Ethiopian Journal of Natural and computational Science(EJNCS)

Ethiop J Nat Comp Sci 2023, Volume 3, Issue 2

Original Research Article

Bioethanol production from the plant *Impatiens tinctoria* A. Rich tuber by using *Saccharomyces cerevisiae* fermentation method

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Article History:

Received: March 2, 2023
Accepted: July 28, 2023
Published: August 10, 2023

Print ISSN 2710-0200
Electronic ISSN 2710-0219

ABSTRACT

The possibility of using bioethanol as an alternative fuel has piqued the interest of biotechnological ethanol production. It's critical to look into the possibilities of using new source of energy that are capable, like fuel that could be utilized instead of or in addition to present fuels and the bioethanol potential from the cellulosic material obtained from the plant *Impatiens tinctoria*, a rich tuber was investigated. For the plant *I. tinctoria* tuber, the impacts of several parameters that determine the concentration of bioethanol were examined. 76.73% w/w carbohydrate concentration were obtained from the pretearted lignoselulosic material which was treated by 2% diluted H2SO4 at 350C. The carbohydrate concentration were determined by phenol-sulphuric acid method. The optimized sample was fermented at a pH of 6.0, a reaction temperature of 32.5°C, and a fermentation time of 4 days, yielding a maximum ethanol level of 10.38% v/v as measured by Pycnometer. It may be concluded that the bioethanol produced from *I. tinctoria* (10.38%) under ideal conditions was highly promising and that it might be employed as a lignocellulosic feedstock for bioethanol production rather than food crops.

Keywords: Bioethanol, *Impatiens tinctoria*, *Saccharomyces cerevisiae*, fermentation, lignocellulosic

INTRODUCTION

Many countries, like Ethiopia, currently import a large amount of petroleum products. Benzene is one of the petroleum products that requires a large amount of foreign currency. There is an industry that mixes benzene with ethanol in a plausible proportion to reduce this problem

on a national scale. Furthermore, there is a requirement for ethanol at various levels (Benti et al., 2021).

People all over the world are dealing with two major issues: the rapid depletion of fossil-fuel reserves and uncontrolled environmental degradation. The possibility of using bioethanol as an alternative fuel has piqued the interest of biotechnological ethanol production. Recent economic and environmental interest has been fueled by price increases and environmental issues caused by fossil fuels.Roots, straw, and fruit peels are amongst the abundant lignocellulosic waste materials in the world (Soleimani, Ghasemi and Shokri, 2015).

Bioethanol can help us reduce our dependence on fossil fuels while reducing net emissions of carbondioxide, the greenhouse gase. Long-term economic and environmental concerns have prompted extensive study into renewable liquid fuel sources to replace fossil fuels. CO2 is released when carbon based fuels such as charkol and fuel are burned, which is the main supplier to climate change (Erdei et al., 2010). They burn cleaner than fossil fuels, and the short cycle of growing plants and consuming the fuel they produce does not emit CO2 (Tekaligne et al., 2015) (Kumar, P. et al., 2009). It's critical to look into the possibilities of employing new power supply that are effective like fuels that could be utilized instead of or in addition to present fuels (Tahir and Aftab, 2010). Bioethanol can be blended with conventional fuel in amounts as high as 5% without requiring engine modifications (Tekaligne, Woldu and Tsigie, 2015) (Talebnia, 2008).

These alternative energy sources should not come from edible sources, as this would increase the cost of these crops, resulting in food insecurity. To address these issues, it is suggested that other agricultural crops such as I. tinctoria tuber, which are not used for human or animal use, be researched.

Vast variety of researches assessed the potential of various plant sources for bioethanol production, playing a key role for growing of renewable energy alternatives. Previous research has investigated bioethanol production from different plant materials such as corn (Kumar and Singh, 2019), sugarcane (Cardona, Quintero and Paz, 2010), switchgrass (Qiao et al., 2012), and various lignocellulosic biomass sources (Bušić et al., 2018).

These studies have focused on optimizing fermentation processes, enzymatic hydrolysis techniques, and genetic engineering approaches to enhance ethanol yield and efficiency. However, despite the extensive research in this field, there remains a need to explore novel and underutilized plant sources for bioethanol production. I. tinctoria tuber, presents an intriguing potential due to its high carbohydrate content and fast growth characteristics. Moreover, I. tinctoria tuber is abundantly available and it is non edible, especially in regions with favorable climatic conditions, making it a promising candidate for bioethanol production (Gidamo, 2023). By focusing on this plant species, we aim to contribute to the diversification of feedstock options for bioethanol production and explore the feasibility of utilizing a previously overlooked resource for sustainable energy generation. The goal of this research study was to create bioethanol on a small scale in the lab and assess the species' potential. The consequences of various parameters are being investigated.

MATERIALS AND CHEMICALS

Materials

Materials used in this study were alcohol meter (Anton Paar Snap Alcohol Meter, Snap 41), incubator (Biosan BK-YC80B), Drying oven (Thermo Fisher Scientific Lab Ovens 50-2500C),

electrical grinder (TOSCANA 1000W), electrical balance (SUN-ACN-320N), UV-Vis spectrophotometer (Abron), fermentation Jar, fractional distillation apparatus, 50mL pycnometer, hydrometer (EISCO), pH meter (Adwa Hungary Jenco 6173, digital), heating mantle (Stuart™ HM2000C), and different size Erlenmeyer flask (pyrexed).

Chemicals

AR grade Methylene blue, Fehling reagents (A and B) Fisher schientific, sulfuric acid 98.08%, D (+) – glucose (anhydrous, FUJIFILM Wako), and calcium hydroxide (AR 96%), *Saccharomyces cerevisiae* yeast, sodium hydroxide (1.0N) which are mostly obtained from Loba chemicals.

Sample Collection and Processing

The plant *I. tinctoria* were collected from the fields of Gayint and Simada towns in South Gondar zone. The plant was collected after identification and authentication of the plant were made by one of the research group members from Biology department. The collected plant material was then transferred to the University of Gondar chemistry department. The sample were freshly washed on running water to remove dust maters.

Feed stock

The primary techniques employed in the production of ethanol from I. tinctoria tubers involved several steps to maximize ethanol yield and efficiency. Initially, samples of the tubers were collected, and then they underwent a pre-treatment phase to enhance their susceptibility to hydrolysis. This phase included grinding and milling the tubers to reduce their size, facilitating subsequent processes. Following pre-treatment, the resulting solution containing sugars underwent yeast fermentation to convert the sugars into ethanol. Subsequently, distillation was carried out to separate and concentrate the alcohol. Additionally, hydrolysis was employed to break down the cellulose and hemicellulose molecules into simpler sugars for further characterization. The entire process aimed at optimizing ethanol production from I. tinctoria tubers while ensuring efficient utilization of the biomass resource. In the sample preparation phase, 13kg of I. tinctoria tuber was manually processed, including size reduction, drying, and grinding. The tubers were meticulously chopped into 3-5 cm pieces with a knife, facilitating drying and grinding procedures conducted in the organic chemistry laboratory at the University of Gondar. These meticulous steps ensured the preparation of high-quality samples for subsequent ethanol production experiments.

After the sample was air dried the sample was chopped and turned to fine material with an electronic grinder. The ground mixed sample's maximum particle size is 1-2 mm. The sample with particles bigger than 2 mm was ground repeatedly until all particle sizes were 1-2 mm. Until the following part of the experiment, the sample ware was stored at room temperature. The surface area of the sample was increased by grinding I. tinctoria tuber into powder, which improves the cellulosic and hemicellulosic materials interaction with the acid, reducing cellulose crystallinity (Tekaligne et al., 2015) (Onuki, S. et al., 2005).

To increase the yield, a 0.5 % sulfuric acid solution was applied to 50g of each tiny I. tinctoria tuber sample in an erlenmeyer flask. For 1 hour, the combination was bought to 125 0c and 25 psi pressure on the heating mantle. The pretreated material was then dried again with at 35°C in an oven before being exposed to further investigation. The pretreatment's goals were to enhance the porocity of the materials by reducing cellulosic materilas and elimination of lignin. That was

confirmed by using the Benedict's test. It exhibits a color change from deep green to red, orange, and yellow (Mishra et al., 2020).

Determination of Moisture Content

The pretreated powder was oven-dried for 48 hours at 60 ^{0}C (to a moisture content of 15%). The oven-drying method was used to determine the moisture content of the samples and weighed. The technique was repeated until the weight remained consistent, indicating that it was free of moisture. After that, the moisture content will be estimated as follows:

$$\% \text{ Moisture content} = \frac{W1 - W2}{W1} \text{ X } 100 \qquad (1)$$

Where W1 is the sample's weight before drying, in grams, and W2 is the sample's weight after drying, in grams.

The first sample weighed 50 grams before drying; after drying in the oven, the sample weighs 42.5 grams.

The moisture content of the sample is converted to percent using this formula.

Moisture content = 50g-42.5g/42.5g/50g X 100 = 15%

Hydrolysis

Individual sugar molecules can be fermented into alcohol using diluted or concentrated acids to break down the cellulose and hemicellulose polymers in lignocellulosic biomass (Tekaligne, Woldu and Tsigie, 2015) (Bušić *et al.*, 2018).

In the triplicated experiment, a quantity of 50g of the pretreated I. tinctoria was utilized. The hydrolysis process was carried out for a duration ranging from 2 to 7 days, at various hydrolysis temperatures including room temperature, 27.5, 30, 32.5, 37.5, and 40 ^{0}C. Additionally, acid concentration varied from 0 to 4%, and the biomass concentration was measured in terms of %w/v, with values of 5.30, 6.14, 7.50, 10.00, 13.20, and 15.7.

The dilute acid hydrolysis method was used for the hydrolysis by introducing the substrate to the pretreated material. To prevent acid evaporation owing to heat, the liquid was placed into glass bottles and sealed with corck. The liquid fraction of the hydrolysate samples was filtered collected, and their sugar content was analyzed using the phenol-sulfuric acid method with D (+)-Glucose as a reference.

Determination of carbohydrate concentration using the Phenol-sulfuric acid method

Glucose is dehydrated to hydroxymethyl furfural in a hot acidic medium and produces a yellow brown coloured result when phenol is added with an absorption of 490nm. Among the quantitative assays for carbohydrate measurement, this method is the simplest and most dependable. (Krishnaveni et.al., 1984). The procedure is straightforward, practical, and the gives repeatable findings. The concentration of sugar is measured using UV-Visible spectroscopy at 490 nm using glucose as a standard for glucose absorbance, and the quantification is done using a calibration curve with glucose as the standard (Dubuois et al., 2008).

Standard and Reagent Solution Preparation

4 g of glucose was dissolved in 100 mL of water to make the stock solution. Then it's time to put together the typical functional solutions. Pipette out 1, 2, 3, 4, and 5 mL aliquots of stock glucose into a 100 mL volumetric flask and dilute to the desired concentration with distilled water. The total sugar content curve was created using known concentrations of standard glucose. The

standard solutions were made in five concentrations: 0.04, 0.08, 0.12, 0.16, and 0.2 g/ml. These concentrations were made by diluting a stock glucose solution.

5 grams of redistilled (reagent grade) phenol were dissolved in 100 milliliters of water. To make 96 % sulfuric acid from 97 % sulfuric acid, take 98.96 mL of 97 % sulfuric acid and mix it with 1.04 mL of distilled water (Krishnaveni et al., 1984).

To each test tube, 1mL of standard solutions was added to determine the calibration curve for standard glucose. Then 5mL of sulfuric acid and 1mL of phenol reagent were added. For 10 minutes, the mixture was left at room temperature. After shaking the contents in the tubes for 10 minutes, they were immersed in a water bath at the range of 25–30 °C for 20 minutes. Next, the absorbance was then measured using a UV-visible spectrophotometer at 490 nm (Abron). The total sugar content of the sample solution was determined using standard curve.

$$Y=AX+B \quad (2)$$

Y = the sample's absorbance, X = standard's concentration, A means the y intercept, and B means slope, and sample's the concentration are determined from the calibration curve using Equation. 3:

$$x = \frac{Y-B}{A} \quad (3)$$

The total sugar content of 50g of samples was determined and reported as gram glucose equivalents (GE) using Equ.5: G. Miliauskas et al., 2004.

$$C= \frac{XVDf}{m} \quad (4)$$

C = the amount of sugar content, g/g sample extract, V = volume extract, Df = dilution factor, m = weight of the sample, and X = concentration of glucose.

Fermentation

The pH value of the hydrolised solution was altered to a new pH value before introducing the yeast. Baker's yeast (*Saccharomyces cerevisiae*) was used to ferment the hydrolysates obtained from the pretreatment and acid hydrolyzed steam portion of *I. tinctoria*. To accommodate yeast development, the hydrolysates were filtered, and theNaOH (4M) nad HCl (2.5M) were applied to the sample to get varing fermentation medium with respect to pH. The pH of the medium was adjusted from 4 to 6.5. *Saccharomyces cerevisiae* was introduced at the intervals of 2, 3, 4, and 5 grams per 100 milliliters.

Fermentation was permitted for 2, 3, 4, and 5 days at room temperature at 27.5, 30, 32.5, 37.5, and 40 degrees Celsius. Using a hydrometer and a pycnometer, the ethanol concentration was calculated based on the density of alcohol distillate at 20°C and expressed in weight percent (w/v) (Igwe, C. et al., 2012).

To preserve anaerobic conditions, the flask mouths were firmly sealed with aluminum foil, with an opening to emit CO_2. Lime water was immersed to the opposite part of the output, which makes the lime water milky, to confirm CO_2 release. Confirmatory tests with K_2CrO_4 were performed to ensure that the distillate was indeed ethanol (Mandal, Kathale and Lecturer, 2012).

Distillation

The fractional distillation method was the final step in the *I. tinctoria* tuber trials' ethanol production. It's a technique for separating two liquids with differing boiling points. The

temperature of the distillation set up was set at 85^{0}C for 3 hours. However, numerous distillations are required to attain great purity. It is the process of purification.

RESULTS AND DISCUSSION

Biomass concentration effect on hydrolysis

The ramification of biomass concentration was examined at 35^{0}C for 72 hours. The greater sugar concentration was obtained at 74.35 %w/w by using the phenol-sulfuric acid method at a 10% biomass density. The concentration of sugar increased while increasing substrate concentration as it is shown in Table 1.

Table 1. Determination of total sugar concentration (% w/w) measured at different substrate concentrations, 2 % H_2SO_4, 3 days and 35^{o}C.

No.	Substrate concentration (%w/v)	Amount of sugar content (%w/w) Phenol sulfuric acid method	remark
1	5.30	41.41 ±0.00	
2	6.14	44.54±1.26	
3	7.50	59.33±1.96	
4	10.00	74.35±2.84	Very high
5	13.20	Out of range	Very high
6	15.70	Out of range	Very high

Effect of acid concentration on hydrolysis

Table 2 shows that phenol-sulfuric acid produced the highest sugar content of 76.73 %, utilizing a 2 % acid hydrolysate of I. tinctoria at 10 %w/v biomass concentration with the lowest yield at 0 % acid concentration (35 %). This demonstrates that 2 % sulfuric acid hydrolysis is more efficient in producing simple sugars than 1, 3, and 4 % sulfuric acid hydrolysis. According to the findings, the amount of sugar gained increases when the concentration of acids increases from 0-2 % to 2-4 % and reduces as the acid concentration climbs from 0-2 % to 2-4 %, according to the findings.

Table 2. Total sugar concentration (%w/w) by varaying H2SO4 concentration for 72 hours at 35 ^{o}C.

No.	Acid concentration (%v/v)	Amount of sugar content (%w/w) Phenol sulfuric acid method	remark
1	0	32.22±1.43	minimum
2	1	68.54±0.97	average
3	2	76.73±0.07	maximum
4	3	62.58±1.29	average
5	4	35.11±2.28	minimum

Fermentation parameters

Numerous variables, including temperature, extent of yeast given, pH and reaction time of reaction, play crucial roles as variables. These parameters significantly impact the bioethanol

production process and are essential for its successful completion. To assess the effects of these variables, the density of the experimental results was measured using a hydrometer and a pyrometer. Subsequently, the alcoholic content of the bioethanol produced was calculated based on these measurements. The ethanol yield was measured at 4g/L yeast extract, pH 6 and 32.5 0c. This comprehensive approach allows for a thorough understanding of the fermentation process and its outcomes in terms of bioethanol production.

Table 3. The yield of ethanol obtained from hydrometer and pycnometer readings using a varaying fermentation time.

No. of days	Specific Gravity		Ethanol yield (%v/v)	
	Hydrometer reading	Pycnometer reading	Hydrometer value	Pycnometer value
2	0.994±0.001	0.9924±0.0011	6.42	6.51
3	0.981±0.003	0.9930±0.0016	8.47	8.71
4	0.986±0.005	0.9899±0.0013	10.16	10.38
5	0.988±0.010	0.9911±0.0011	9.83	10.12
6	0.991±0.004	0.9915±0.0015	9.24	9.49
7	0.997±0.006	0.994±0.0001	8.76	8.92

DISCUSSION

Distillation yields were determined, and the concentration of ethanol (% v/v) was measured using a hydrometer and a pycnometer, taking into account various parameters.

Fermentation time, pH, and temperature are the most important factors in increasing bioethanol output. It was discovered that 35°C and a pH of 6 were the best conditions for obtaining a high bioethanol output. Table 3 and the following chart (Figure 1) shows the outcomes of fermentations held within different days.

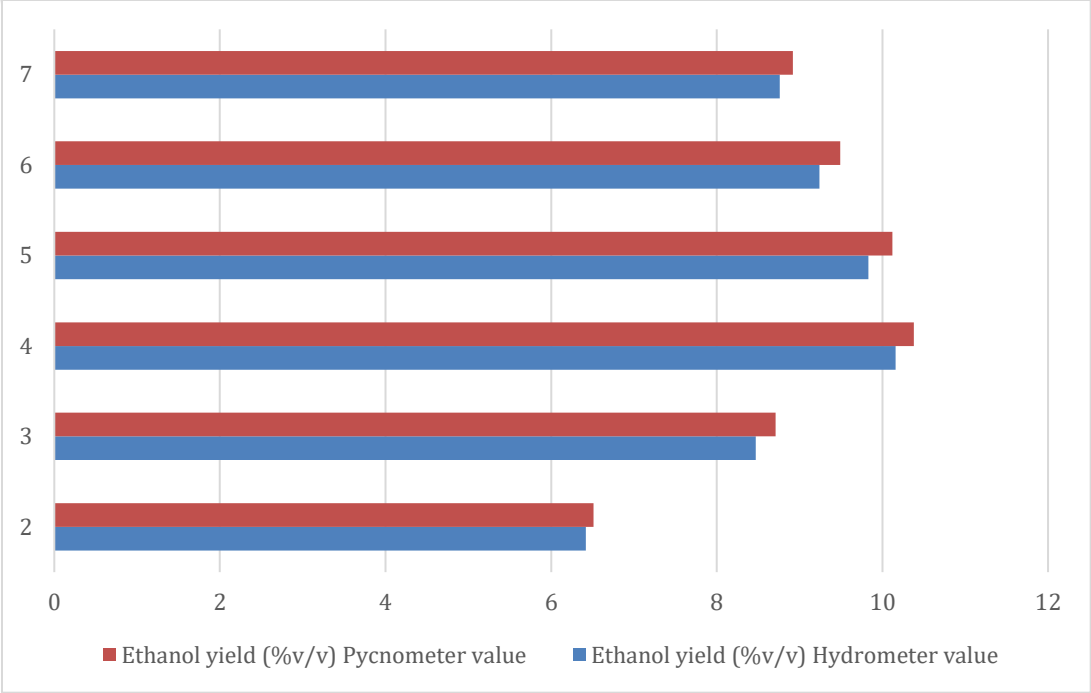

Figure 1. The yield of ethanol obtained from hydrometer and pycnometer readings using a varying fermentation time.

This chart (Figure 1) and Table 3 shows that after 4 days of fermentation, the highest amount of ethanol was produced (10.16 and 10.38 % from Hydrometer and Pycnometer measurements, respectively). As the time period was extended, the amount of ethanol produced decreased. The difference between the alcoholic content measured with a Hydrometer and a Pycnometer isn't substantial. The concentration of bioethanol increased as fermentation time increased, then fell as fermentation time increased. According to Table 3 and the above chart, increasing the fermentation days to 6 and 7 days reduces the concentration of bioethanol production to 9.24 and 8.76, respectively, when measured using a hydrometer. The bioethanol yield began to decline, after four fermentation days. It could be owing microorganisms' use of starch for ethanol generation. It could also be because the hydrolysate contains high quantities of metabolic inhibitors that can prevent fermentation. In comparison to the other ethanol ethanol yields obtained from edible sources and by byproducts in the same hydrolysis way, this sudy showed higher ethanol product. From maize cobs and groundnut shells 8% (Akpan *et al.*, 2005), rice husk 3.802% (Osuigwe, Bolade and Agboola, 2018) and 1.033% from mango peel and 1.1% from plantain peel (Jagessar *et al.*, 2023) The amount of ethanol produced by hydrolyzing *I. tinctoria* under optimal conditions (10.16 and 10.38 % from Hydrometer and Pycnometer measurements, respectively) was extremely good. The results suggests that ethanol produced from *I. tinctoria* may be used in place of other agricultural products such as corn, cassava, and mango juice. *I. tinctoria* can prevent food disaster by taking the place of food crops in the manufacture of bioethanol because it is not a plant that is edible to humans.

CONCLUSION

According to the findings of the study, *I. tinctoria* is a potential source of carbohydrates or sugar, which is a necessary prerequisite for the production of bioethanol from plants. The

production of bioethanol from plants is influenced by a variety of factors. The primary parameters that impact the yield of bioethanol generated are fermentation duration, acid concentration, temperature, and pH.

Thee concentration of ethanol obtained by hydrolysis of the *I. tinctoria* using optimum conditions (10.16 and 10.38 % from Hydrometer and Pycnometer measurements, respectively) was highly satisfactory.

RECOMMENDATIONS

Even though the plant *L. tinctoria* has higher sugar concentration the yield of bio ethanol is minimum. To achieve greater yield of bio ethanol product there should be optimized condition related to temperature, PH, acid concentration and fermentation time. In addition to these, further elemental and impurity analysis should be conducted to determine the quality of bioethanol produced from the plant.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the University of Gondar for financial support.

Author contributions

The main manuscript was evaluated and written by Birhanu Ayalew, the first and corresponding author. The article and data were evaluated by Ayalew Temesgen, the second author. The remaining writers contributed significantly to various aspects of the study. The final manuscript was read and approved by all members.

Competing interests

The authors state that they have no known competing financial interests or personal ties that could have influenced the research presented in this study.

REFERENCES

Ali, M.N. and Khan, M. (2014). Production of bioethanol fuel from renewable agrobased cellulosic wastes and waste news papers, *Int. J. Engin Sci Techno.*, 3(2): 884-889

Akpan, U.G., Kovo A.S., Abdullahi, M., and Ijah, J. J. (2005). The production of Ethanol from Maize Cobs and Groundnut Shells. AU J.T., 9(2): 106-110

Benti, N.E., Gurmesa, G.S., Argaw, T., Aneseyee, A.B., Gunta, S., Kassahun, G.B., Aga, G.S. and Asfaw, A.A. (2021). The current status, challenges and prospects of using biomass energy in Ethiopia. *Biotechnology for Biofuels*, 14(1): 1-24. doi:https://doi.org/10.1186/s13068-021-02060-3.

Bušić, A., Marđetko, N., Kundas, S., Morzak, G., Belskaya, H., Ivančić Šantek, M., Komes, D., Novak, S. and Šantek, B. (2018). Bioethanol Production from Renewable Raw Materials and its Separation and Purification: a Review. *Food Technology and Biotechnology*, 56(3): 289-311. doi:https://doi.org/10.17113/ftb.56.03.18.5546.

Cardona, C.A., Quintero, J.A. and Paz, I.C. (2010). Production of bioethanol from sugarcane bagasse: Status and perspectives. *Bioresource Technology*, 101(13): 4754–4766. doi:https://doi.org/10.1016/j.biortech.2009.10.097.

DUBOIS, M., GILLES, K., HAMILTON, J.K., REBERS, P.A. and SMITH, F. (1951). A Colorimetric Method for the Determination of Sugars. *Nature*, 168: 167–167. doi:https://doi.org/10.1038/168167a0.

Erdei, B., Barta, Z., Sipos, B., Réczey, K., Galbe, M. and Zacchi, G. (2010). Ethanol production from mixtures of wheat straw and wheat meal. *Biotechnology for Biofuels*, 3(1): 1-9. doi:https://doi.org/10.1186/1754-6834-3-16.

Gizachew Haile Gidamo (2023). Antioxidant activity and mineral content of Impatiens tinctoria A. Rich (Ensosila) tuber, an Ethiopian medicinal plant. *Scientific Reports*, 13(1): 14998 doi:https://doi.org/10.1038/s41598-023-41824-9.

Jagessar, R.C, Garraway, R. (2023). The production of bioethanol from mango (Mangifera indica) peel and plantain (Musa paradisiaca) peel via water pretreatment, dilute acid hydrolysis and fermentation supported by Saccharomyces cerevisiae. *American Journal of Research Communication.* Vol 11(4): 1-21 www.usa-journals.com, ISSN: 2325-4076.

Kumar, D. and Singh, V. (2019). Bioethanol Production From Corn. Corn, 3rd edn. *Elsevier*. pp.615–631. doi:https://doi.org/10.1016/B978-0-12-811971-6.00022-X.

Kumar, P., Barrett, D.M., Delwiche, M.J. and Stroeve, P. (2009). Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Ind. Eng. Chem. Res.,* 48(8): 3713–3729. doi:https://doi.org/10.1021/ie801542g.

Krishnaveni, S., Balasubramanian, T. and Sadasivam, S. (1984). Sugar distribution in sweet stalk sorghum. *Food Chemistry*, 15(3): 229–232. doi:https://doi.org/10.1016/0308-8146(84)90007-4.

Mandal, P. and Kathale, N. (2012) Production of ethanol from Mahua flower (Madhuca latifolia L.) using Sacchatomyces serevisiae. *ABHINAV*, 1(9): 6–10.

Mishra, M.S., B, C., Chatterjee, T. and Singh, K. (2011). Production of Bioethanol from Jatropha oil seed cakes via dilute acid hydrolysis and Fermentation by Saccharomyces cerevisiae. *Int. J. Biotechnol. Appl.,* 3(1): 41–47. doi:https://doi.org/10.9735/0975-2943.3.1.41-47.

Martín, M. and Grossmann, I.E. (2011). Energy optimization of bioethanol production via gasification of switchgrass. *AIChE Journal*, 57(12): 3408–3428. doi:https://doi.org/10.1002/aic.12544.

L. Veeranjaneya Reddy and O. Vijaya Sarathi Reddy (2007). Production of Ethanol from Mango (Mangifera indica L.) Fruit Juice Fermentation. *Research Journal of Microbiology*, 2(10): 763–769. doi:https://doi.org/10.3923/jm.2007.763.769.

Osuigwe, J. M., and Bolade O.A. (2018). Bioethanol production from rice husk using different pretreatments and fermentation conditions. *3 Biotech*, 8(1):15. doi: 10.1007/s13205-017-1033-x.

Shiva Soleimani (2012). Ethanol production by Zymomonas mobilis PTCC 1718 using low cost substrates. *Afri.J. Microbiol. Res.,* 6(4): 704-712. doi:https://doi.org/10.5897/ajmr11.576.

Shinnosuke , O., Jacek A. , K., J. Hans, van L., William S. , J., David , G. and Lingshuang , C. (2008). Ethanol production, purification, and analysis techniques: a review. In: ASABE Engineering a Sustainable Future. *2008 ASABE Annual International Meeting . ASABE*, p.085136.

Arifa Tahir, Madiha Aftab and Tasnim farasat (2010). Effect of Cultural Conditions on Ethanol Production by Locally Isolated Saccharomyces cerevisiae BIO-07. *J. Appl. Pharm.,* 3(2): 72–78. doi:https://doi.org/10.21065/19204159.2.72.

Talebnia, F., Pourbafrani, M., Lundin, M., Taherzadeh, M.J., (2008). Optimization Study of Citrus Waste Saccharification by Dilute-acid Hydrolysis, *BioResources*, 3(1): 108-122.

Tekaligne, T.M., Woldu, A.R. and Tsigie, Y.A. (2015). Bioethanol production from finger millet (Eleusine coracana) straw. *Ethiopian Journal of Science and Technology*, 8(1): 1-14 doi:https://doi.org/10.4314/ejst.v8i1.1.