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Production of Bioethanol from *Cordia dichotoma* and *Physalis minima*

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ABSTRACT

The experiment was conducted from September, 2022 to June, 2024 in the Department of Food Safety and Quality Assurance, College of Food Science and Technology, Acharya N. G. Ranga Agricultural University, Pulivendula. Bioethanol, an eco-friendly and renewable biofuel, can be efficiently produced from agricultural and fruit wastes. The present study aimed to produce bioethanol from the fruits of Cordia dichotoma and Physalis minima, which are abundantly available, cost-effective, and underutilized. Twelve yeast isolates were obtained from fruit wastes such as banana, apple, sapota, jamun, jackfruit, dragon fruit, pineapple, pomegranate, grapes, guava, papaya, and mango, collected under sterile conditions. The isolates were identified as yeasts based on morphological and biochemical characteristics. Sugar utilization tests revealed that all isolates fermented glucose, fructose, and sucrose, while only some (CAY, MY, DFY, JY, PGY, SY, BY) utilized maltose. Among them, isolates CAY, MY, and BY showed the highest ethanol yields with reduced residual sugars during glucose fermentation and were selected for further fermentation studies using Physalis minima and Cordia dichotoma powders as substrates. The CAY isolate exhibited maximum fermentation efficiency, producing 18.644 g l^{-1} ethanol from Physalis minima and 28.024 g l^{-1} from Cordia dichotoma, with minimal residual sugars. These findings indicate that Cordia dichotoma and Physalis minima fruits can serve as promising, low-cost substrates for sustainable bioethanol production, contributing to renewable energy development and reducing reliance on fossil fuels.

KEYWORDS: Bioethanol, reducing sugars, *Cordia dichotoma*, *Physalis minima*, yeast

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Bioethanol is a sustainable energy source and clean-burning fuel, which gives higher thermal efficiency and power than conventional gasoline (Hansen et al., 1985). Overuse of fossil fuels and consequent environmental issues have become a concern recently for the production of alternative fuels. Microbial conversion of renewable cellulose and lignocellulosic feedstock to biofuel such as bioethanol is very effective in minimizing the problems associated with the world environment (Cho et al., 201, Vadalà et al., 2023). Industrialization and the continuous growth of the world population are the causes of the rapid utilization of fossil fuels as an energy source. This contributes to an increase in energy costs and the release of massive amounts of greenhouse gases that have adverse effects on the environment (Naik et al., 2010, Suri et al., 2022, Costa et al., 2019). *Physalis minima* fruit has various common names like Sunberry, Gooseberry, Ground cherry, and Wild Gooseberry, which are in the Solanaceae family. Organic matter-enriched soil is a suitable habitat for *Physalis minima* and was spread in the non-agricultural grasslands (Pradeepkumar et al., 2022). India is also one of the countries in which different plant-based medicinal fields, such as Siddha, Ayurveda, and Unani, are used for treatments. Since ancient times, the history of using medicinal plants in India has been for the treatment of various diseases. Plant-based compounds with diverse molecular structures are superior to synthetic chemical drugs. So, the research field is trying to identify new plant-based molecules with the structure for a novel drug design (Patwardhan et al., 2005; Newman et al., 2016). *Cordia dichotoma*, first belonging to the family Boranginaceae, is a medium-sized tree with a short, usually crooked trunk and bearing globose, grows in India, Sri Lanka, and other warmer countries. It is one of the traditionally medicinally important deciduous plants. The whole plant of *Cordia dichotoma* is used as food. Immature fruits are pickled and are also used as a vegetable mixture of flowers and curd, applied two times in a day, used to protect the body against heavy sun heat waves (Pawar et al., 2015, Jamkhande et al., 2013). The medicinal attributes of *Cordia dichotoma* have been known for a long time. The fruits of the plant have been used as cooling, astringent, emollient, expectorant, anthelmintic, purgative, and diuretic. Several pharmacological properties have been reported. *Cordia dichotoma* fruits show the presence of pyrrolizidine alkaloids, coumarins, flavonoids, saponins, terpenes, and sterols. The fruit contains about 70% pulp; the pulp contains 100 g: water 6 g, protein 35 g, fat 37 g, and carbohydrates 18 g. The seed contains 100 g: water 32 g, fat 46 g; the principal fatty acids are also present (Ganjare et al., 2019; Jamkhande et al., 2013; Patra and Basak, 2017).

Yeasts, particularly those belonging to the genus *Saccharomyces*-and most notably *Saccharomyces cerevisiae*-are the preferred microorganisms for industrial bioethanol production (Azhar et al., 2017). Among the various microorganisms capable of ethanol production, S. cerevisiae is recognized as the principal bioethanol producer, capable of generating up to 20% (v/v) ethanol through fermentation of different carbon sources (Coe et al., 2007). A review of the literature further emphasizes that pretreatment of cellulosic waste biomass is a crucial step in achieving high-quality bioethanol with efficient yields (Sarao et al., 2022, Boluda-Aguilar et al., 2010). Pretreatment processes may involve physical, chemical, physicochemical, or biological methods, and their effectiveness significantly influences both fermentation time and overall ethanol productivity (Choi et al., 2013, Kumar et al., 2009, Mahato et al., 2021). In this context, the present study was undertaken to develop a sustainable, laboratory-scale strategy for bioethanol production through fermentation of *Cordia dichotoma* and *Physalis minima* biomass.

2. MATERIALS AND METHODS

The experiment was conducted from September, 2022 to June, 2024 in the Department of Food Safety and Quality Assurance, College of Food Science and Technology, Acharya N. G. Ranga Agricultural University, Pulivendula, to study the production of bioethanol from the fruits of *Cordia dichotoma* and *Physalis minima*, which are abundantly available and cost-effective

2.1. Raw materials

A total of twelve fruits (banana, custard apple, dragon fruit, guava, grapes, jamun, jack fruit, mango, pineapple, papaya, pomegranate, sapota) waste were collected from the local markets of Pulivendula. *Physalis minima* was collected from agricultural lands and *Cordia dichotoma* was collected from local fields.

2.2. Collection of samples

Different fruit waste samples were collected from the local fruit juice market and used for the production of lactic acid by isolating the yeast cells.

2.2.1. Isolation of yeast isolates

The samples are diluted and streaked on a PDA plate and incubated at 37°C for 24 to 48 hrs. Colonies will be selected based on their morphological appearance and again, repeated streaking will be done on PDA plates to purify the isolates. The streaked plates will be incubated at 37°C for 24 to 48 hours (Niepel et al., 2017).

2.2.2. Identification of yeast cells

The morphology of yeast cells will be determined by Gram

staining and observed under the microscope, which shows oval and budding cells (Agarwal et al., 2011).

2.2.3. Preservation of yeast cells

The individual colonies will be stored in PDA agar plants or in glycerol and stored at 4°C for further analysis (Liang et al., 2023).

3. PHYSICOCHEMICAL PROPERTIES PHYSALIS MINIMA AND CORDIA DICHOTOMA

3.1. pH

The pH of *Physalis minima* and *Cordia dichotoma* was measured by using pH meter (Anonymous, 1990) TSS. The refractometer is used to measure the total soluble solids of food products. It is a sample instrument used for measuring the concentration of aqueous solutions such as gases, liquids, and translucent solids. The commonly used scale for a refractometer is the "Brix scale (Anonymous, 1990).

3.2. Moisture content

To moisture content of *Physalis minima* and *Cordia dichotoma* was estimated using by tray dryer.

Moisture content (%)=(Initial weight-Final weight/Initial weight)×100.

3.2.1. Estimation of reducing sugars

The reducing sugars were estimated by the Di-nitro salicylic acid (DNSA) method as described by Miller (1959).

3.3. Preparation of DNSA reagent

One gram of 3, 5-dinitrosalicylic acid was dissolved in a small amount of 2N NaOH and 30 g of sodium potassium tartrate was added and made up to 100 ml with 2N NaOH.

3.4. Preparation of a stock solution of glucose

The standard stock solution was prepared @ 1 mg ml^{-1} by dissolving 100 mg of D-glucose in distilled water and the final volume was made up to 100 ml of distilled water.

3.5. Procedure

The representative samples of 0.1 ml from each treatment and replication were taken into thin-walled boiling test tubes and 0.9 ml of distilled water was added. A reagent blank containing one ml of distilled water was also prepared. Similarly, standards were also included, ranging from 100 µg to 1000 µg concentration of glucose. DNSA reagent @ 0.5 ml was added to each sample, mixed well and kept in a boiling water bath for 5 minutes. The samples were cooled and the final volume was made up to 25 ml using a volumetric flask. Absorbance in terms of optical density of the standard and the samples was read at 540 nm using a UV-visible spectrophotometer.

3.6. Estimation of ethanol

Ethanol was estimated by the calorimetric method as described by Capture et al. (1968), further confirmed by Gas Chromatography (Wang and Li, 2003).

3.7. Preparation of the reagent

3.7.1. Potassium dichromate ($K_2Cr_2O_7$)

Potassium dichromate ($K_2Cr_2O_7$) of 34 g $K_2Cr_2O_7$ was dissolved in 500 ml of distilled water and 325 ml of sulphuric acid was added and the volume was made up to 1000 ml with distilled water to give 0.23N $k_2cr_20_7$.

3.7.2. Preparation of stock solution

A standard stock solution of 100 percent pure analytical grade (containing 789 mg ml^{-1}) ethanol was prepared by dissolving 12.6 ml of ethanol in 100 ml of distilled water, which resulted in 100 mg ml^{-1} of standard ethanol.

3.7.3. Procedure

One ml of the representative samples from each treatment was transferred to a 250 ml round-bottom distillation flask connected to the condenser and was distilled with 30ml of distilled water. The sample was distilled at 75°C. The distillate was collected in 25 ml of 0.23 N $K_2Cr_20_7$ reagent, which was kept at the receiving end. The distillate containing alcohol was collected till a total volume of 45 ml was obtained. Similarly, standards (20–100 mg ml^{-1} ethanol) were mixed with 25 ml of $K_2Cr_20_7$ separately. The distillate of samples and the standards were heated in a water bath at 60°C for 20 minutes and were cooled. The volume was made up to 50 ml with distilled water and the optical density was measured at 600 nm using a spectrophotometer. The standard curve was plotted considering the concentration against absorbance.

4. RESULTS AND DISCUSSION

This chapter contains the results obtained for isolation, identification and preservation of yeast isolates, physicochemical properties and production of bioethanol from *Physalis minima* and *Cordia dichotoma*. The obtained results were represented in suitable forms and discussed.

4.1. Isolation of yeast isolates

In this present, a total of 15 yeast isolates were isolated from 20 different fruit waste. All the isolates were cultured on PDA agar plates by the streak plate method and initially subjected to morphological characteristics. Ananth et al. (2019) reported that they isolated yeast from rotten fruits like papaya, pomegranate, grapes and milk.

4.2. Identification of yeast cells

Based on the morphological characteristics of yeast, among the 15 isolates, 12 isolates were found to be Gram-positive and oval in shape. The microscopic view of the identified isolates was a violet oval shape and budding. Pilap et al. (2022) reported that the colonies were chosen based on morphology, size and colour for purification.

4.3. Physico-chemical properties

4.3.1. pH

The pH in the *Physalis minima* was recorded as 5.8 and *Cordia dichotoma* was recorded as 6.0 (Table 1).

4.3.2. Moisture content

The moisture content of *Physalis minima* was recorded as 77.05% and *Cordia dichotoma* was recorded as 64.20% (Table 1).

4.3.3. Total soluble solids (TSS)

The total soluble solids recorded in *Physalis minima*, 11.5 Brix and *Cordia dichotoma* were 13 Brix, respectively (Table 1).

Table 1: Physico-chemical properties of *Cordia dichotoma* and *Physalis minima*

Sl. No.	Parameters	*Cordia dichotoma*	*Physalis minima*
1.	Moisture content (%)	64.20	77.05
2.	pH	6.0	5.8
3.	TSS (brix)	13	11.5
4.	Reducing sugars (mg g^{-1})	17.7	5.67

4.4. Reducing sugars

The reducing sugars recorded in *Physalis minima* were 5.67 mg g^{-1} and in *Cordia dichotoma* were 17.7 mg g^{-1} (Table 1).

4.5. Screening of yeast isolates for ethanol yield and residual reducing sugar from glucose fermentation

The best isolate from the sugar utilization test was used to screen for ethanol production by taking carbon as an energy source. The isolates are BY, CAY, MY, JY, PGY, DFY, and SY. Among the 7 isolates, CA showed the highest ethanol production, 38.47 g l^{-1} and the least residual reducing sugars, 2.35 g l^{-1} followed by BA and MA. The least ethanol production and high reducing sugars were shown by JY, PGY, DFY, and SY. Therefore, the three isolates were taken for bio-ethanol production from *Cordia dichotoma* and *Physalis minima* because of high ethanol production as shown in Table 2 and Figure 1. Pilap et al. (2022) stated that the fermented yeast strains were screened for their ability to ferment glucose using the Durrham tube assay.

Table 2: Screening of yeast isolates from glucose fermentation

Sl. No.	Yeast isolates	Ethanol yield (g l^{-1})	Residual reducing sugars (g l^{-1})
1.	CAY	38.463	2.347
2.	BAY	31.203	2.990
3.	MAY	30.513	2.800
4.	JAY	29.533	3.480
5.	DFY	6.597	9.270
6.	PGY	15.300	6.220
7.	SAY	6.437	9.373

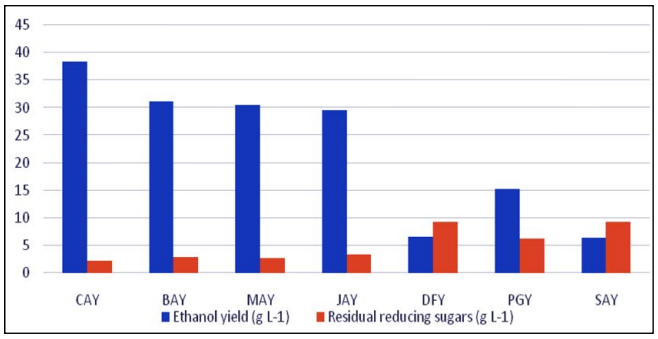

Figure 1: Graph for screening of yeast isolates

4.6. Production of bio-ethanol from fermented cordia dichotoma fruit powder

By taking the three best isolated form screening, i.e., CAY, BAY, and MAY, performed fermentation for 5 to 6 days. After 5 days of fermentation, the gas production was stopped, and we screened for bio-ethanol production. The maximum bio-ethanol production CAY recorded 28.02 g l^{-1} and less reducing sugars 3.37 g l^{-1} and bio-ethanol production in MAY (Table 3 and Figure 2). Further, the most efficient isolate CAY for bio-ethanol production was sent for molecular identification and identified as *Hanseinaspora guilliiermondii*. Similarly, Domingue et al. (2015) reported that the bioethanol production from sugarcane juice, coconut milk, pineapple juice, and tuna

Table 3: Production of bio-ethanol from *Cordia dichotoma*

Sl. No.	Yeast isolates	Ethanol yield (g l^{-1})	Residual reducing sugars (g l^{-1})
1.	CAY	28.024	3.37
2.	BAY	20.858	3.93
3.	MAY	18.972	4.72

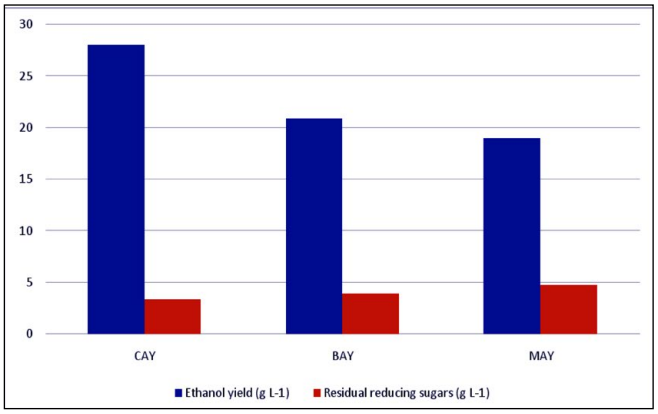

Figure 2: Graph for the production of bioethanol from *Cordia dichotoma*

juice as carbon sources for bio-ethanol production using *Saccharomyces cerevisiae*.

4.7. Production of bio-ethanol from fermented Physalis minima

By taking the three best isolated form screening, i.e., CAY, BAY, and MAY, we performed fermentation for 5 to 6 days. After 5 days of fermentation, the gas production was stopped, and we screened for bio-ethanol production. The maximum ethanol production recorded in CAY is 18.64 g l^{-1}, and less bioethanol production was recorded in the MAY isolate (Table 4 and Figure 3). Evcan et al. (2015) reported the bio-ethanol production from apple pomace using *Saccharomyces cerevisiae*.

Table 4: Production of bio-ethanol from *physalis minima*

Sl. No.	Yeast isolates	Ethanol yield (g l^{-1})	Residual reducing sugars (g l^{-1})
1.	CAY	18.644	2.840
2.	MAY	8.202	4.904
3.	BAY	18.184	2.488

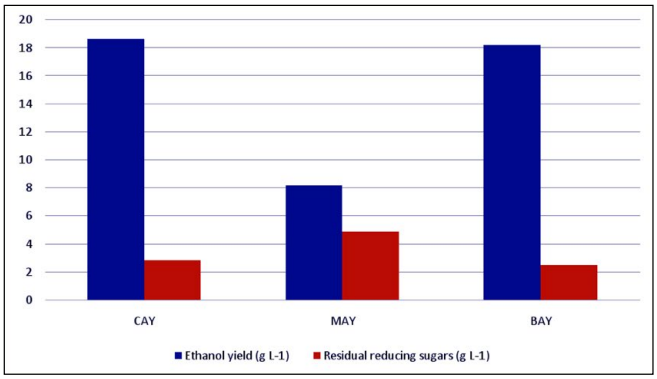

Figure 3: Graph for the production of bioethanol from *Physalis minima*

4. CONCLUSION

Based on the present study, twelve yeast isolates were obtained from various fruit wastes and identified as yeasts through morphological and biochemical characterization. Among them, seven isolates were evaluated for glucose utilization, showing significant variation in ethanol production. The CAY isolate showed the highest ethanol yield, producing 18.64 g l^{-1} from *Physalis minima* and 22.02 g l^{-1} from *Cordia dichotoma*. These results indicated that *H. guilliermondii* (CAY) was an efficient and sustainable strain for bioethanol production from underutilized fruit wastes.

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