (1.2%) and Barley (11.1%) [11].

The percentage of protein is slightly higher in lemon grass, which is almost in same range with Kim and Day [22] finding of protein present in wheat (12.5%) and corn (10.2%). Lipids and fibres are less in all the samples, but much less in lemon grass. This indicates that the percentage yield of essential or volatile oil in grass is lower. When compared to other research by Health and Ageing Australian Governmeny [23] and Mosier and Ileleji [24] show that lipid present in corn is almost in same range with lipid present in present studies, however, higher than wheat (1.0%), Barley (1.8%), and lemon grass (1.83%).

According to Nasrullah and Touseef, [13], the production of Bioenergy from various biomass sources usually needs pretreatment, hydrolysis (sachharification) followed by simple fermentation process using various strains of yeast and bacteria [25,27]. The selection of strain for bioenergy production is made by considering their productivity, tolerance to ethanol, fermentation inhibitors and severe pH and temperature conditions [25]. In most of the fermentation processes, *S. cerevisiae* is used which is an efficient bio-ethanol and bioenergy producer due to its high tolerance to ethanol, low optimum pH range and anaerobic conditions requirement [27,28].

Pre-treatment is performed mildly to obtain high sugar yield with low inhibitor concentrations in the hydrolysates. This was performed mildly in order to obtain a high yield of bioethanol as pointed out by Shinnosuke et al. [30]. This reduced the inhibitory activity of the grass samples against the yeast (*S. cerevisiae*) and fungi (*Aspergillus niger*) used for the enznymatic activities due to their antimicrobial potentials [25].

The use of fungi (*Aspergilus niger*) for the hydrolysis of the sample would alter the enzymatic hydrolysis when compared to bacteria because fungi have fast tendency of breaking down lignocellulose compound than bacteria. A chemical (acid or alkaline) is not used for the hydrolysis in order to avoid any conversion or reaction to take place when compared to enzymatic hydrolysis which does not change or alter the form of the samples.

The hydrolyzed sample of lemon grass would not inhibit fermentation process. When compared to pre-treatment of several sugar degradation products such as 5-hydromethyl-furfural and furfaral are degraded product of hexose and pentose sugar released into the hydrolyzed sample with weak organic acids and Phenolic compounds leading to inhibition of yeast and enzymes; which effect cell growth during fermentation and more than the ethanol formation [11,27].

According to Yang et al. [29], large amount of food waste (vegetables and fruits) can be utilized to produce bioethanols, it will lead to significant economic and environmental benefits [25,30]. Useable bioenergy are produced by removing excess water content from the liquid wash by distillation process. The final ethanol product is limited to 95-96% due to the formation of a low boiling water – ethanol [31-33].

5. CONCLUSION

The end product of bioenergy after Gc Ms analysis shows the presence of ethyl alcohol in all the samples under different pH range. In addition, other compounds where present in all the samples play a vital role as biofuel due to fuel properties each of the compounds possessed. It was due to the present of organic compounds in the grass samples such as crude fibres, protein and lipids. This is the process of fermentation and enzymatic activities resulted in the formation of the following constituent: 1,2- propanediamine, Hydrazine carboximide acetic acid, acetyldehyde and ethyl alcohol.

6. RECOMMENDATION

Base on the findings, the production of biofuel can be achieved and encourage using grass species which are non-edible food crops without competing with food crops.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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