The Applied Biology & Chemistry Journal
https://www.theabcjournal.com
Original Research
eISSN: 2582-8789

Production of bioethanol from amla (*Emblica officinalis* Gaertn.)

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Received March 28, 2021
Revised July 01, 2021
Accepted July 10, 2021
Published September 15, 2021

Abstract: On account of the increasing demand for valuable herbal products, an attempt was made to produce a functional fermented Ethanol from Amla. This study investigates the potential of ethanol production from Amla *(Emblica officinalis* Gaertn*)*. In the present study, Amla juice was extracted, filtered, fermented and it shows a suitable medium for the growth of *Saccharomyces cerevisiae* on yeast peptone dextrose medium for the production of ethanol. Ethanol was separated by fractional distillation and then estimated at 4, 6, 8 and 10 days of the fermentation process by iodometric method for 30ºC. The ethanol percentage estimated by the iodometric titration method was high on the 10th day, and it was found to be 1.63 gm% compared to all days. So, the outcome of this study reveals that amla fruit can be used as a crucial constituent for the yield of ethanol with a higher commercial value.

Keywords: amla; *Emblica officinalis* Gaertn.; ethanol; fractional distillation; iodometric method; solid-state fermentation

1. Introduction

Over the last several decades, biofuel production trends from biomass are gaining attention to face the expected energy crisis of the planet. Ethanol is generally used as biofuel and is produced due to the fermentation process from sugars, starches or cellulose biomass, including fruit wastes [1, 2]. Ethanol has been in use as an alternative fuel for a long time, and these days, it is broadly used as biofuel in the transport sector [3]. Since the 1980s, the curiosity to use bioethanol has increased, and it is currently being used in several countries. Bioethanol can be classified into three groups based upon the feedstock from which it has been derived. First-generation bioethanol is obtained from the feedstock rich in sucrose (sweet sorghum, sugarcane, sugar beet and other fruits) or starch (potato, wheat, corn, rice, sweet potato, cassava and barley). Second-generation, bioethanol is produced from lignocellulose biomass such as straw, bagasse, grasses, wood and other agricultural residues. Third-generation bioethanol is derived from algal biomass, which includes aquatic photosynthetic plant-like organisms [4]. Amla is understood as Indian gooseberry (*Emblica officinalis* Gaertn), is indigenous, belongs to the family Euphorbiaceae in the Indian subcontinent and a crop of economic significance. Within the world, India ranks first in the area and production of this crop [5]. The fruit carries many polyphenolic substances, which has antioxidant property and good radical scavenging activity [6]. The fruit contains alkaloids (Phyllantidine and phyllantine), auxins and minerals. About 500-700 mg/100 gm of pulp are often extracted from this fruit and may be utilised in several medicines. This fruit contains a high percentage of vitamin C, tannin, medicinal properties, and mineral contents of amla, which offers enormous processing scope. All the valuable natural constituents of amla, Indian gooseberry, *E. officinalis* Gaertn., with curative value, are often simply isolated in water after dispensing the berries in hot water. Enriching the extract with the sugar made it a suitable medium for growing *Saccharomyces cerevisiae* and fermenting the sugar into ethanol [7]. For a successful fermentation, the nutritional requirement of the microorganisms for their growth must be met by the amla as a substrate being fermented. Since, the challenge for ethanol manufacturing is increasing, various non-conventional raw materials are being investigated for this purpose [8, 9]. Microorganisms like yeasts play a vital role in the making of ethanol by fermenting a vast array of sugars to ethanol. These have been used for brewing the beer for thousands of years and are perhaps the oldest domesticated organism [10]. So, this information has boosted us to explore amla as the potential substrate for ethanol production employing fermentation process.

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2. Experimental

2.1 Collection of fruits

The amla fruits used in this study were collected from the local market (Sakkardara Market, Nagpur) and juice vendors, washed in sterile distilled water and air-dried. The fresh fruit was used within 1 hour after collection.

2.2 Preparation and extraction of sample of amla juice

The amla fruit was grated and the seeds were separated. The juice from the grated remainder was obtained by squeezing it in a sterilised muslin cloth. About 200 ml juice was obtained from 400 gm of amla. The juice was then stored in an airtight bottle to prevent invasion of any kind. As per the experiment, fresh juice was used.

Citation: Deolekar MA, Shende T (2021). Production of bioethanol from amla (*Emblica officinalis* Gaertn.). *T Appl. Biol. Chem. J*; 2(3):66-69. https://doi.org/10.52679/tabcj.2021.0011

2.3 Microorganisms

Saccharomyces cerevisiae (NCIM 3227) (Baker's yeast) were obtained from (National Chemical Laboratory; NCL), Pune and was used for fermentation. The selection of given yeast was made due to their ability to produce a higher amount of alcohol by fermenting sugars. It provides a significant yield of alcohol when fermented under optimal conditions. Maintenance of pure culture of *S. cerevisiae* was done by sub-culturing with the respect to time period in Saboraud dextrose broth. The broth was inoculated on Saboraud's dextrose agar slants for 2-3 days. On incubation for 2-3 days at 30°C, white colonies of *S. cerevisiae* were observed, which was confirmed by simple crystal violet staining.

2.4 Preparation of the inoculum

The yeast peptone dextrose (YPD) medium containing yeast extract, peptone and dextrose was inoculated with 1% of *Saccharomyces cerevisiae* and incubated for 4-5 days at 30°C and used as inoculum for solid-state fermentation.

2.5 Solid-state fermentation

200 ml of amla juice was taken into four different flasks and were sterilised by autoclaving. 1% of inoculum was added under aseptic condition. The flask was incubated for four days, six days, eight days and ten days respectively in each flask containing sterilised fermentation medium containing $(NH_4)_2SO_4$, $MgSO_4$ and KH_2PO_4. The flasks were incubated under the static condition at room temperature (30°C). The flasks were sealed to maintain the anaerobic conditions and kept in stable form under solid-state fermentation till each day of the interval [11]. Ethanol was estimated after the 4^{th}, 6^{th},8^{th} and 10^{th} day interval of the incubation period of the fermentation process by the iodometric method**.**

2.6 Separation of ethanol by fractional distillation

Fractional distillation method was used to separate alcohol (ethanol) and water. This system relies on the very fact that the compounds within the mixture have different boiling points. As ethanol boiled at a lower temperature (78.5°C) than water, the alcohol vapourised while nearly all water remains a liquid. A good distillation column produced a mixture of 95% alcohol and 5% water. This ratio represents the purest form of ethanol possible with distillation and is broadly accepted as an industry standard. The fermented mixture was poured into the round bottom flask. The fractional distillation apparatus was assembled by attaching the fractioning column to the round bottom flask. The condenser was attached to the fractioning column and placed in the distillate capturing flask to capture the distillate. Placed the Bunsen burner below the round-bottom flask and heated the mixture to above the boiling point of ethanol (about 80^0 C). Maintain the mixture at a constant temperature until the boiling has come to an end. At this point, the process of distillation was completed. This process was done on the 4^{th}, 6^{th}, 8^{th} and 10^{th} day, respectively, for each flask, and then further proceeded for the iodometric method of each flask after their interval days of incubation.

2.7 Estimation of ethanol yield

The total alcohol was determined by using the iodometric principle. For estimation of alcohol content, the following procedure was adopted. By the iodometric method, the percentage of ethanol contained in the flask was determined. In a clean, dry test tube, 1ml of distillate was taken (extract) from each flask and 5ml of $K_2Cr_2O_7$ was added. Now, it was kept in an ice bath for 5 min. Then concentrated H_2SO_4 was mixed slowly with continuous stirring. The flask was then kept in a boiling water bath for 5-7 min. Flasks were allowed to cool, and the contents were transferred into respective flasks, each containing 1 gm of potassium iodide. Flasks were washed with distilled water, and washing was added to the flask and mixed well. The flasks were kept stable for 5min. Then, in each flask, 1-2 drops of the starch indicator were added. The burette was filled with 0.05N thiosulphate, and it was used for the titration process. During the titration process, the colour changed to brown and reached the endpoint when the colour of the solution in each flask turned to pale yellow. Titration was repeated thrice for each flask, and the observations were noted. The percentage of ethanol was calculated by using the following formula $N_1V_1 = N_2V_2$.

$$Volume\ of\ dichromate\ solution = \frac{Normality\ of\ hypo\ solution\ x\ Volume\ of\ hypo\ solution}{Normality\ of\ dichromate\ solution}$$

3. Results and discussion

3.1 Simple staining for *S. cerevisiae*

The morphological characterization of yeast was subjected to simple staining (crystal violet staining) developed by Teresa Thiel. Spherical or Budding yeast cells were observed (**Figure 1**).

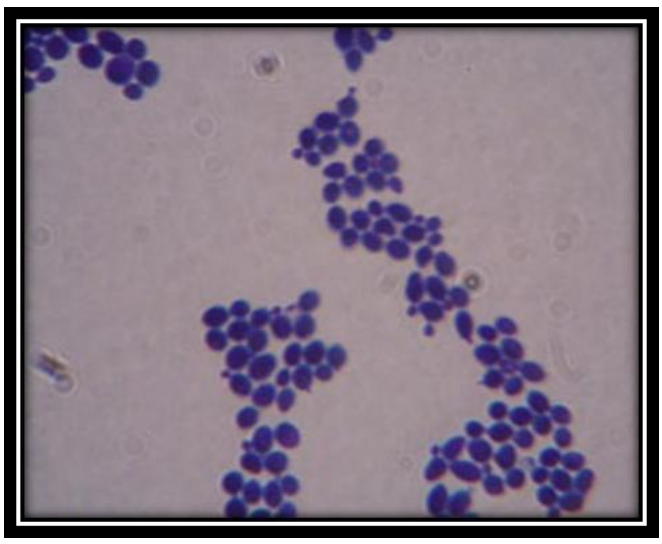

Figure 1: Showing *Saccharomyces cerevisiae* by crystal violet staining.

3.2 Ethanol yield

In the present investigation, the production of ethanol from amla fruit using *Saccharomyces cerevisiae*. The objective is to identify suitable for fermentation of amla for the yield of ethanol production. Amla used in this study was collected from local markets and fruit juice vendors. The Iodometric method was developed to estimate the production of bioethanol (**table 1**). It was found that there is no ethanol production till the 4th day; it was gradually increased after the 5th day; titration was done in which the flask containing distillate was treated with thiosulphate solution till the colour turned pale yellow. On the 6th day, it was found to be 1.59 gm%, followed by the 8th day 1.62 gm% and on the 10th day, it was found to be 1.63 gm% which was the highest yield of ethanol. The distillation process was carried on different days; **figure 2** represents the maximum yield of ethanol at the 10th day incubation period. An overview of the current study exhibited that the fermentation of amla fruit features a sizeable economic potential for bioconversion into ethanol. It is a modest and easy, high-yielding and economically achievable method.

Table 1: Percentage yield of ethanol from amla

Duration of Fermentation (in days)	Ethanol (gm%)
4	-
6	1.59
8	1.62
10	1.63

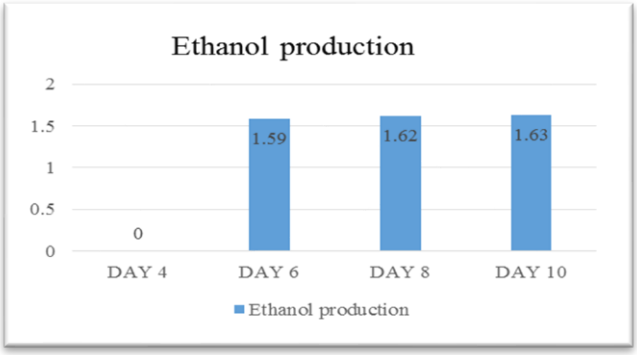

Figure 2: Bar graph showing the gm% yield of ethanol after different duration of fermentation.

The results correlated thereupon with the previous studies wherein different fungi were used for the bioconversion of ethanol. In a study, Karsch et al. (1993) demonstrated the potential of *Zymomonas mobilis* and *Saccharomyces cerevisiae* for aerobic and anaerobic ethanol production from glucose. The optimum values for ethanol production were 94% for *Z mobilis* and 88% for *S cerevisiae*, both under anaerobiosis with corresponding biomass production of 2.5 g/L and 6.5 g/L and total acid content of 16 mol/ml and 12 mol/ml respectively. In some cases, apple pomace was used to produce bioethanol employing three different fungal strains *viz. Saccharomyces cerevisiae*, *Aspergillus foetidus* and *Fusarium oxysporum.* The yield percentage of ethanol was determined by the iodometric titration method and was found to be the maximum (1.3702 gm%) with the combination of three fungi, *Saccharomyces cerevisiae*, *Aspergillus foetidus and Fusarium oxysporum*, in comparison to *S. cerevisiae* (1.326 gm%) and *A. foetidus* and *F. oxysporum* combinedly (1.292 gm%) [12]. In some cases, the solid-state fermentation (SSF) process was used for the efficient conversion of sugar substrate to bioethanol [13]; the same procedure was applied in the present study. In the present study, amla fruit was used as a noble raw material for ethanol mass with a high yield. Thus, amla fruit has enormous encouraging economic potential for bioconversion into ethanol.

4. Conclusion

The current study was conducted using amla fruit as a substrate for bioethanol production and the efficiency of locally isolated yeast (*Saccharomyces cerevisiae).* The iodometric titration estimation of the percentage yield of ethanol was 1.59 gm% on the 6th day and 8th day 1.62 gm%. The highest yield of ethanol was 1.63 gm% on the 10th day. Further studies related to ethanol production from amla fruit will exhibit substantial bio-based economic growth. The future perspective and goal of this research should focus on the investigation of the practicable use of Indian gooseberry for making ethanol through a simple method of titration. Using fruit carbohydrates for ethanol production is an outstanding illustration of the beneficial potential of fruit and fruit wastes for building a sustainable society.

Declarations

Acknowledgements: The authors are grateful to the Department of Microbiology, Kamla Nehru Mahavidyalaya and the management of Kamla Nehru Mahavidyalaya for providing all the needful facilities for the successful completion of the research project.

Author Contribution: MD has contributed to the conception of the presented idea for the article; MD and TS did literature search and data analysis; and both the authors have contributed in preparing the manuscript.

Funding: Not Applicable

Conflict of interest: No potential conflict of interest is being reported by the authors.

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