***Original Research Article***

**Sustainable Biodiesel Production from Aphanamixis polystachya (Pithraj) Seeds: A Promising Non-Edible Feedstock for Renewable Energy**

**Abstract: -**

This study investigates the feasibility of producing biodiesel from the non-edible seeds of Aphanamixis polystachya (Pithraj) seed emphasizing its potential as a sustainable feedstock for renewable energy. The extracted oil underwent a two-step transesterification process to yield biodiesel, which was then characterized for its physicochemical properties, including density, viscosity, pour point, acid value, and calorific value. “Gas Chromatography–Mass Spectrometry (GC-MS)” analysis identified diethyl phthalate as a significant component, with a retention time of 18.932 minutes, contributing to 22.35% of the total chromatographic area. The biodiesel exhibited a density of 0.84 g/cm³ at 15°C, a pour point below -18°C, an acid value of 0.41 mg KOH/g, and a calorific value of 38.90 MJ/kg. These properties align well with international biodiesel standards, suggesting that *Aphanamixis polystachya* seed oil is a promising non-edible feedstock for sustainable biodiesel production.

**Keywords:** *Aphanamixis polystachya*, Pithraj, biodiesel, diethyl phthalate, GC-MS, renewable energy

**Introduction**

The escalating global demand for energy, coupled with growing concerns over environmental degradation and fossil fuel depletion, has intensified the search for alternative, sustainable energy sources. Biodiesel, a renewable, biodegradable, and non-toxic substitute for petroleum diesel, has emerged as a pivotal solution. It offers substantial environmental advantages, including reduced greenhouse gas emissions and biodegradability, while simultaneously promoting energy security and rural economic development.

Among the vast array of biodiesel feedstocks, this trend is supported by recent reviews highlighting the environmental and socio-economic advantages of using underutilized non-edible oils for biodiesel production [1, 2, 3]. Non-edible plant oils are gaining traction due to their ability to avoid the food-vs-fuel conflict. One such promising candidate is the Pithraj tree (*Aphanamixis polystachya*), a deciduous species native to South and Southeast Asia, known for its high oil content and widespread availability in marginal lands. Its seeds, often underutilized, possess considerable potential as a non-edible feedstock for biodiesel production.

In terms of the environment, society, and economics, biodiesel has the potential to support worldwide sustainable development. Studies further confirm that biodiesel from non-edible feedstocks contributes to emission reduction and enhances energy resilience in developing regions [4, 5].The development of biodiesel is complicated because it involves the possibility of external issues that could jeopardize sustainable development. Every nation's development depends heavily on its supply of energy, and as part of their sustainable development strategy, nations can expand their energy portfolios to include biodiesel as a viable choice. Positive socioeconomic effects from the production and use of biodiesel as an alternative fuel may eventually result in sustainable development. Biodiesel, produced from renewable and sustainable feedstocks such as plant-derived oils, is both **biodegradable and non-toxic to the environment**, offering a cleaner alternative to fossil fuels[6] The development of biodiesel has the potential to yield socioeconomic benefits for rural communities and agricultural sectors specifically [7] Advancements in green technologies play a pivotal role in addressing environmental challenges [8] by fostering sustainable economic progress and enhancing environmental stewardship.[9] It has been observed that developed countries have significantly improved environmental quality through the adoption of advanced technologies, which have contributed to substantial reductions in pollution. In the context of the United States, the adoption of green energy sources is seen as an effective way to reduce environmental degradation. Studies also highlight that while fossil fuels continue to elevate emissions, the use of biomass energy in transportation can significantly cut down environmental impact. To successfully transition away from fossil fuels, it is essential to formulate strategies for the large-scale production of biomass-based transport fuels—such as methanol, ethanol, bio-crude, biodiesel, and methane—along with offering economic incentives to make these alternatives viable and attractive.[10] edible oils are used in almost of the methods used to produce biodiesel [11]

This study explores the viability of Aphanamixis polystachya (Pithraj) seed oil as a sustainable, non-edible feedstock for biodiesel production through Soxhlet extraction and a two-step acid- and base-catalyzed transesterification process. It aims to characterize the physicochemical properties of the resulting biodiesel, benchmark them against international fuel standards (ASTM D6751 and EN 14214), and identify key chemical constituents using Gas Chromatography–Mass Spectrometry (GC-MS). Additionally, the study focuses on optimizing reaction parameters to maximize biodiesel yield and quality. The significance of this research lies in its contribution to sustainable energy development by utilizing a renewable feedstock that does not interfere with food supply chains, promotes rural economic opportunities, and offers a cleaner alternative to fossil fuels. Despite growing interest in non-edible biodiesel sources, comprehensive studies on Pithraj seed oil remain scarce, particularly those involving standard compliance, optimized production parameters, and in-depth GC-MS profiling. This research addresses these gaps, reinforcing the potential of Pithraj oil as a promising candidate in the global shift toward renewable energy.

**Materials and Methods**

**2.1 Oil Extraction Process:** The seeds of *Aphanamixis polystachya* (Pithraj) were collected from Department of Biotechnology Campus, Rajarshi Shahu Mahavidyalaya, Latur, (Latitude**:** 18°24'51" N, **Longitude**: 76°32'4" E) Maharashtra. India Upon collection, the seeds were exposed to sunlight for several hours to remove surface moisture and eliminate insect infestation, a critical step in ensuring proper storage and oil yield [12]

**2.2 Seed Preparation:** Following sun-drying, the *Aphanamixis polystachya* (Pithraj) seeds were manually separated from their outer shells. They were then washed thoroughly with warm distilled water to eliminate dirt and other surface contaminants. The cleaned seeds were oven-dried at 55°C to remove residual moisture, as excessive water content can hinder solvent penetration during extraction [13]

**2.3 Soxhlet Extraction of Pithraj(*Aphanamixis polystachya* )** **Oil:** The dried seeds were finely powdered using a clean pestle and mortar. Approximately 5 g of the seed powder was placed in a thimble and subjected to solvent extraction in a Soxhlet apparatus using 250 ml of n-hexane. The extraction was carried out at 60°C for 5 hours, during which the solvent continuously recycled through the sample to ensure maximum oil recovery [14]

**2.4 Oil Content Determination:** After the extraction process, the solvent was evaporated, and the residual oil was dried in an oven at 60°C to remove traces of solvent. The oil content was calculated using the mass difference method:

**W₄ = W₁ + W₂ − W₃**,

Where *W₁* is the weight of the empty container, *W₂* is the weight of the container with oil and sample, and *W₃* is the weight of the sample used. After the extraction process, the solvent (n-hexane) was evaporated by heating the sample at approximately 70°C under atmospheric conditions, ensuring complete solvent removal. Alternatively, solvent evaporation may be conducted at 60°C under reduced pressure to avoid thermal degradation of the oil.

**2.5 Filtration of Crude Oil:** The extracted crude oil from Aphanamixis polystachya (Pithraj) was filtered using Whatman No. 42 filter paper to remove any suspended solids or particulate matter.

**2.6 Determination of Free Fatty Acid (FFA) Content:** The FFA content in the filtered Pithraj(*Aphanamixis polystachya* ) seed oil was analyzed using an acid-base titration method. Potassium hydroxide (KOH) was used as the titrant. The FFA content was calculated using the formula:

**FFA (wt%) = (56.1 × M × V × 100) / W**,

Where 56.1 is the molecular weight of KOH, *M* is the molarity of the KOH solution, *V* is the volume of titrant used, and *W* is the weight of the oil sample [15]

**2.7 Biodiesel Synthesis:** Biodiesel was synthesized using a two-step transesterification process. The first step involved acid-catalyzed esterification, followed by alkali-catalyzed transesterification to maximize yield and quality. The percentage yield of biodiesel was calculated by using formula.

**Biodiesel Yield (%) =** (Mass of biodiesel / Mass of oil used) × 100.

**2.8 Biodiesel Production Process**

**2.8.1 Acid-Catalyzed Esterification**: Concentrated sulfuric acid (H₂SO₄) and methanol were mixed in a 1:6 molar ratio and added to the oil in a 1:1 ratio (acid to oil) in a reaction flask. The mixture was heated at 60°C for one hour. After the reaction, it was allowed to settle for 2 hours, leading to the formation of two layers: a methanol-water layer on top and esterified oil at the bottom.

**2.8.2 Alkali-Catalyzed Transesterification**: The esterified oil (30 ml) was heated to 120°C and then cooled to 60°C. A separate catalyst solution of NaOH or KOH dissolved in methanol (0.50 to 1.25 w/w %) was prepared and mixed with the oil. The transesterification reaction was conducted in a 250 ml round-bottom flask equipped with a magnetic stirrer, reflux condenser, and thermometer. Reaction conditions were varied to optimize biodiesel yield.

**2.8.3 Separation**: The reaction mixture was transferred to a separating funnel for decantation. The heavier glycerol layer settled at the bottom and was separated, while the lighter biodiesel layer was collected.

**2.8.4 Washing**: The crude biodiesel was washed with warm distilled water to remove impurities such as residual catalysts and soap. The washing process involved three cycles of settling for 8 hours each or overnight.

**2.8.5 Drying**: After washing, the biodiesel was dried in an oven at 110°C for 1.5 to 2 hours to eliminate residual moisture, ensuring fuel stability and preventing microbial contamination.

**2.8.6 Yield Calculation**: The final mass of the biodiesel was measured and compared with the initial oil mass to determine the percentage yield, confirming the efficiency of the transesterification process.

**2.9 GC-MS Analysis:** Gas Chromatography–Mass Spectrometry (GC-MS) analysis was performed to identify the chemical constituents present in the biodiesel sample obtained from Aphanamixis polystachya (Pithraj) seed oil. The analysis was conducted at Punyashlok Ahilyadevi Holkar Solapur University (**Latitude**: 17.6590° N, **Longitude**: 75.9064° E) using a standard auto sampler method. The instrument was equipped with a capillary column and coupled to a mass selective detector. A 1.00 mL aliquot of the purified biodiesel sample was injected into the system.

**Results and Discussion: -**

## **3.1: Physicochemical Parameters of *Aphanamixis polystachya* (Pithraj) Seed Crude Oil and Esterified Oil**

Table 1 provides a comprehensive overview of the physicochemical properties of Pithraj(*Aphanamixis polystachya* ) **seed oil,** highlighting its potential for biodiesel production and industrial applications. The favorable characteristics—such as low acid value, acceptable density, low pour point, and good calorific value—indicate that the oil is suitable for transesterification processes and subsequent biodiesel use.

### **Table 1: Physicochemical Parameters of Pithraj(*Aphanamixis polystachya* )** **Seed Oil**

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Units** | **Values** |
| Color | - | Amber / Pale Yellow |
| Density at 15°C | g/cm³ | 0.84 |
| Pour Point | °C | < -18 |
| Acid Value | mg KOH/g | 0.41 |
| Free Fatty Acid (FFA)  (0.546 × Acid Value) | % | **0.22** |
| Calorific Value | MJ/kg | 38.90 |

The **percentage of Free Fatty Acids (FFAs)** in both crude and esterified Pithraj (Aphanamixis polystachya ) seed oil was determined using **acid-base titration with potassium hydroxide (KOH).**

### **Table 2: Comparison of Physicochemical Properties of Biodiesel from Pithraj (*Aphanamixis polystachya* ) seed** **Oil, Biodiesel Standards, and Commercial Diesel**

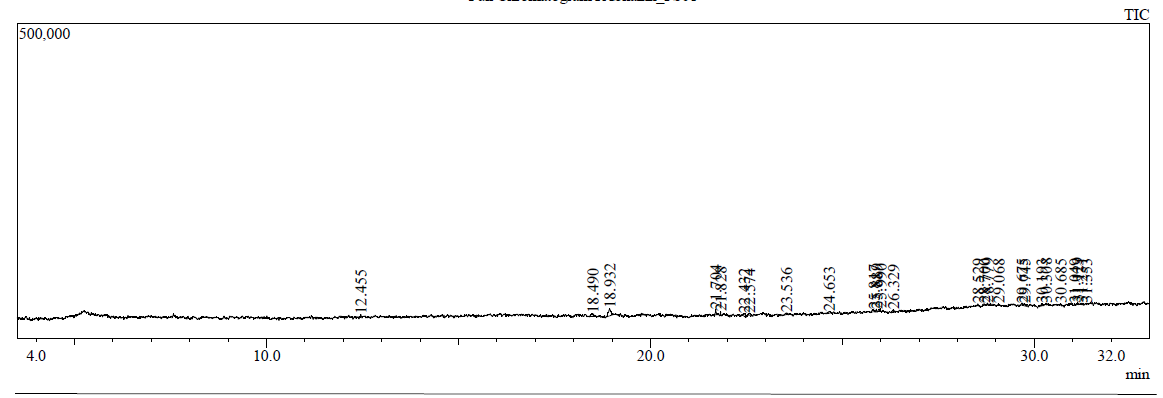
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Properties** | **Unit** | **Biodiesel from Pithraj Oil** | **Biodiesel Standards** | **Commercial Diesel** |
| Pour Point | °C | 9.0 | 15–16 / -10 | 10 |
| Flash Point | °C | 145 | 100–170 | 70 |
| Density at 15°C | g/cm³ | 0.84 | 0.86–0.90 | 0.8445 |
| Acid Value | mg KOH/g | 0.41 | Max. 0.80 | 0.34 |
| Calorific Value | MJ/kg | 38.90 | 40.20 | 44.50 |

## **3.2: Biodiesel Yield and Extraction Optimization** the biodiesel yield from Pithraj seed oil was optimized using solid base catalysts, specifically sodium hydroxide (NaOH) and potassium hydroxide (KOH). For the NaOH catalyst, the highest biodiesel yield was achieved under the conditions of a methanol-to-oil molar ratio of 8:1, a catalyst concentration of 1 wt.%, a reaction temperature of 65°C, and a reaction time of 1.5 hours. In contrast, the KOH catalyst provided optimal yield at slightly milder conditions, with a methanol-to-oil molar ratio of 6:1, the same catalyst concentration of 1 wt.%, a lower reaction temperature of 60°C, and a shorter reaction time of 1 hour. These optimized parameters were crucial in enhancing transesterification efficiency and maximizing biodiesel output from the crude oil.

## The oil yield from Pithraj seed was analyzed at different extraction times—2, 4, and 6 hours—to determine the optimal duration for maximum efficiency. At 2 hours, the oil yield was 33.5%, which significantly increased to 44.12% at 4 hours. A further increase to 46.03% was observed at 6 hours; however, the difference between the 4-hour and 6-hour yields was only 2.04%, which was not considered statistically significant. Therefore, a 4-hour extraction time was selected as the most efficient and practical duration for oil extraction, balancing both yield and operational efficiency.

## **3.3: GC-MS Analysis and Identification of Key Compound**

The GC-MS analysis of the biodiesel derived from Pithraj(*Aphanamixis polystachya* ) seed oil revealed a diverse profile of compounds, confirming the success of the transesterification process. The sample was first vaporized and carried through the gas chromatography (GC) column by an inert gas (helium) under programmed temperature conditions, enabling separation based on the volatility and polarity of the compounds. The separated compounds were then introduced into the mass spectrometer, where they were ionized and detected through their molecular fragment patterns across a defined m/z range. The resulting chromatographic peaks were matched against the NIST17 and Wiley spectral libraries for compound identification. A total of 25 major peaks were recorded, with the most prominent peak observed at a retention time (tR) of 18.932 minutes. This peak, with a similarity index of 90%, was identified as **Diethyl Phthalate (C₁₂H₁₄O₄),** accounting for **22.35%** of the total chromatographic area, thus indicating its significant presence and suggesting efficient ester formation during biodiesel production. As per the Health and Environmental Research Online (HERO) database (Wu, 2008, pp. 455–460), Diethyl Phthalate is a known characteristic marker for biodiesel combustion analysis. Other notable compounds identified included **Neophytadiene** (RT: 21.704 min), **Ergost-9(11)-ene-3, 6, 20-triol diacetate** (RT: 22.574 min), and **Oxazol-5(4H)-one derivatives** (RT: 25.990 min), each contributing additional structural diversity with varying similarity indices and retention times. The identification of these esters and hydrocarbon derivatives affirms the presence of complex bio-compounds typically found in biodiesel. Overall, the GC-MS analysis substantiates the successful chemical conversion of Pithraj seed oil into biodiesel, reinforcing its viability for further physicochemical characterization and practical applications.



**Figure 1:** Figure 1: GC-MS total ion chromatogram of biodiesel derived from Pithraj seed oil showing key peaks including Diethyl Phthalate (RT: 18.93 min, 22.35%), Neophytadiene (RT: 21.70 min), and other compounds. This confirms successful transesterification and complex ester formation."

### **Discussion**

The present study highlights the viability of Aphanamixis polystachya (commonly known as Pithraj. Aphanamixis polystachya seed oil as a promising non-edible feedstock for sustainable biodiesel production. The oil yield of 51% (w/w) demonstrates its potential as a commercially feasible raw material for large-scale biofuel applications. Utilizing a three-step conversion process—saponification, acidification, and esterification—the study achieved a maximum biodiesel yield of 97%, confirming the effectiveness of the method, especially under optimized conditions (7.5 wt% FFA, 70 °C, 90-minute reaction time), aligning with recent advances in biodiesel process optimization [16]

#### **Physicochemical Properties and Fuel Standards**: The resulting biodiesel meets major international fuel specifications, including ASTM D6751 and EN 14214. Its density (0.84 g/cm³ at 15 °C) and low pour point (below −18 °C) make it suitable for use in diverse climatic conditions. The low acid value (0.41 mg KOH/g) indicates minimal free fatty acid (FFA) content, enhancing combustion efficiency and storage stability. Although the calorific value (38.90 MJ/kg) is slightly lower than conventional diesel (~44.5 MJ/kg), it is still within an acceptable operational range and supports efficient engine performance. These fuel properties align with recent findings on other non-edible oils like Citrullus colocynthis and Jatropha curcas, which also demonstrate comparable combustion characteristics [17, 18].

#### **GC-MS Analysis:** Gas Chromatography–Mass Spectrometry (GC-MS) analysis confirmed the successful transesterification process. Diethyl phthalate was identified as the major compound, accounting for 22.35% of the total chromatographic area. While this ester-like compound has also been observed in biodiesel derived from other non-edible sources, its presence warrants further evaluation to determine its effects on long-term engine emissions and fuel combustion characteristics. Recent research suggests that biodiesel combustion profiles can vary based on feedstock-specific ester compositions, impacting exhaust particulate matter and NOx emissions

#### **Sustainability and Feedstock Viability**: The use of non-edible Aphanamixis polystachya (Pithraj) seeds circumvents the ongoing food-versus-fuel debate and supports a sustainable energy future.

#### Recent life-cycle and techno-economic analyses emphasize that non-edible feedstocks, when optimized, offer long-term sustainability and cost-effectiveness for biodiesel production [19, 20, 21]. *Aphanamixis polystachya* is abundant in South Asia and adaptable to marginal land, making it ideal for decentralized biodiesel production in rural regions. Such approaches not only enhance energy security but also offer economic upliftment through local employment and agro-industrial integration

#### **Comparative Analysis and Catalytic Methods**: Compared to more expensive enzymatic and heterogeneous catalytic processes, the chemical catalysis method used in this study proves cost-effective while delivering high yields and acceptable fuel quality. While enzymatic methods such as those applied to *Citrullus colocynthis* oil—are environmentally benign and highly selective, their high operational costs limit scalability. Similarly, animal fats have been used as low-cost feedstocks but often result in lower conversion rates and suboptimal cold flow properties. In contrast, Aphanamixis polystachya (Pithraj) oil-based biodiesel presents a balanced approach with promising economic and environmental performance [22, 23]

**Conclusion:**

In conclusion, the research focused on optimizing the extraction of oil from Pithraj seeds for biodiesel production. This comparative study underscores the potential of Bio-Diesel from Pithraj seeds as a viable alternative to traditional diesel fuels. Its favorable physicochemical properties, including viscosity, pour point, flash point, density, acid value, and calorific value, position it as a promising candidate for sustainable and eco-friendly energy solutions. The findings contribute valuable insights to the field of biodiesel production, demonstrating the feasibility and compatibility of Pithraj seed oil as a renewable resource for biofuel production.

**Disclaimer (Artificial intelligence)**

**Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.**

**Acknowledgment**

The authors express their sincere gratitude to **Dr. M. H. Gavhane**, Principal of Rajarshi Shahu Mahavidyalaya, Latur,(Autonomous) for his constant support and encouragement throughout this research work. We also extend our heartfelt thanks to **Dr. Sachin Kulkarni**, Director and Head of the Department of Biotechnology, for providing the necessary laboratory facilities, guidance, and academic support essential for the successful completion of this study.

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