**‘Seed oil a valuable resource and can also run many economies!’ – Agronomy and conversion of *Hydnocarpus* species seed oil to biodiesel**

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**Abstract:** Due to increased demand and depletion of fossil fuels, there arises the necessity of devising alternative efficient energy sources such as biofuels. For the past few years, there is a change in the approach to the production of diesel from plant biomass as a prospective source. However, it has been a strive to inquisitive shift to non-edible oils from non-crop plants. *Hydnocarpus* species is one such unexplored plant that stores different types of free fatty acids (FFAs) in its seeds. In the book chapter, a comprehensive review is reported on the benefits of *Hydnocarpus* species seed oil as a feedstock, methods for conversion of seed oil to biodiesel, and characteristics of biodiesel. In addition, we attempted to address the conversion of seed oil to biodiesel mainly by transesterification by acid/base/enzymes-based transesterification. The use of *Hydnocarpus* seed oil as a biodiesel feedstock can contribute to the reduction of greenhouse gas emissions and promote sustainable development. The use of *Hydnocarpus* seed oil as a natural antimicrobial agent can provide an alternative to synthetic antimicrobial agents, which are associated with adverse effects such as toxicity and the emergence of antibiotic-resistant bacteria. This book chapter is also an attempt to find multiple ways of realizing the utility of endangered plants in different fields of agronomy such that it helps in the conservation of plant species as well.

**Keywords:** *Hydnocarpus* species; transesterification; free fatty acids, plant conservation; FTIR

1. **Introduction**

Alternative energy sources are being increasingly sought after due to the depletion of oil resources and the negative environmental impact of fossil fuels. The world's reserves of fossil fuels and raw materials are limited, so there is growing research interest in renewable, non-polluting fuels (Holechek et al.,2009). Achieving energy independence is crucial to the success of developing economies. In addition to exploring alternative energy sources, there is a growing focus on biofuels (Sandesh & Ujwal 2021). Biofuels such as biodiesel, bioethanol, and biogas are studied as substitute energy resources. Biofuels are renewable fuels made from domestic feedstock or by-products of industrial processing of agriculture or food products (Linda & Thomas, 2022). Biodiesel is the most popular biofuel for the transport sector. It can be blended with petroleum fuel in any ratio to create a biodiesel blend. Biodiesel can be used in conventional heating equipment or diesel engines with minimal modification (Bhatia, 2014). Several non-edible plant oils used as cooking oils and byproducts of edible oils are emphasized as important biodiesel feedstock (Sujata et al., 2022). The non-edible vegetable oils extracted from *Jatropha curcas*, *Madhuca indica* (Butter tree), *Croton gratissimus* (Lavender croton), *Pongamia pinnata* (Karanja tree), *Ricinus communis* (Castor oil plant), seeds are found to be suitable for biodiesel production (Tumba et al., 2021, Meher et al., 2006, Senthil et al., 2003). The efficiency of these seed oils as biodiesel is investigated as biodiesel feedstock.

Biodiesel is one of the promising alternative resources that have gained attention in various nations everywhere throughout the world on behalf of its sustainability, better gas emission, and its biodegradability (Fazal et al., 2013). Of the various alternate fuels under consideration, biodiesel, derived from vegetable oils and nonedible oils is the most promising alternative fuel to diesel due to the following reasons. Biodiesel can be used in the existing engine without any modifications. It has the benefits of being nontoxic, biodegradable, and essentially free of sulfur and carcinogenic ring components. Biodiesel is insoluble in water and has a light to dark yellow, clear liquid physical appearance. Biodiesel is basically light musty or soapy in odor and it is more biodegradable than fossil diesel (Sayyed et al., 2013). Biodiesel is a renewable liquid transportation fuel that can be used to replace petroleum-derived diesel without making modifications to existing engines or fuel distribution networks. Biodiesel comprises alkyl esters of fatty acids and is typically produced from triglycerides (e.g., soybean oil) and an alcohol (e.g., methanol) in the presence of either a base or acid catalyst (Canakci et al., 1999; Ma & Hanna, 1999; Wahlen et al., 2008). This process is called transesterification, forms fatty acid methyl esters (FAME) where the properties of the biodiesel are dependent upon the fatty acid composition of the feedstock oil (Knothe 2005; Knothe et al., 2005; Mc Cormick et al., 2001).

1. ***Hydnocarpus* as feedstock**

*Hydnocarpus* is a genus of medium to large trees in the Family Achariaceae; the genus was previously placed in the family Flacourtiaceae. These species are found in India, China, Indonesia, Malaysia, and the Philippines. In India, many biodiesel species are grown, which can yield oil as a source of alternative fuel *Hydnocarpus wightiana* is found to be one of the most suitable species grown as an alternative energy source. This tree grows in high rainfall regions, commonly found in evergreen forests of the Western Ghats of Karnataka, Kerala, the coast of Maharashtra, Assam, and Tripura, and planted on the roadsides in hilly areas. *Hydnocarpus wightiana* seed contains 61% - 71% kernels which contain about 63% pale-yellow oil when a mechanical expeller is used for the recovery of oil from the seeds (Krishnamurthy et al., 2018). *Hydnocarpus pentandrus* is an evergreen tree that is 5 - 25 m tall, with a diameter of up to 3 m. Bark rough, brown, usually fluted, branchlets and shoots are brownish pubescent. Leaves simple, alternate, variable, lanceolate-ovate, elliptic-ovate, oblanceolate to oblong, about 6 - 25 x 3.5 - 10 cm across, base usually inequilateral, cuneate to obtuse.

Margin entire or sub serrate, apex abruptly or long acuminate, dark green above, paler beneath, lateral veins 5-8, on either side of the midrib, upturned, gradually diminishing apically towards the margins, impressed above and prominent beneath, minutely appressed pubescent on midrib and veins above when young, later glabrescent when mature, minutely pubescent beneath, connected further with super adjacent secondaries by series of cross veins, petiole usually swollen at both ends, brownish pubescent, about 7 - 15 mm long, stipules linear, minute, pubescent, caducous.

Male flowers: greenish, in 2-3 raceme fascicles, densely brownish tomentose, about 6 mm long, peduncles about 1 cm long and pedicels about 7 mm long, both brown tomentose, sepals 4-5, imbricate, unequal, outer broadly ovate-orbicular, inner suborbicular, slightly connate at the base, concave, apex obtuse, densely brown ferruginous, about 2.5 mm across, petals 4-5, ovate-suborbicular, connate at the base, margins ciliate, pilose inside near the base, smaller than sepals (Oken, 1841).

Stamens 5, filaments filiform, free, broadened at base, about 2 mm long, anthers 2 loculed, reniform, basifixed, pollen fleshy, tricolporate, ovary rudimentary densely pilose.

Female flowers: Solitary or binate on short axillary pilose peduncles, about 8 mm long, usually from the older nodes, sepals and petals similar to male flowers but bigger, staminodes 5, linear-oblong, appressed to ovary apex obtuse, ovary superior, ovoid-ellipsoid, faintly penta angular, apex protruded, creamy yellow tomentose, carpels 5, connate, unilocular, stigma 5, usually equaling the number of placentae.

Fruit: indehiscent berry, obovoid-globose about 5-10 cm across, reddish brown, hard pericarp, thick, tomentose. Seeds 15-20, oblong-ovoid, about 1.7-2.2 x 1-1.5 cm across, embedded in pulp, irregularly compressed, testa hard, oily endosperm, longitudinally striated (Ganeshaiah, [India Biodiversity Portal](https://indiabiodiversity.org/species/show/275987)).

*Hydnocarpus pentandrus* seed is a nonedible multipurpose seed that has components providing it with the medicinal properties and fatty acids present in them that contribute to biodiesel production. Being the species belonging to the vulnerable species as the survey conducted in 2014 there is an increasing need to protect the species. The evergreen tree is found mainly in the Western Ghats. The applications of it make it relevant for protection thereby leading to sustainable development and helping in restoring the species diversity and the ecosystem. Different species of *Hydnocarpus* being used in Siddha, Ayurveda, and folk medicines are present in India.

1. **Methods of Biodiesel synthesis**

**a. Extraction of oil from seeds of *Hydnocarpus***

India has many species of biodiesel crops that can provide oil as an alternative fuel source. Among these species, *Hydnocarpus pentandrus* is the most suitable for this purpose. *Hydnocarpus pentandrus* is a medium-sized tree that belongs to the Achariaceae family. It grows up to 10 m in height and is found in the moist deciduous forests of the Western Ghats in India. The seeds of this tree contain 61% - 71% kernel, which yields about 63% pale yellow oil when a mechanical expeller is used to recover the oil from the seeds (Kumar & Kumar, 2012). The seeds of *Hydnocarpus* are considered non-edible feedstock making it suitable for biodiesel production as it reduces the dependence on edible feedstock in the increasing demand for biodiesel. *Hydnocarpus pentandrus* seed oil is a promising feedstock for biodiesel production due to its high oil content and favorable fatty acid composition. The oil has a high percentage of unsaturated fatty acids, which makes it a good source of biodiesel with good fuel properties. Additionally, the use of *Hydnocarpus pentandrus* seed oil for biodiesel production can provide economic benefits to rural communities by creating new sources of income and employment opportunities (Yadav & Gupta, 2012).

The extraction of biodiesel from *Hydnocarpus* seed oil can be done through various methods such as solvent extraction, mechanical pressing, expeller method, and supercritical fluid extraction. The method used in our work was the expeller method. The expeller method is a mechanical method of extracting oil from seeds by pressing them under high pressure. Here are some advantages and disadvantages of using the expeller method for extracting *Hydnocarpus* seed oil:

**Advantages:**

- The expeller method is a relatively simple and inexpensive method of oil extraction.

- The oil produced using this method is generally of good quality and can be used for a variety of purposes.

- The expeller method does not involve the use of solvents or other chemicals, making it a more environmentally friendly method of oil extraction.

 **Disadvantages:**

- The expeller method is not as efficient as other methods of oil extraction and may leave behind some residual oil in the seed cake.

- The high pressure used in the expeller method can generate heat, which may degrade some of the oil's nutritional and medicinal properties.

- The expeller method may not be suitable for extracting oil from seeds that are very hard or have low oil content.

The efficiency and yield of the expeller method for extracting *Hydnocarpus pentandrus* seed oil may vary depending on the specific conditions used, such as the pressure and temperature applied during the process. However, studies have reported that the expeller method can yield between 33-38% of oil from the seeds. While this yield is lower than other methods such as solvent extraction, the expeller method is still a viable option for producing high-quality oil. (Khatoon et al., 2015)

**b. Transesterification of seed oil to biodiesel**

Biodiesel can be produced from the following methods: 1. Pre-heating, 2. Blending/Dilution, 3. Pyrolysis, 4. Micro-emulsification, 5. Trans-esterification. Among these processes, trans-esterification is considered the best method of reduction of viscosity of vegetable oils as per concern of economy and quality. The trans-esterification process is being widely used to produce biodiesel (Wang et al., 2023). The transesterification process is generally and globally used method to modify vegetable oil into respective esters (biodiesel) (Math and Hedge 2016).

Transesterification is one of the organic treatments where an alcohol group in ester is substituted. It can also be a treatment of vegetable oil/fat with alcohol to give ester and glycerol. The applicability of transesterification is not restricted to the laboratory. Several relevant industrial processes use this reaction to produce different types of compounds. Transesterification of vegetable oils, a triglyceride reacts with three molecules of alcohol in the presence of a catalyst, generating a mixture of fatty acids alkyl esters, and glycerol. Oils (triglycerides) + Methanol → Biodiesel + Glycerol

The overall process is a sequence of three following reactions, in which di-glycerides and mono-glycerides are made as intermediates. Transesterification is a flexible reaction thus excess alcohol is used to increase the yields of the alkyl esters and to permit its phase separation from the glycerol formed. Conversion of vegetable oil to biodiesel is affected by several parameters, namely (i) Reaction timing, (ii) Reactant ratio (Molar ratio of alcohol to vegetable oil), (iii) Type of catalyst, (iv) Amount of catalyst, and (v) Temperature of reaction.

Transesterification is catalyzed by Bronzed acids. These catalysts provide very high yields in alkyl esters, but the rate of reaction is slow, needful, usually, temperatures above 100 °C and more than 3 hours to reach complete conversion. H2SO4 is a commonly used acid catalyst in Acid catalyzed transesterification which gives the mechanism of an acid-catalyzed process.

**Base-Catalyzed Transesterification**: The base-catalyzed or alkaline-catalyzed transesterification of vegetable oils proceeds faster than the acid-catalyzed reaction. Because of the above reason, together with the fact that the bases are less corrosive than acidic catalysts (Elangovan et al., 2016).

Biodiesel production mainly depends on free fatty acids and it has to be reduced by less than 2% to increase the yield of the biodiesel (Yakshith et al 2015). The oil is mainly extracted by mechanical expeller as it increases the yield of the production of oil from the dried seeds (Nde et al., 2020). The esterification reaction is mainly carried out to decrease the free fatty acid content. The acid-catalyzed reaction is mainly done as it offers some advantages of eliminating separation, corrosion, and environmental problems (Mehmood & Ian et al., 2014). The water can prevent the conversion of free fatty acids to esters from going to completion. After dewatering the esterified oil was fed to the transesterification process (Bobade & Khyade et al 2012).

To produce biodiesel, alcohols like methanol and ethanol are mostly preferred as these are less costly and easily available. Sodium hydroxide (NaOH) and potassium hydroxide (KOH) are used as catalysts. The parameters such as the temperature of the reaction, proportion of alcohol to vegetable oil, catalyst used and mixing intensity influence the transesterification process. After transesterification, the viscosity of vegetable oil reduces and can be used in a diesel engine in pure or blended form (Atabani et al., 2013). The production of biodiesel can be achieved through a non-catalytic supercritical reaction method, which offers the advantage of higher yields. However, this approach is characterized by its high energy requirements due to the need for elevated temperature and pressure conditions.

Additionally, it necessitates a high alcohol-to-oil molar ratio, which can lead to equipment corrosion. Furthermore, the process poses challenges in terms of catalyst recovery, as well as product separation and purification, resulting in lower efficiency in these aspects (Lourinho et al., 2014). Enzyme-catalyzed transesterification is highly regarded for its efficiency, primarily due to its insensitivity to free fatty acid (FFA) and water contents in the feedstock. This method also offers a simple procedure for product purification, as it generates no by-products. Another advantage is that the enzyme catalyzes both esterification and transesterification reactions simultaneously. Additionally, the process allows for easy product separation and catalyst recovery. Furthermore, enzyme-catalyzed transesterification can achieve higher yields under mild reaction conditions (Stamenkovic et al, 2011). To achieve the maximum yield of biodiesel, it is necessary to use an excess amount of alcohol in relation to the triglyceride being reacted to. Stoichiometrically, one mole of triglyceride produces three moles of biodiesel and one mole of glycerol. By using an excess of alcohol, the reaction is driven toward the production of biodiesel, increasing the overall yield (Shahid & Jamal et al. 2011).

The reduced yield of biodiesel observed at low catalyst concentrations can be attributed to the incomplete conversion of triglycerides into biodiesel. In other words, when the catalyst concentration is low, a significant portion of the triglycerides remains unreacted, resulting in a lower yield of biodiesel (Jeong et al., 2009). During the transesterification process, the esterified oil sample reacts with the methanol and base catalyst to form methyl esters and glycerol (Hashemzadeh & Sadrameli 2019). The primary goal of the transesterification reaction is to decrease the viscosity of crude oil. This is important because high viscosity can lead to deposit formation within the engine and hinder the proper atomization of fuel when injected into the combustion chamber (Subhadip & Aniket 2023). Methyl esters were purified by washing with warm water (Ong et al. 2014). The composition of specific fatty acid categories plays a crucial role in determining the fuel quality of biodiesel (Beyene et al., 2022). Crude biodiesel often contains various impurities including soap, water, glycerides, excess catalyst, and untreated alcohol (Pradhan et al., 2012).

*Hydnocarpus pentandrus* seed oil is known to contain a variety of fatty acids, including both saturated and unsaturated fatty acids. Here is a general overview of the fatty acid profile of the oil:

- Palmitic acid (C16:0): 20-30%

- Stearic acid (C18:0): 15-25%

- Oleic acid (C18:1): 25-35%

- Linoleic acid (C18:2): 10-15%

- Linolenic acid (C18:3): 1-2%

The fatty acid profile of the oil may vary depending on factors such as the location of the plant and the extraction method used. However, the fatty acid profile of *Hydnocarpus pentandrus* seed oil suggests that it may have potential health benefits, such as reducing inflammation and improving heart health (Lima et al., 2005). *Hydnocarpus pentandrus* seed oil thus has several potential applications, including as a biodiesel feedstock and as an antibacterial agent (Sahoo et al., 2014).

**IV. Characterization tests for biodiesel**

There were two other important analytical methods that helped in biodiesel characterization, Fourier Transform Infrared (FTIR) Spectroscopy Analysis and Gas Chromatography–Mass Spectrometry (GC-MS). These methods determine the synthesis of crude oil to fatty acid methyl ester and the quality of the biodiesel produced. FTIR and GC-MS are both analytical methods that can be used to determine the composition of biodiesel. FTIR can identify the functional groups present in biodiesel, while GC-MS can separate and detect individual compounds in the sample. Together, these methods can provide information on the quality and purity of biodiesel, which is important for ensuring that it meets industry standards. This works by measuring the absorption of infrared radiation by the sample. Different functional groups absorb radiation at different frequencies, so FTIR can be used to identify the types of chemical bonds present in the sample (Islam and Islam, 2011). In biodiesel these peaks arise from the methyl (CH3) and methylene (CH2) groups in the fatty acid chains. The region between 1740 - 1750 cm-1.This region shows the C=O stretching which is present in the ester functional group. The new peak at 1197 as compared to the Hydnocarpus oil shows the C-O stretching region of the methyl esters confirming the presence of methyl esters for structural elucidation (Salaji & Jayadas 2021).

GC-MS stands for Gas Chromatography-Mass Spectrometry. It works by separating the components of a sample based on their physical and chemical properties. The sample is first vaporized and then passed through a column that separates the components based on their interactions with the column material. The separated compounds are then detected and identified by mass spectrometry. GC-MS can be used to identify individual compounds in the sample and determine their concentrations. (Islam and Islam, 2016). The major components of *Hydnocarpus pentandrus* oil are converted to fatty acid methyl esters. *Hydnocarpus pentandrus* oil contains around 61.17% of saturated fatty acid which mainly comprises Lauric acid, Myristic acid, Palmitic acid, Stearic acid, etc., and around 25.693% of unsaturated fatty acid which comprises Arachidic acid, Oleic acid, Hydnocarpic acid, etc. The fatty acid composition of the *Hydnocarpus pentandrus* oil and the biodiesel formed was analyzed by the GC-MS analysis. The oil had the presence of acids like Lauric, Myristic, and Palmitic acids as the presence of this saturated fatty acid in the precursor oil used to produce biodiesel was changed to corresponding methyl ester. Biodiesel is characterized by GC – MS Analysis (Thanh et al., 2010; Yin et al., 2012). The fatty acid profile can also be obtained by the GC- MS analysis where the fatty acid profile of the oil is used in biodiesel production.

**V. Physical properties of Biodiesel**

The physical properties of biodiesel play an important role in determining the quality of diesel (Ismail & Ali 2015).

**Viscosity**: This test measures the thickness or flow resistance of the biodiesel. Viscosity is an important parameter for determining the fuel's ability to flow through fuel lines and injectors. Higher viscosity can lead to clogging and poor performance.

**Specific gravity and density**: These tests measure the mass per unit volume of the biodiesel. Specific gravity and density are important for determining the fuel's energy content and for calculating fuel consumption. They can also be used to determine the volume of fuel needed for a given weight or energy output.

**Cetane value**: This test measures the ignition quality of the biodiesel. A higher cetane value indicates that the fuel ignites more easily and burns more cleanly. This can lead to better fuel efficiency and lower emissions.

**Cloud point**: This test measures the temperature at which the biodiesel begins to form solid crystals. The cloud is an important parameter for determining the fuel's ability to flow and operate at low temperatures. A lower cloud point indicates better cold weather performance.

**Saponification value**: This test measures the amount of potassium hydroxide required to neutralize the free fatty acids in biodiesel. The saponification value is an important parameter for determining the quality of the feedstock used to produce biodiesel. Higher saponification values can indicate the use of lower-quality feedstocks.

**Acid value**: This test measures the amount of acid present in the biodiesel. The acid value is an important parameter for determining the fuel's stability and resistance to oxidation. Higher acid values can indicate that the fuel is more prone to degradation and may have a shorter shelf life.

Overall, these characterization tests are important for ensuring that the biodiesel meets industry standards and performs well in engines. Crude vegetable oil has high viscosity which has a higher value than the acceptable diesel fuel values. For this reason, they cannot be directly used safely as fuels in a compression ignition engine, at least not without prior heating. Viscosity is indirectly proportional to the temperature and only for relatively small blending ratios (Sujata et al., 2022). Crude vegetable oils have the highest viscosity than biodiesel and biodiesel have higher viscosity compared to fossil diesel, crude vegetable oil has a viscosity 10 to 17 times higher than that of Biodiesel (Sayyed et al., 2013). Increasing the degree of unsaturation results in a decrease in kinematic viscosity and as the temperature of oil is increased its viscosity decreases and it is therefore able to flow more readily. Double bond orientation also impacts kinematic viscosity (Bryan et al., 2009).

The increased saturated fatty acid increases the cloud point and the cetane number and improves stability (Demirbas et al., 2009; Moser et al., 2009). The results showed medium stability as the cloud point is found to be 1.8 and 47 is the cetane number. The high saturated fatty acids support viscosity (Jakeria et al., 2014), and the viscosity is found to be 39.9 mm2/s. It has a relation in determining the physical properties of biodiesel as saturated fatty acid methyl esters can increase the cetane number and cloud point, but more polyunsaturated fatty acids can reduce the cetane number, cloud point, and stability (Moser et al., 2009).

Many researchers studied the effect performance of biodiesel and its blends in diesel engines such as brake power, brake-specific fuel consumption, and exhaust emission (Silitongaa, et al., 2016). The calorific value of biodiesel tends to decrease as the mass fraction of oxygen decreases with an increase in the carbon chain length of fatty acids, assuming no change in saturation level. A higher calorific value indicates a higher energy content of the biodiesel. The calorific value generally increases as the molecular weight of the biodiesel increases, but it decreases as the number of double bonds in the molecules increases (Beyee et al., 2022). The acid value gives a high percentage of free fatty acid and depicts the number of corrosive acids and oxidation substances present in the oil (Kapilan et al., 2009).

Biodiesel can be an alternative to petroleum diesel or can be used as blends. Switching to the nonedible feedstock also reduces the prevailing pressure on the edible feedstocks. This species is one of the sensitive species that needs to be protected thus providing effective value to the protection by the sustainable development of the species by identifying multiple applications. The *Hydnocarpus* is an evergreen multipurpose tree that can find immense applications in various fields of treatment procedures as well as usage of the fuel produced from the oil from the seeds that can be used as blends in petroleum oils. Being the species that need a prior importance of protection this can be possible by finding out multiple applications which can lead to the sustainable balance of the ecosystem.

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