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Extraction of Alcohols From Non-Edible Agricultural Weed, Lignocellulouic Feedstock - *Alternanthera caracasana*

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ABSTRACT

Since natural resources for fossil fuels are getting harder to find, bioethanol has become one of the most promising and attractive liquid fuels. Bioethanol, which can be made from lignocellulosic materials, is getting more and more attention because it is easy to get, cheap, environmentally friendly, and beneficial. Bioethanol is the perfect fuel substitute in our view. Bioethanol is a cleaner-burning fuel with the potential to match gasoline's performance in internal combustion engines. Accordingly, a study was performed on Alternanthera caracasana as a possible source of bioethanol because of its availability and abundance in nature. It is commonly called Khaki weed and in very few cases this is used as a feedstock in animal husbandry and poultry. A. caracasana can be easily used to extract lignocellulosic material because of its abundance and non-usability. This lignocellulosic material can be subjected to fermentation for the production of bioethanol. The extracted bioethanol was characterized with FTIR and GC-MS for purity and percentage.

Keywords: *Biofuel, Bioethanol, Fermentation, Alternanthera caracasana*

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INTRODUCTION

Environmentally sustainable energy sources are desperately needed because of the quickly expanding industrialization. The majority of the world's wealth is derived from nonrenewable fossil resources including natural gas, coal, and oil. They are employed in the production of fuel, power, and other things (Sarkar *et al*., 2012). With an increase in population, modes of transportation, and technological development, fossil fuel use rises, resulting in a depletion of natural raw materials. This circumstance causes to hike. In addition, greenhouse gases, which change the climate and worsen air pollution, are produced by fossil fuels. We now need to look for alternative energy sources due to factors such as the daily increase in global energy consumption, global warming, the depletion of fossil fuel resources, and the rising expense of fuels derived from petroleum. These sources need to be affordable, productive, and efficient while emitting fewer pollutants or having no adverse impacts (Nigam & Singh, 2011; Lhawang *et al*., 2021; Sirigeri *et al*., 2022). As a biofuel, plant biomass can be regarded as a good alternative fuel source. Due to its advantages of being easily available, affordable, and advantageous for the environment, bioethanol, which may be produced from lignocellulosic biomass, is growing in popularity (Chiaramonti, 2007).

Bioethanol can be used as a clean fuel in cars in the following forms: E85 (85 percent ethanol and 15 percent gasoline), E100 (100 percent ethanol with or without a fuel additive), and oxy-diesel (usually an 80/10 mixture of diesel, ethanol, and additives and blending agents). Additionally, if it is continuously generated in large quantities, ethanol can be utilized to generate energy. In contrast to the United States, where corn is mostly used to make ethanol, sugarcane molasses is used as the main raw material in India (Krishnan *et al*., 2020).

One can categorize biofuels as first- or second-generation (Agarwal, 2007). Second-generation biofuels are often produced from lignocellulosic biomass, which includes grasses, wood, and stems.

Under research are numerous second-generation biofuels, including biohydrogen, bioethanol, and mixed alcohols (Agarwal, 2007). "First-generation" bioethanol is produced through the fermentation of raw sugars, whereas "second-generation" bioethanol is produced through the use of raw lignocellulosic materials. Preliminary research is being done on the "third generation" of algae-based bioethanol (Rajeswari *et al*., 2022). In addition, cellulose plant material represents an untapped supply of fermentable sugars for a major application, particularly as non-food agricultural by-products like rice and wheat straw, bagasse, rice husk, etc.

Lignocellulosic Biomass (LB) like wood (soft and hardwood), agricultural by-products (Corn, wheat, and rice straw), grass, and waste, are captivating and durable alternatives. It is also

utilized to produce bio-alcohol because it is available in large quantities. In addition, it is less expensive compared to food ingredients (based on sugar and starch). 442 billion liters of bio-alcohol can be produced annually using LB. The production of biofuels from lignocellulosic raw materials has the potential to replace approximately 30% of conventional fuels currently consumed by the United States (Hashim *et al*., 2022).

Furthermore, second-generation ethanol is created from lignocellulosic feedstock, In this study, the main goal is to identify the non-edible plant for the synthesis of bioethanol and the objective was the extraction of bioethanol through a fermentation process (Devi *et al*., 2022). The study examined the plant *A.caracasana* as well as extracted bioethanol by fermentation using *S. cerevisiae.*

A.caracasana is a lignocellulosic plant with a high growth rate such plants can generate bioethanol that can be used as fuel for vehicles. The characteristic feature of this plant is rapid growth is essential to fulfilling the demand for daily usage of fuel. Lignin degradation is a crucial process in the extraction of bioethanol. Since *A.caracasana* is not a source of food for the human population, the extraction of bioethanol from it will not interrupt the production of food. Furthermore, the use of ethanol as an automobile fuel will benefit the environment by cutting emissions.

It is possible to reduce food consumption by producing bioethanol from lignocellulosic biomass such as *A.caracasana*, which is abundant. *A.caracasana* sugar concentration enables it to create ethanol. Having a high sugar content in plants means that bioethanol production has a lot of capability. Different pretreatment methods can be utilized in the conversion of sugar to ethanol, such as hydrolysis. A larger sugar extraction can be achieved using these pretreatment procedures, which break down hemicellulose and lignin. physical pretreatment is a typical pretreatment method that is both simple and effective at breaking down lignin. Fermentation follows the pretreatment of the pretreated material. During the fermentation process, sugar is transformed into ethanol (Krishnan *et al*., 2020).

Bioethanol

Bioethanol fuel contributes significantly to environmental protection since it slows global warming and conserves fossil fuels. It is an alcohol produced from carbohydrates by fermentation. Producing bioethanol from biomass or waste is one method for reducing both crude oil use and environmental pollution. Additionally, lignocellulosic biomasses, such as maize, sugar, molasses, etc., derived from edible sources like trees and grasses are used to produce ethanol feedstocks. Both bioethanol and ethanol have the same physical and chemical properties, but they are made from different materials. In its pure state, bioethanol is indeed a clear, odorless, colorless liquid. It freezes at 112°C and boils at 78°C (Lau *et al*., 2009) **(Figure 1)**.

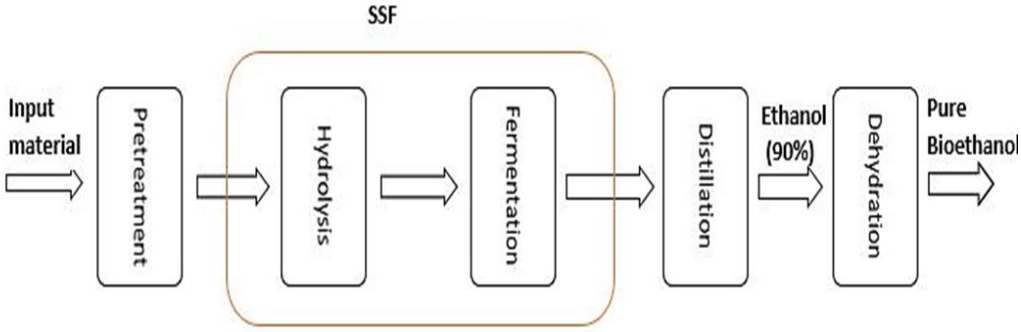

Figure 1. Production of bioethanol fuel schematic representation (Haq *et al*., 2016)

MATERIALS AND METHODS

Alternanthera Caracasana commonly known as the khaki weed was chosen as a raw material in this work because it is the most widely grown weed in southern states. Since this weed directly does not compete with the food market, it makes *A.caracasana* a good choice for bioethanol production. *A.caracasana* consists of cellulose, hemicellulose, lignin, organic compounds, and some trace compounds; it is a flowering plant species of the family Amaranthaceae it is called a tough weed in some places. *A.caracasana* weed – Samples were collected in and around the JSS AHER campus in December 2021 **(Figure 2)**.

a)

b)

Figure 2. *Alternanthera caracasana*

Pretreatment was carried out by cutting into tiny chunks and thereafter drying for about 7 days, then the dried sample was powdered in a grinder and the sample was preserved at room temperature in a sealed zip lock bag. The pretreatment and biofermentation process are shown in **Figure 3**. 5g of sample was weighed and to that 100 of 1% HCl (hydrochloric acid) was added, after that the sample was hydrolyzed in an autoclave at 105ºC for 15mins, and the hydrolyzed sample was filtered by filter paper with 110mm (Whatmann cat no 1033.110), under LAF(laminar airflow) to avoid further contamination of the sample. The filtered supernatant was cooled to room temperature and further carried out for fermentation. The yeast *S.carevisiae* (saccharomyces) or brewer's yeast was used for fermentation, for 100ml of sample; around 2g of yeast was added and incubated at 37ºC for 76 hours, and pH was adjusted before keeping for fermentation (Saadon *et al*., 2022).

Distillation is one of the fundamental sample purification techniques used virtually all around laboratories and industries. The liquid product is transferred to a distillation column to distill the alcohol to almost azeotrope levels. Filtered and distilled with the aid of a heat supply, the sample was set in a round bottom flask, condenser, and distillate flask, 100ml ethanol was selected, and it was distilled in the same round bottom flask by heating the ethanol. It is distilled at a boiling point of 78ºC at a temperature range of 20-30ºC, and the resulting distillate is collected. Nevertheless, this purity falls short of the customary minimum purity standard of 99 percent. Thus, the hydrous ethanol must undergo dehydration to become anhydrous ethanol. This was traditionally performed using a molecular sieve. The sample was collected in an airtight container and characterized for chemical analysis.

Analysis of reducing sugars

The reducing sugars recovered from *A.caracasana* were analyzed by the DNS method. 3, 5-dinitrosalicylic acid (2-hydroxy-3,5-dinitrobenzoic acid, DNS) combines with some reducing compounds as well as reducing sugars to absorb 540 nm light strongly. Forms as an aromatic compound (in the case of glucose). The free carboxyl (C = O) group presence is identified by this procedure, sometimes known as reducing sugar. This includes the ketone functional groups in fructose and, for example, the oxidation of aldehyde groups in glucose. In alkaline conditions, 3,5-dinitrosalicylic acid (DNS) is changed into 3-amino-5-nitrosalicylic acid at the same time.

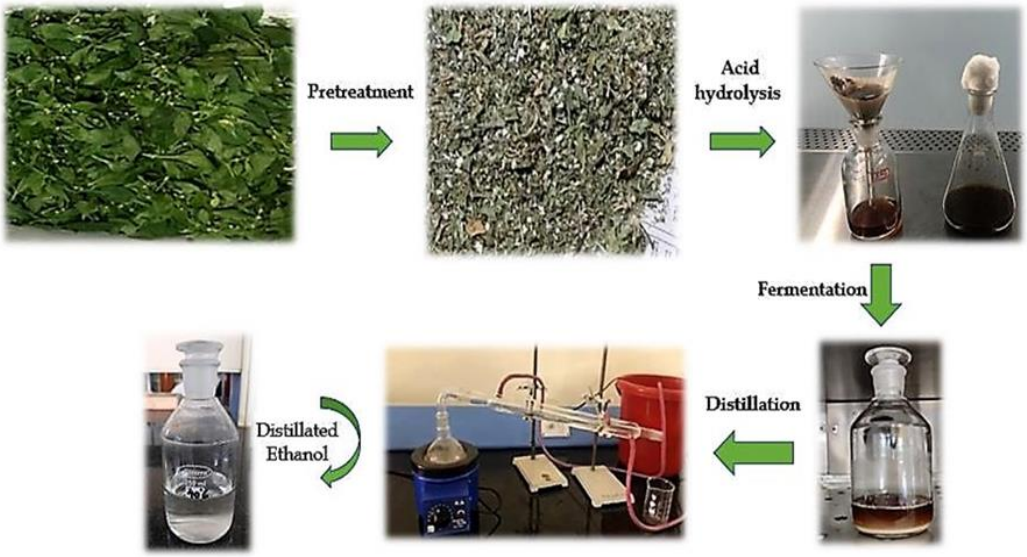

Figure 3. Schematic representation of the bioethanol process

RESULTS AND DISCUSSION

Sugar content analysis

The DNS method was used to analyze the sample's sugar content, here the minimum reaction time required for DNS to react was taken about 10-15mins at 100ºC; consequently, we concluded that 20min is the most effective time to stabilize the color development of the standard curve as shown in **Figure 4**. Now, as the amount of reduced sugar increases, so does the brightness of the orange color. At 540 nm, the optical density of this orange color could be measured. By measuring the absorbance of non-concentration of reducing sugar and

amplifying its optical density, a standard curve was made. Based on this standard curve, the concentration of undetermined reducing sugar was obtained.

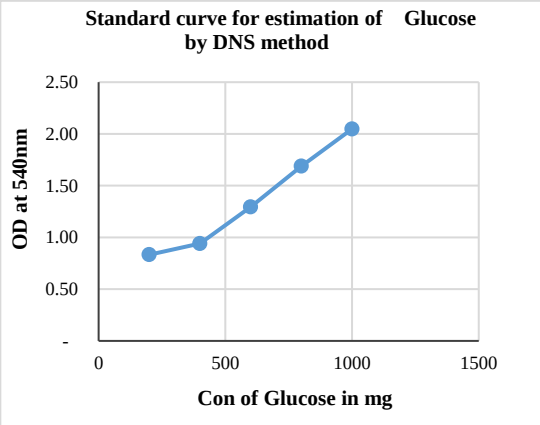

Figure 4. Graph indicating the presence of glucose content by DNS method.

This pretreated biomass was hydrolyzed such that the cellulose and hemicellulose polymers within lignocellulosic biomass, which can be fermented to ethanol, are broken down by dilute or concentrated acids. The acid hydrolysis was done by adding 1% Conc.HCL to 5g of pretreated biomass and was autoclaved for about 15 minutes at a temperature of 150ºC which resulted in 0.414mg of reducing sugars from 5g of sample.

Alcohol analysis

The sample supernatant analysis was carried out through a few identification tests for ethanol after autoclave and the results are presented in **Table 1**.

Table 1. Results compilation for identifying tests of ethanol

Sl.No.	Test Name	Result
1	Organoleptic	Volatile liquid with a characteristic pleasant odor
2	Miscibility	The sample is completely miscible with water
3	Boiling point	78ºC
4	pH	Neutral (7)
5	Specific gravity	0.9005 at 25ºC-30ºC
6	Combustion test	The pale blue color observed
7	Jones Oxidation	A green to blue color cloudy suspension is formed
8	Triiodomethane test	A pale yellow precipitate is observed
9	Density	23.500gms

FTIR

Fourier transforms infrared (FTIR) spectroscopy is a technique that employs a mathematical procedure (Fourier transform) to transform raw data (interference program) into a real spectral range. The Fourier transform infrared method is used to find out whether a sample lets or lets go of infrared light. It finds out if the sample has both inorganic and organic materials. Using spectroscopic data in automated spectroscopy software, major chemical groups in the sample will be determined from the range 600-4000cm^{-1} based on the infrared absorption frequency.

Two samples were analyzed in FTIR one is a predistillation sample and the other is distilled, the peak shape and its position in the corresponding region determined the functional group by Fourier transform infrared spectroscopy (FTIR) plot for bioethanol in the sample.

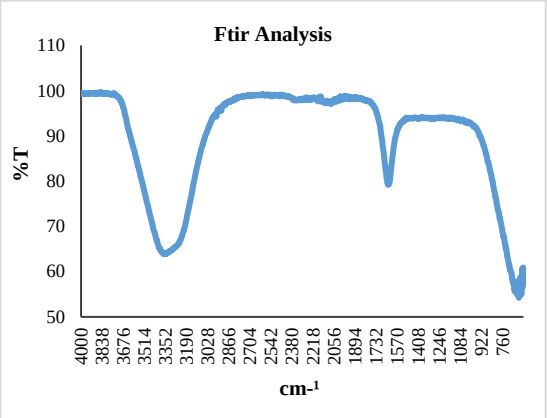

Figure 5. FTIR spectrum graph indicating the presence of functional compounds.

FTIR results obtained in this study showed some common peaks. The sample was measured in the frequency range 4000-450 cm^{-1}, with a spectral resolution of 50cm^{-1}. The spectra would be obtained with an average of 4 scans, analysis was performed on both the samples. The absorption spectrum in **Figure 5** and band in **Table 2**: Shows peaks between the range of 3350.57- 3350.17 cm^{-1} which indicates OH stretching vibrations, presence of alcohols, phenols, 1638.05-1637.23cm^{-1} indicates alkene group, 604-651 cm^{-1} indicates aryl and alkyl halides.

Table 2. FTIR absorption bands.

Frequency, cm^{-1}	Bond	Functional group
3350.57- 3350.17 cm^{-1}	O-H stretch	Alcohol/Phenol
1638.05-1637.23 cm^{-1}	C=C stretch	Alkene
604-651 cm^{-1}	C-l stretch	Aryl and Alkyl halides

GC-MS

A PerkinElmer Auto System XL gas chromatograph equipped with a quadrupole mass spectrometer (Model: Turbomass Gold, Waltham, MA, USA), and an RTx-5 Cross bond 5 percent diphenyl/ 95 percent dimethyl polysiloxane capillary column (30m x 0.25mm x 0.25mm film thickness; Restek, USA) was utilized to analyze an ethanolic extract of *A. caracassana* using gas. Helium gas was used as the carrier, and the gas chromatography and mass spectrometer conditions were 40°C for 2 minutes. For an injection volume of 1 L ramp was adjusted from 5 °C/min to 260 °C/min. The split ratio was 20:1, and the operation lasted 5 minutes. The intake line and source temperatures were 200°C and 180°C, respectively. 10 L of the sample was diluted in 990 L of n-Hexane. As a blank in sample preparation and GC-MS analysis, HPLC-grade n-hexane was

utilized. For chemical identification and quantification, the peak area percentage of the chromatograms was employed. Using the NIST 2011 Mass spectral library and their fragmentation pattern, the major constituents of the ethanolic extract were determined.

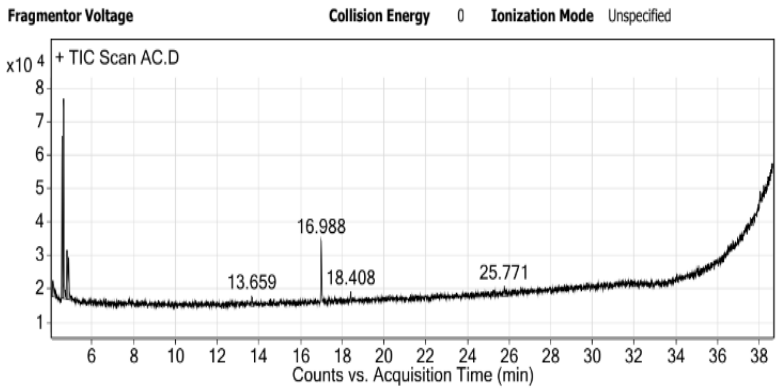

Figure 6. Graph of GCMS Analysis

Examination using GC–MS generally gives a complete profile of the phytochemical properties of ethanol extract is given in **Figure 6**. The major compound was 2-hexanone (100%) with minor compounds 3-hexanol (31.36%), 2-hexanol (32.46%), Propanoic acid, 3-hydroxy-, hydrazide (8.5%), 3-Isopropylbenzaldehyde (38.74%), Cyclohexasiloxane, dodecamethyl-(5.2%) and Amphetamine-3-methyl (19.12%) present. The retention time and percent area of compounds present in ethanol extract are listed in **Table 3**.

Table 3. Chemical composition of *A.caracasana* sample extract at different retention times.

Sl.No.	Compound Name	Retention Time	Area %
1.	2-Hexanone	4.642	100
2.	**3-Hexanol**	**4.802**	**31.36**
3.	**2-Hexanol**	**4.877**	**32.46**
4.	Propanoic-acid,3-hydroxy-, hydrazide	13.659	8.5
5.	3-Isopropylbenzaldehyde	16.988	38.74
6.	Cyclohexasiloxane, dodecamethyl	18.408	5.2
7.	Amphetamine-3-methyl	25.771	19.12

CONCLUSION

It is anticipated that lignocellulosic biomass will be one of the primary essential resources for the production of economically feasible bioethanol. Even though prospective bioethanol production from sugar and starch is higher than that from lignocellulose (g ethanol/g substrate), such conventional sources of energy are inadequate for global bioethanol production. Agricultural residues are regenerative, affordable, and abundant in this sense. Agricultural residues need not necessitate distinct energy, land, or water requirements, as they will not contain the same amount of nutrients. To make bioethanol production economically feasible, several challenges must be addressed.

Its four primary factors are feedstock, conversion technology, hydrolysis technique, and fermentation setup. Primary issues for feedstocks include cost, availability, handling, and harvesting. Concerning conversion technology, the challenges include biomass treatment, appropriate and cost-effective pre-treatment technology to extract associated hemicellulose and cellulose from the lignin compound, and biomass processing. The objective of a hydrolysis procedure is to design a method for depolymerizing cellulose as well as hemicellulose into highly concentrated fermentable monomers.

The actual purpose of this study is to, Identify a non-edible plant for the synthesis of bioethanol hence a commonly available tough weed that is grown in lots nearby roads, cleared areas, tracks, and in some places that are sandy, rough, and often well-traveled was selected. thus *A.caracasana* was chosen and the process included in bioethanol production was pretreatment where the weed was trimmed into small pieces naturally dried under sunlight and grinded. To enhance the efficiency of subsequent processing and decrease the crystallinity of cellulose. Later the sample was hydrolyzed at 105ºC for 15 minutes using hydrogen chloride and DNS method analysis was done for reducing sugars. where the content of glucose in the sample was estimated at 0.414mg at 540nm further it was fermented for about 76 hours at 37ºC and distilled using a simple distillation unit at boiling point 78ºC at a temperature range from 20-30ºC and distillate was collected. Yet, this purity falls short of the minimum purity criteria, which is normally 99 percent. To purify the hydrous ethanol into anhydrous ethanol, it is required to dehydrate the sample, Thus, this dehydration was done by using molecular sieves in the standard procedure.

Characterization of the distilled sample was done by FTIR where the sample was measured in the frequency range 4000-450 cm^{-1} with a spectral resolution of 50 cm^{-1}. In **Figure 5**, the peak range of 3350.57-3350.17 cm^{-1} indicates the presence of 63% of the OH group. And in the characterization of GC-MS minor compounds like 3-Hexanol (31.36%), and 2-Hexanol (32.46%) were observed.

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ETHICS STATEMENT: None

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