Stability of biodiesel produced from *Pongamia pinnata* oil by natural antioxidants

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ABSTRACT

Pongamia pinnata oil is non-edible oil extracted from seeds of *Pongamia pinnata*. The present study investigates the potential of pongamia oil as an alternative source of biodiesel which envisages it in food security, as biodiesel is produced from non-edible seed oil. Biodiesel was produced from *Pongamia Pinnata* oil by transesterification reaction, using Fungal Lipases isolated from *Aspergillus Spp*. The mole fraction of methanol and pongamia oil was 5:1 ratio. The temperature, speed of mixing and reaction time used were 45°C, 150 rpm and 24hr, respectively. Biodiesel (methyl ester) relatively becomes unstable on storage due to release excessive free radicals, caused by atmospheric oxidation and also microbial oxidation. This necessitates the evaluation of natural antioxidants for their antimicrobial activity. Commercial antioxidants are mainly used in food processing and preservation industries, in animal feeds ,beer production, in disease prevention etc. Hence commercial antioxidants can be spared for food security, whereas natural antioxidants which can be easily extracted from plants, which are cost effective can be used for maintaining stability of biodiesel. Natural Antioxidant were extracted from different medicinal plants like Mango, Neem, Turmeric, Tinospora, curry leaves and microbes like *Aspergillus* spp. These antioxidants were added to biodiesel in the ratio of 1:50.The standard antioxidants used were Butylated Hydroxyl Toulene (BHT) and Gallic acid (GA).The different parameters like viscosity, density and acid value were measured. The results showed that biodiesel with BHT, mixture of BHT and GA, plant extracts such as Tinospora (Ethanolic extract) [T (EA)], mixture of Mango and Neem (MN) and Curry Leaves (Ethanolic extract [dry leaves]) [ECL(D)] were the best antioxidants to maintain biodiesel stability. The measured value of viscosity, density and acid value of biodiesel were in the range of 5-6 CST, 0.92-0.94 kg/m^3 and 15-17 mg KOH/ g of oil respectively. The resulted values were compared with standard values; these were found within acceptable range. The results showed that biodiesel substituted with standards such as BHT, mixture of BHT and GA, curry leaves and mixture of both Mango and Neem extracts were stable up to 120 days, whereas Biodiesel substituted with plant extracts such as Turmeric was stable up to 15 days. The thermal stability of biodiesel substituted with BHT was tested at 0 and 15 days which should relatively good stability with density and viscosity being very close to standard values. The antioxidant activity of organism extracts were less compared to plant extracts. The usage of crude fungal lipase enzyme and natural antioxidants for production of biodiesel and maintenance of its stability has proved to be very cost effective. The antioxidants used to maintain biodiesel stability, can also be evaluated for antibacterial activity. The natural antioxidants were evaluated for antibacterial activity against *E. coli, Micrococcus, Bacillus subtilis*, *Proteus vulgaris* etc. In these antioxidants [ECL(D)] and Turmeric (Ethanolic extract) were evaluated as the best antioxidants for antibacterial activity, which shows 3.9 cm and 3.6 cm zone of inhibition and are more active against *E. coli, Micrococcus* and *Bacillus subtilis*. The organism which is inhibited by most of the antioxidants was *E. coli*.

Keywords: stability, *Aspergillus spp*., biodiesel, lipase, antioxidants, antimicrobial activity.

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Abbreviations: WO-Waste Oil; CL- *Murraya Koenigii*; T- *Curcuma longa*; A- *Tinospora cordifolia*; M-Methanol; EA- Ethyl Acetate; E- Ethanol; BHT - Butylated Hydroxy Toluene; BHA- Butylated Hydroxy Anisole; GA - Gallic acid; B+ A(SOX) - Biodiesel + Tinospora; B + CLM(D) - Biodesel + Dry Curry Leaves Methanolic Extract ; B + T(EA) -Biodiesel + Turmeric Ethanol Extract; B + MN - Biodiesel + Mango Neem; B + ECL(D) - Biodiesel +Dry Curry Leaves Ethanolic Extract; Asp – *Aspergillus* spp.

INTRODUCTION

With the increasing price of petroleum fuel as supplies are depleted, the need for alternative fuel sources has steadily increased. Biodiesel is an alternative fuel, derived from vegetable oil, non-edible oil or waste cooking oil that can be used directly or blended with petroleum diesel at any percentage without engine modification [1]. Biodiesel is a renewable fuel and presents advantages of producing approximately 80% less carbon dioxide emissions, and almost 100% less sulfur dioxide [2]. In addition, BD has a relatively high flash point (150 ^{0}C) that makes it less volatile and safer to transport or handle than petroleum diesel. Furthermore, it provides lubricating properties, which reduce engine wear and extend engine life. At the same time, BD has physical properties and energetic content close to those of petroleum diesel, which allows its efficient function in conventional diesel engines without any modification [3]. The main problem facing the conventional processes of biodiesel production, using alkali catalyst, which is usually used in an industrial scale, is that it presents problems of separation of catalyst and unreacted methanol from biodiesel and is not suitable for oils containing high free fatty acids (FFA) contents, such as WO. On the other hand, biodiesel produced from biocatalyst eliminates these disadvantages and produces biodiesel with a very high purity [2-4] and hence can be used with WO. Further, all FFA present in the WO can be converted to BD using this approach [4]. Different lipases isolated from microbes, such as *Aspergillus spp, Candida antarctica*, *Pseudomonas cepacia* and *Thermomyces lanuginosus,* have been employed as biocatalysts in the production of biodiesel from vegetable oil but there are only a few enzymatic studies that involve the use of waste cooking oil [3-4].The biodiesel production was carried out using pongamia pinnata oil. The *Pongamia pinnata* is known for its multipurpose benefits and as a potential source of biodiesel [5]. It has been recognized as "Biodiesel" as several parameters of diesel and *Pongamia pinnata* oil are comparable. The *Pongamia pinnata* seeds contain about 40% oil, which can be converted to biodiesel by transesterification method. Pongamia biofuel requires no engine modification, when blended with diesel in proportions as high as 20 percent [6]. As biodiesel chemically is an ester molecule there is every possibility that in the presence of air or oxygen it will be hydrolyzed to alcohol and acid. Presence of alcohol will lead to reduction in flash point and presence of acid will increase total acid number. All these make methyl ester relatively unstable on storage and cause damage to engine for biodiesel [6]. Vegetable oils contain natural antioxidants. The most common are tocopherols, which are hindered phenolic chain breaking antioxidants. Chain breaking antioxidants are highly reactive with free radicals and form stable compounds that do not contribute to the oxidation chain reaction [6-7]. Natural antioxidants can be added to biodiesel to improve the fuel's antioxidant behavior. The efficiency and necessary quantity of antioxidants are strongly dependent on the feedstock and biodiesel production technology. These additives have different effects on biodiesel, depending on the feedstock however, fuel properties such as viscosity, acid value, density, and others are not affected by the antioxidants [8-9]. *Tinospora cordifolia, Magnifera indica*, *Azadirachta indica, Murraya koenigii, Curcuma longa* are sources of several bioactive components which has chemical constituents of different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides which results in antioxidant properties [13-16]. However much less studies have been carried out on biodiesel stability by

natural antioxidants. Hence, the present study was undertaken to investigate the production of biodiesel by fungal lipases and further investigates the biodiesel stability by natural antioxidants.

MATERIALS AND METHODS

Screening of Lipase producing fungi

The fungal isolates were inoculated on media of the following composition (g/L) Nutrient broth (HiMedia), 8.0; sodium chloride, 4.0; agar, 10.0. The medium was adjusted to pH 7.0, autoclaved and cooled to 60ºC. Olive oil (31.25 ml) and 10 ml of Rhodamine B solution (0.001%, w/v) was added with vigorous stirring and emulsified by mixing for 1 min. The medium was allowed to stand for 10 min at 60ºC to reduce foaming. The medium was poured into sterile petriplates [17]. To determine the ability of fungi to utilize different oils as a sole source of carbon, Rhodamine B agar containing different oils (1%, v/v) was prepared. The fungal strains were point inoculated on the Rhodamine B agar plates at room temperature for 48 h. Lipase production is detected by irradiating the plates with UV light at 350 nm. Fungal colonies that have lipolytic activity showed zone of orange fluorescent halo [17].

Enzymatic method of Transesterification

Lipase producing fungi *Aspergillus* spp., which showed highest lipase activity at 48- 72 hours (30°C for 3 days) on a rotary shaker (170rpm), was inoculated in lipase production medium (LP-medium containing 7.5% soybean flour, 2% glucose,0.1% KH_2PO_4,0.1% $NaNO_3$,0.05% $MgSO_4$ and 1%(v/v) olive oil with ph 7.0±2) [19]. The culture broth was centrifuged at 5000 rpm for 15 min. After that supernatant (4%) is collected and added to methanol solvent (methanol =0.277Xoil) and mixed with *Pongamia pinnata* oil. This mixture was agitated for 45 min-1hour at 250 rpm at the temperature of 70ºC and then added to separating funnel and kept for overnight. The fat or oil used is converted to esters during this stage. Glycerin and methyl esters are the two major products created after the reaction is completed. Glycerin is the denser of the two products and the obtained biodiesel is washed using 28% of distilled water and 1% of tannic acid (1 liter) [18].

Isolation of antioxidants from medicinal plants and microbes

Open Shaker Method

The Leaves of *Tinospora cordifolia*, *Magnifera indica*, *Azadirachta indica* , *Cucurma longa*, *Murraya koenigii* were cleaned and dried at 40°C in hot air oven [12]. Then the dried leaves were ground separately into fine powder using a mixer. 25 g dried powder of each plant's leaf was weighed and transferred into a beaker. 200ml of solvent (i.e. 80% methanol (1:6), 70% ethanol (1:10), 80% ethyl acetate (1:10)) was added into the beaker and the mixture was shaken using open shaker for 72hr at room temperature. Each extract was filtered using Whatman No.42 filter paper. The filtrate was collected and evaporated under pressure until all the solvent had been removed by distillation. The extracts were filled in the bottles and stored at 4°C for further use [20].

Soxhlet Extractor Method

The Leaves were shade-dried and pulverized to a coarse powder. Equal quantities of the powder that is 15g were exhaustively extracted with absolute ethanol (v/v), methanol (v/v), ethyl acetate solvents respectively in soxhlet apparatus at 40°C. The extract was evaporated under pressure until

all the solvent has been removed by distillation. The extracts were filled in the bottles and stored at 4°C for further use [21-22].

Preliminary Phytochemical Analysis of Isolated Plant Extracts

Test for Terpenoids (Salkowski test)

To 0.5 g each of the extract, 2 ml of chloroform was added. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for Anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Flavonoids

Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow coloration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminum solution were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for Saponins

0.5 g of extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate, was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other.

The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani Test)

0.5 g of extract diluted to 5 ml in water was added to 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer [23-24].

Quantitative analysis

Determination of total phenolic content

100 mg of the sample dissolved in 0.5 ml of water was mixed with 2.5 ml FCR (Folin ciocalteau reagent) (diluted1:10, v/v) followed by 2 ml of Na_2CO_3 (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at 30°C for 90 min. Results were expressed as Gallic acid equivalents (mg of Gallic acid/g dried extract) from Gallic standard curve [25-26].

Linoleic Assay Model System

0.284g of linoleic acid was mixed with 0.284g of Tween-20. 50 ml of sodium phosphate buffer (pH 7.0) was added, emulsified for 1 min. 500µl of extract was mixed with 5 ml of emulsion, from this 0.2 ml was taken and diluted to 5ml with methanol. The absorbance was measured at 234nm (i.e. at time t=0).The sample was kept at 50°C and absorbance was measured at 234nm for the interval of every 2 hrs to determine the antioxidant activity [27].

Stabilization studies of biodiesel by adding antioxidants

Transparent glass bottles were autoclaved at 121˚C for 30 minutes to maintain initial sterilization and the setup was made such that mouth of bottle was plugged with cotton having a 6mm tubing so that air passes through it. 50ml of biodiesel was added to each bottle under sterile conditions and 1ml of antioxidants extracts showing good phenolic content was added. The parameters like density, viscosity and total acid number was checked at interval of every 15 days [28]. The density of Biodiesel was measured by using specific gravity bottle, viscosity by Viscometer and acid value measurement was carried out by titration method. 1ml of sample was taken in conical flask and 20ml of neutral ethanol was added and kept in water bath until it dissolves. Further, it was titrated against 0.1N KOH using phenolphthalein indicator and blank experiment was done similarly without the sample [6].

Determination of Antimicrobial Activity

Bore well method

Nutrient agar plates were prepared under sterile conditions and different bacterial strains (*E. coli, Micrococcus, Bacillus subtilis, Proteus vulgaris, S. aureus, M. leutius, P. aeruginosa*) were inoculated by spread plate method. Bore wells were etched in plates using sterile borer. Different plant and microbial extracts were added to the wells and incubated for 24 hrs at 30°C. The zone of inhibition was observed and the diameter was measured in cm [29-31].

RESULTS AND DISCUSSION

The lipase produced from fungi (screened using Rhodamine B agar plates) was used for biodiesel production of *pongamia pinnata* oil by enzymatic method , similar transesterification reactions was carried out using *Aspergillus spp* and *mucor spp* in reference papers [17,18]. Various physicochemical parameters like viscosity, density and acid value of biodiesel substituted with different antioxidants was evaluated and compared with standard values. The results of Viscosity (Figure1) and Acid value (Figure 2) showed that biodiesel substituted with standards such as BHT, mixture of both BHT and GA, curry leaves and mixture of both Mango and Neem extracts were stable up to 120 days, whereas Biodiesel substituted with plant extracts such as Turmeric was stable up to 15 days [6].

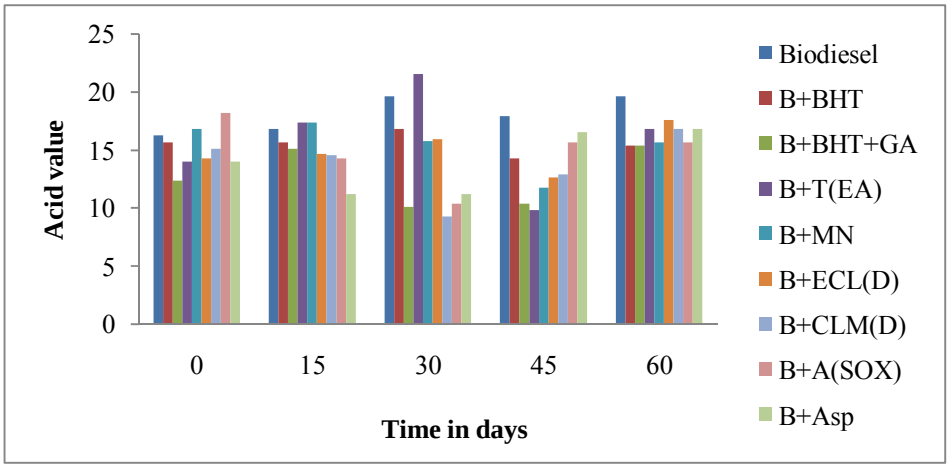

Figure 1. The above Histogram represents comparison of Acid values of Biodiesel and Biodiesel substituted with various standard antioxidants and natural antioxidants.

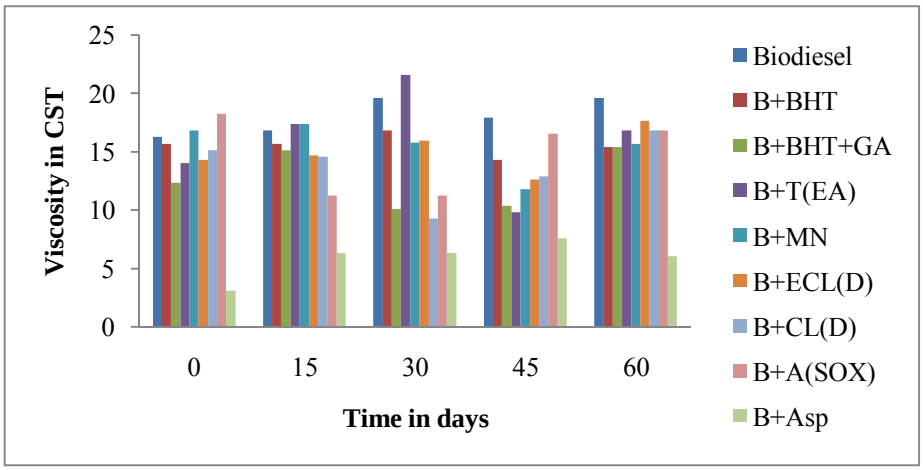

Figure 2. The above Histogram represents comparison of Viscosity of Biodiesel and Biodiesel substituted with various standard antioxidants and natural antioxidants.

Antioxidants were extracted from different medicinal plants like *Murraya koenigii , Magnifera indica, Tinospora cordifolia, Azadirachta indica, Curcuma longa* and a weed *Chromolaena odorata* using different solvents like methanol, ethylacetate and ethanol by open shaker and soxhlet extractor. Extraction done by using soxhlet extractor is more efficient than open shaker method. The phytochemical analysis was carried out for the plant extracts which include the qualitative tests for tannins, terpenoids, reducing sugars, saponins, anthraquinones, alkaloids and cardiac glycerides, similar analysis was carried out in reference paper [32]. The qualitative tests showed positive results for all dry and fresh samples of leaf extracts except MN (M&E solvents) and CO (Ethanol)(both fresh & dry) samples showed negative results for Flavanoids , MN(Ethyl acetate) (dry) for Tannins. Cardiac glyceride test showed negative results for fresh and dry leaf extracts except for MN (M), CL (EA) (fresh) showed positive results (Table 1, 2). The negative results are due to the inability of the solvent to extract the particular components.

Table 1. Qualitative analysis of dry plant solvent extracts.

Qualitative test	70% ethanol (1:10) dry					80% methanol (1:6) dry					70% ethylacetate (1:10) dry				
Plant samples	MN	A	T	CO	CL	MN	A	T	CO	CL	MN	A	T	CO	CL
Tannins	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reducing Sugars	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavanoids	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Antraquinones	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycerides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. Qualitative analysis of fresh plant solvent extracts.

Qualitative test	70% ethanol (1:10) fresh					80% methanol (1:6) fresh					70% ethylacetate (1:10) fresh				
Plant samples	MN	A	T	CO	CL	MN	A	T	CO	CL	MN	A	T	CO	CL
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reducing Sugars	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavanoids	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Antraquinones	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycerides	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+

Quantitative method for estimation of total phenolic content was done for all leaf extracts (fresh and dry)extracted from different solvents. The study revealed that, for dry samples, ethanol solvent extracted highest amount of phenol from MN(100mg/ml), CO(58mg/ml), CL(71mg/ml) respectively and ethyl acetate from A (97mg/ml) and methanol from T(75mg/ml). From this we infer that highest amount of phenol has been extracted from MN, for fresh samples, Ethanol solvent has extracted highest phenols from MN (160mg/ml), CL (100mg/ml) and methanol from CO (136mg/ml). (The

values were compared with reference [33-34]). From this we conclude that ethanol is the best solvent. MN showed highest phenolic content than other leaf samples. Fresh samples have highest phenolic content then dried samples (Table 3, 4). Antioxidant screening for all leaf extracts was done by Linoleic assay model system. The absorbance measured at 234nm decreased with time (for the interval of every 2hrs), which indicates decrease in oxidation which in turn shows increase in antioxidant activity.

Table 3. Determination of Total phenolic content for Fresh plant extracts.

Solvents	Plant samples	OD at 765nm	Conc. of Gallic acid (mg/ml)
Ethyl acetate	MN	0.382	57
	A	0.638	97
	T	0.125	18
	CO	0.363	45
	CL	0.232	40
Ethanol	MN	0.619	100
	T	0.225	77
	A	0.493	36
	CO	0.406	58
	CL	0.438	71
Methanol	MN	0.466	78
	T	0.474	75
	A	0.131	20
	CO	0.223	32
	CL	0.214	30

Table 4. Determination of Total phenolic content for Dry plant extracts.

Solvents	Plant samples	OD at 765nm	Conc. Gallic acid (mg/ml)
Ethyl acetate	MN	0.922	148
	CO	0.542	85
	CL	0.525	83
Ethanol	MN	1.02	160
	CO	0.521	80
	CL	0.659	100
Methanol	MN	0.888	138
	CO	0.827	136
	CL	0.62	93

The thermal stability of biodiesel is usually determined using a Rancimat method. Since the latter requires very complex setup and continuous heating system, which in turn increases power consumption [35], Thus thermal stability of Biodiesel was evaluated by just heating the biodiesel at regular intervals after every 15 days. The various properties were measured (Table 5, 6) which was found to be in line with standard values. Hence the biodiesel was found to be thermally stable upto 15 days. The different antioxidants used to maintained biodiesel stability were also evaluated for their antimicrobial activity using bore well method. The maximum zone of inhibition of antioxidant Extracts in cms (Table 7) was shown by the curry leaves ethanolic extract (dry), and turmeric ethanolic extract which were found to be more effective against *E.coli, Bacillus subtilis* and *Micrococcus.* Similar antimicrobial activity against *Bacillus stearothermophilus, Klebsiella*

pneumonia, Micrococcus luteus, Salmonella typhi was analyzed in reference paper [36-37]. In present work the most inhibited microorganism was *E. coli*.

Table 5. Screening for Antioxidant Activity.

Solvents	Plant samples	OD at 234 nm		
		0^{th} hr	2^{nd} hr	4^{th} hr
Ethyl acetate	MN	1.327	1.105	0.903
	A	0913	0.809	0.753
	T	0.665	0.662	0.552
	CO	1.114	0.954	0.813
	CL	1.250	1.374	0.554
Ethanol	MN	3.00	3.00	1.380
	A	0.398	0.326	0.202
	T	1.342	1.146	1.096
	CO	1.653	1.568	1.415
	CL	0.954	1.018	0.862
Methanol	MN	1.467	1.813	1.156
	A	0.908	1.073	0.929
	T	1.166	0.993	1.166
	CO	0.230	0.103	0.230
	CL	0.209	0.088	0.209

Table 6. Thermal Stability.

Sample	Density	Viscosity	Acid value
Positive control	920	6.69	16.830
		Before heating T = 0 days	
Biodiesel	912	5.468	09.817
B+BHT	920	4.642	12.622
		After heating T = 0 days	
Biodiesel	917.96	5.141	11.781
B+BHT	932	4.946	16.830
		Before heating T = 15 days	
Biodiesel	920	5.295	16.830
B+BHT	917	5.036	17.952
		After heating T = 15 days	
Biodiesel	920	5.340	14.023
B+BHT	919.9	5.878	18.516

Table 7. Antimicrobial Activity of Antioxidants.

Test Organisms	Zone of inhibition of antioxidant Extracts in cm						Zone of inhibition of Standard antibiotics in cm	
	A (SOX)	CLM (D)	T(EA)	MN	CL(E) (D)	Asp	Tetracycline	Streptomycin
E. coli	3.9	1.7	2.0	0.0	1.2	0.0	3.0	2.7
Micrococcus	2.1	1.6	3.4	2.3	1.5	1.2	2.7	2.8
B. subtilis	3.8	2.1	3.6	2.0	1.1	0.0	3.0	2.9
P. vulgaris	1.7	1.3	0.0	0.0	1.2	0.0	2.0	1.6
S. aureus	1.9	1.3	2.5	0.0	0.0	0.0	1.6	2.7
M. leutius	2.7	2.5	2.6	0.0	0.0	0.0	3.0	2.7
P. aeruginosa	2.0	2.0	2.5	1.5	1.3	1.1	3.0	2.7

CONCLUSION

The fungal strains proved to be good lipase producers as they showed zone of clearance around the colony using Rhodamine B for screening. Enzymatic tranesterification of *Pongamia pinnata* oil produced biodiesel and various physical parameters (like density, viscosity and acid value) were studied. Antioxidants extraction by soxhlet extractor was found to be more efficient than open shaker method. The Phytochemicals were qualitatively analysed. Quantitative method showed highest phenolic content for fresh leaf samples then dried samples and Ethanol being the best solvent for extraction of phenols. Linoleic assay model system revealed the extracts to posses Antioxidant activity. Biodiesel substituted with standards such as BHT, mixture of both BHT and GA, curry leaves and mixture of both Mango and Neem extracts were stable up to 120 days, whereas Biodiesel substituted with plant extracts such as Turmeric was stable up to 15 days. Hence the combination of Antioxidants showed good results for maintaining stability of Biodiesel. Antioxidants extracted from Plants showed more stability than organisms extracts. Further, antibacterial properties were also exhibited by the plant extracts against various bacteria and *E.Coli* being the most inhibited bacteria. Present research work emphasizes on biodiesel stability by natural antioxidants as a perspective of food security as commercial antioxidants can be diverted for the stability of industrially important food products rather than using for biodiesel stability.

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