PROJECT TITLE: Production of biodiesel from seeds of the Indian medicinal plant *Hydnocarpus pentandrus* and study of antibacterial potential of the seed oil

Name of the College: Kristu Jayanti College (Autonomous), Bengaluru

Department: Microbiology, Life Sciences

Project Team Members:

1. Name: Anjumol Babu

Email id: 21msmb05@kristujayanti.com

Contact No. : 9072240580

2. Name: Merin V Ninan

Email id: 21msmb21@kristujayanti.com

Contact No. : 7902636085

3. Name: Pooja B

Email id: 21msmb24@kristujayanti.com

Contact No. : 6363320804

4. Name: S Ashika

Email id: 21msmb30@kristujayanti.com

Contact No. : 8921266008

Project Guides:

1 .Name**:** Dr. P Roopa

Email id: roopa.p@kristujayanti.com

Contact No. : 9447496986

2. Name: Dr. C M Reena Josephine

Email id: reena.j@kristujayanti.com

Contact No. : 9943241061

Keywords

Esterification, Transesterification, agar well diffusion method

Introduction

Hydnocarpus pentandrus is an evergreen tree mainly found in the Western Ghats of Karnataka, Kerala, the coast of Maharashtra, Assam, Tripura. (K.N Krishnamurty et al., 10.1016/j.energy.2018.04.068) It can be considered as the multipurpose tree as the seeds can be used for the biodiesel production as well as the species of the *Hydnocarpus pentandrus* is used in Ayurvedic and Sidha treatments. The shifting towards the allopathic medicines are readily available and potentially effective but cause side effects and some adverse effect to the human body(Minakshi Rajput et al.,2019:8(1) ;01-5) Finding applications of the traditional medicines against various microorganisms helps in identifying in use in new drug discoveries.

The world fossil fuel reservoirs are limited and there is an ever increasing demand for energy. Also in the recent years, diminishing fossil fuel, global warming and the environmental pollution problem have become major global issues. (Abbazaadeh A et al., 2012:63; 138-48). The seeds being non edible make a suitable alternative for the petroleum diesel it reduces the pressure on the edible feedstocks which can be used for the production of the biodiesel. Biodiesel is an alternative fuel for diesel engines that is gaining attention all over the world. Its primary advantages of being one of the most renewable fuels available, on toxicity and biodegradability make it a suitable alternative in most diesel engines without making engine modifications.(Yakshith P.C .,2015 ISSN:2278-0181).

Hydnocarpus pentandrus is one of the vulnerable species so this project put forward d the idea of protecting the species and the need for the sustainable development by finding multiple applications of the particular species in different fields thereby creating a balanced ecosystem.

Objectives of the project

- Collection of fully grown seeds and eliminating seeds with poor quality and also immature seeds because it affects on the yield of the oil produced.
- Authentication of the collected seeds for the species identification by collecting a sample seed for the same.
- Extraction of oil by expeller method as it produces maximum yield of the oil
- The oil is collected and used for biodiesel production and to checking antibacterial potency against *Staphylococcus aureus* and *Pseudomonas aeruginosa* by agar well diffusion method
- Biodiesel characterization

Methodology

- *Hydnocarpus pentandrus* belongs to the family of Achariaceae, the seeds of the plant were collected and oil is extracted from it by expeller method.
- Authentication of the seed sample was done FRLHT Bangalore.
- The extracted oil was subjected to base titration in order to check the free fatty acid value of the oil.
- The oil should have free fatty acid value less than 2%, or else it should be esterified before transesterification.
- By base titration method, the free fatty acid value of oil before esterification was found to be greater than 2%.

- Esterification process is to be done. About 100 ml oil is measured and pre-heated in a conical flask in water bath set at 60 degree Celsius.
- To the oil sample 1% v/v of H_2SO_4 and 20 ml of methanol is added and the reaction is carried out in the magnetic stirrer.
- After esterification process the reaction mixture is transferred to separating funnel in which two layers are formed and the esterified sample is separated from the bottom formed catalyst layer.
- Then the esterified sample was washed till remains of catalyst are completely removed.
- Then base titration was repeated and the free fatty acid value of esterified sample was found to be less than 2%.
- Therefore transesterification procedure was carried out.
- To the esterified sample required amount of NaOH and methanol was added and reaction is carried out in the magnetic stirrer for 1 hour.
- The reaction mixture is added to separating funnel after cooling. Then two layers are formed, top layer is methyl esters (biodiesel) bottom layer is glycerol after 11 hours of settling due to gravity separation.
- The biodiesel sample is separated and washed with warm distilled water until the pH of the water turns 7.
- After washing, the biodiesel was boiled at 100°C for 1 hour inorder to remove the water content.
- The biodiesel yield was calculated:

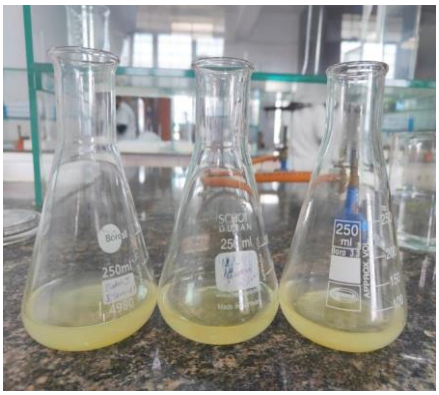
$$\text{Biodiesel yield (\%)} = \frac{\text{weight of methyl ester produced (g).}}{\text{Weight of oil used (g).}} \times 100$$

- Biodiesel characterization was done by GC-MS and FTIR.
- The physical properties of the biodiesel like iodine value, saponification value, Viscosity, calorific value, cloud point, cetane number was also estimated.

- The antibacterial activity of the extracted seed oil is estimated by agar well diffusion method.
- The broth cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was prepared for antibacterial activity.
- Different concentrations of oil is made and added to the wells and incubated at 37°C for 24 hours.
- The zone of inhibition was calculated after incubation.

Hydnocarpus pentandrus tree

Hydnocarpus pentandrus seed



base titration

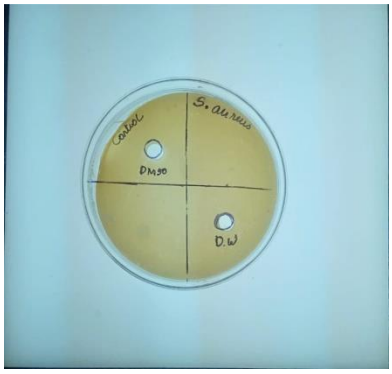
preheating

reaction in magnetic stirrer

Esterification

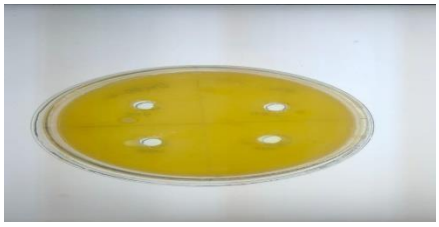
Transesterification

Product- Fatty acid methyl esters

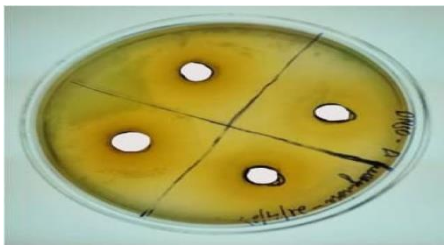


Control *S.aureus*

Contol *P.aeruginosa*



S.aureus



P.aeruginosa

Results and Conclusion

The Hydnocarpus pentandrus is known to have antimicrobial activity (Prashith Kekuda et.,al 2017). Thermophysical properties are important in biodiesel characterization and combustion modelling(Parag Saxena et al.,2012). The biodiesel was formed via transesterification reaction. The yield of the biodiesel formed depends of fatty acid profile of the hydnocarpic pentandrus oil and the catalyst concentration used in the both reactions mainly transesterification reaction. The physical properties of the biodiesel play an important role in determining the quality of the biodiesel produced .such as acid value, iodine value, ,saponification value, density ,cetane value,viscocity,specific gravity cloud point. The GC MS analysis of the sample shows the relative conc. of the fatty acid methyl esters. In FTIR results the peak 1743.14 cm^{-1} confirms the presence of fatty acid methyl esters.

Table 1. Biodiesel characterization

Property specifics	**Units**	**HPOME**
Acid value	g/mol	1.4364
Iodine value	g/ml	10.1
Saponification value	KOH/mg	47.685
Density	Kg/m^3	0.88
Viscosity	Poise.s	0.0995
Cetane number	-	47
Calorific value	Kcal/kg	9345
Cloud point	^0C	1.8

Table 2 Antibacterial effect of seed oil against *Pseudomonas aeruginosa*

concentration of oil (µg/ml)	zone of inhibition
n250	0.4
500	0.6
750	0.8
1000	1

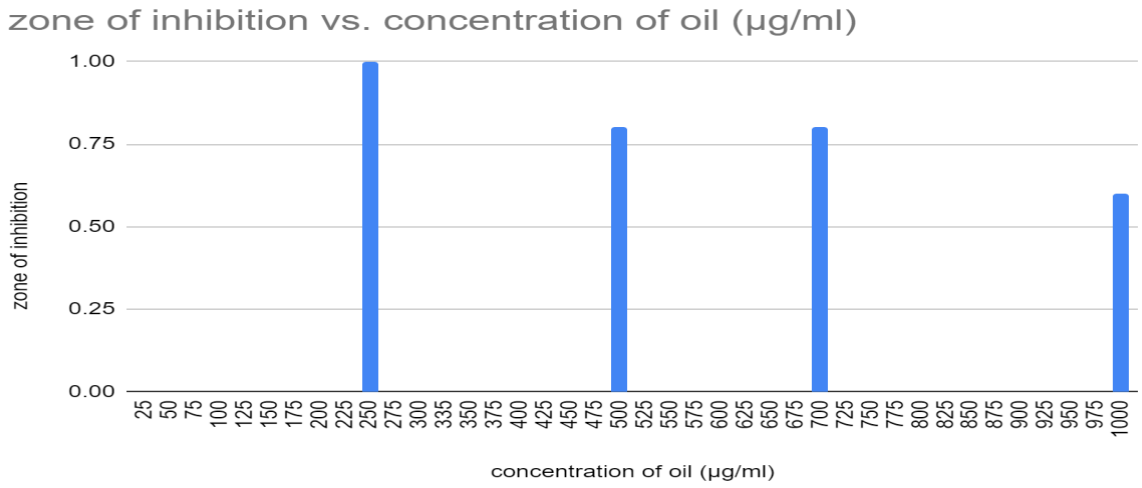


Table 3 Antibacterial effect of seed oil against *Staphylococcus aureus*

concentration of oil (µg/ml)	zone of inhibition
250	No zone
500	No zone

750	No zone
1000	No zone

Table 4 GC MS analysis of HPO

Compound Name	**Common Name**	**Component area**	**Relative concentration**
Octanoic acid	Caprylic acid	30723164.6	0.003942686713
n-Decanoic acid	Capric acid	24755545.2	0.003176865418
Dodecanoic acid	Lauric acid	260318364.2	0.03340651164
Tetradecanoic acid	Myristic acid	230761212	0.02961345865
Hexadecanoic acid	Palmitic acid	2360652977	0.3029412903
Pentanoic acid	Valeric acid	1552605.7	0.0001992450304
Decanedioic acid.	Sebacic acid	747605899	0.09593985135
Heptadecanoic acid	Margaric acid	8711141.7	0.001117895994
cis-9-octadecenoic acid	Oleic acid	3734291011	0.4792194992
Octadecanoic acid	Stearic acid	294111859.7	0.03774321222
Cis-13-Eicosenic acid	Erucic acid	24243701.4	0.003111180786
Eicosanoic acid	Arachidic acid	50419834.5	0.006470349463
Trans-butenedioic acid	Fumaric acid	6246854.4	0.0008016553686
(5R,8R,8aS)-8-Ethyl-5-((Z)-pent-3-en-1-yl)octahydroindolizine	Hydnocarpic acid	18049621.2	0.002316297901
		7792443792	

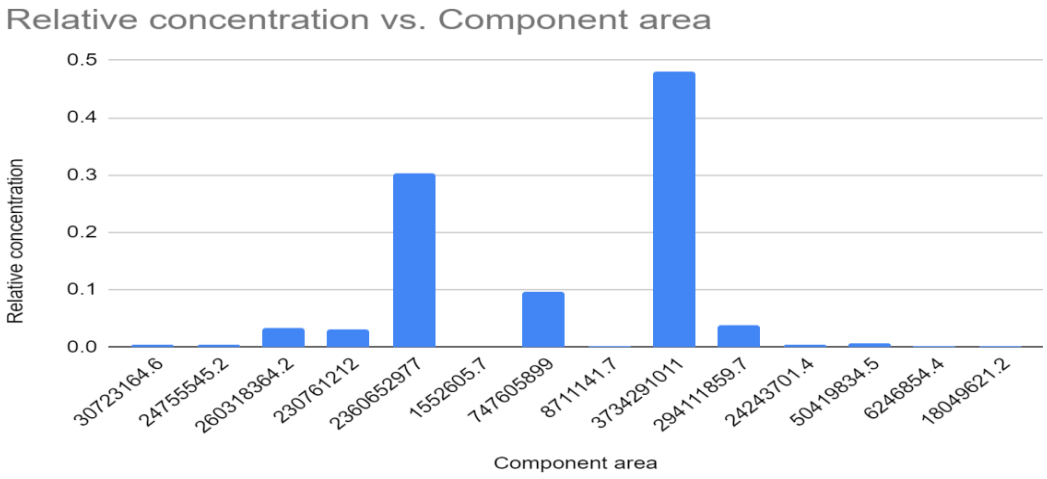


Table 5 GC MS Analysis of HPOME

Compound name	**Coumpound area**	**Relative concentration**
Octanoic acid methyl esters	627858373	5.932717401
Decanoic acid methyl esters	534162459.3	5.047372231
Dodecanoic acid methyl esters	4779208311	45.15937594
(7H) Thiopyrano[3,4-C] isoxazole,3,3a,4,5- tetra hydro- 7- (1-methylethyl)	7047173.1	0.06658967734
Palmitic acid, 9-hexadecenyl ester	66979355.1	0.6328968484
Pentanoic acid methyl esters	31181493.3	0.2946380837
Pentanamide,2-(dimethyl amino-3-methyl-N-methyl propyl dioxide,3-phenyl-2-oxa-6,9-diazabicyclotetra enyl	33101057	0.3127762968
2-Heptanone, 6-(3-acetyl-2-methyl-1-cyclopropen-1-yl)-6-methyl	167868112.5	1.586208156
Oleic acid, butyl ester	610442754.5	5.768154902
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	1622738127	15.33346872
Cis-13-Eicosenoic acid methyl esters	335212415.9	3.16746677
Eicosanoic acid, methyl ester	1756806741	16.60030091
Fumaric acid, 3,5-dimethylphenyl 2,2,3,3-Tetrafluoropropyl ester	10374926.5	0.09803406249
	10582981299	

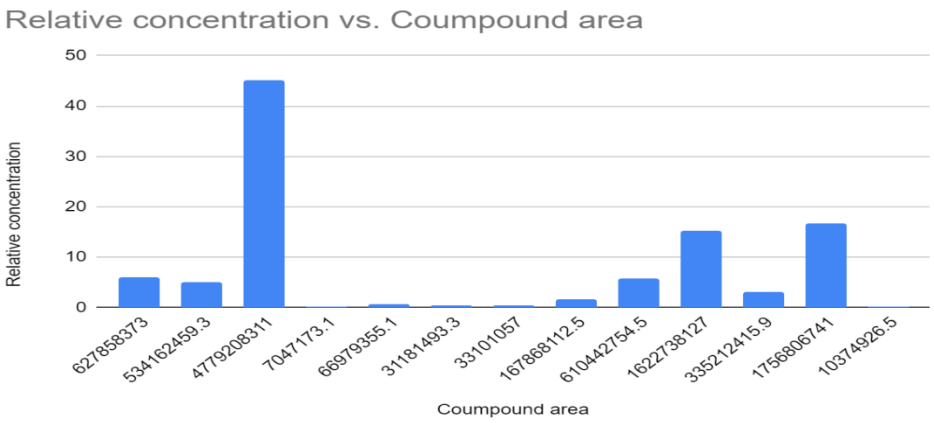
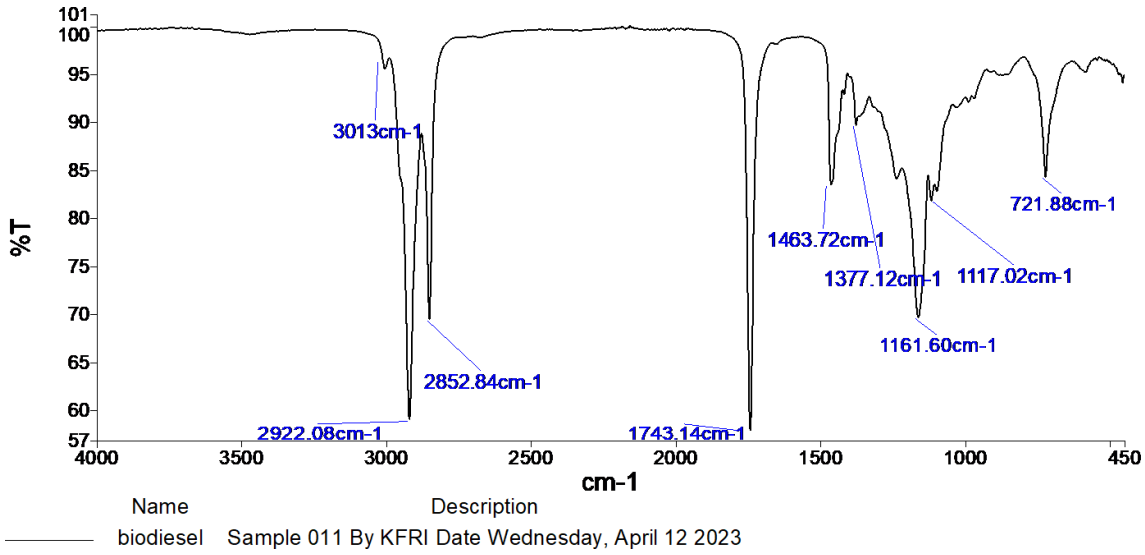


Fig. 1. FTIR Analysis of HPOME



Innovation of the project

Biodiesel can be an alternative to petroleum diesel or can be used as blends. Switching to the non edible feedstock is also reduces the prevailing pressure on the edible feedstocks. The seed which is used to produce biodiesel is a multipurpose seed so it finds a wide range of applications in case of antimicrobials .This particular species is one of the sensitive species that needs to be protected thus providing the effective value to the protection by the sustainable development of the species by identifying multiple applications.

Scope for future works

The *Hydnocarpus pentandrus* is an evergreen multipurpose tree can find immense applications in various fields of treatment procedures as well as usage of the fuel produced from the oil from the seeds can be used as blends in the petroleum oils. The seeds are proven to have various clinical applications can may lead to drug discovery.

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.