Abstract
Rapid urbanization and increase in population have evoked tremendous attention for biofuels production to combat shortage of fuels, environmental concerns, foreign exchange savings and socioeconomic issues. In recent years biodiesel production from agro-industrial feedstocks such as waste vegetable oil, animal fat, grease, non-edible fruit oils etc., acquired prominent place to fulfill the gap between production and demand. The present investigation has been undertaken to explore a novel and environmentally friendly process for developing biodiesel production technology by subjecting dried fruits of Lagerstroemia speciosa to mild ultrasonication at 33KHz for 20 min at 35±2ºC for obtaining high lipid yield, precursor for the production of biodiesel by transesterification. The biodiesel compounds 2,4-di-tert-butylphenol, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid (Z,Z) methyl ester, 9-octadecenoic acid methyl ester, methyl stearate, cis-11,14-eicosadienoic acid methyl ester, 18-methylnonadecanoate were recognized as the main compounds in GC-MS analysis.

Keywords: Biodiesel, Lagerstroemia speciosa, dried fruits, ultrasonication, transesterification

Introduction
Fossil fuels such as petroleum, diesel, gasoline and liquefied petroleum gas occupied a prominent place in transportation industry (1). In order to meet the transportation demands of growing population, natural resources are explored extensively. Global fossil fuels consumption expected to decline 1000 million tonnes from 4000 million tonnes by 2050 due to the diminution of fossilfuel resources (2). Over time, exponential utilization of fossil fuels has generated an imbalance in the carbon cycle, dramatically increasing greenhouse gas and contributing to climate change (3). To combat shortage of fuels, environmental concerns, foreign exchange savings and socioeconomic issues related to the rural sectors of all countries in the world has urged a research interest for biofuels such as bioethanol, biobutanol, biodiesel, green diesel, bio methanol, dimethyl ether, bio-oil production (3-10). In recent years biodiesel production has evoked tremendous attention due to the advantage of environmental compatibility, biodegradable and alternative to petroleum fossil fuel (11). Biodiesel is a non-petroleum based alternative diesel fuel comprised of monoalkyl esters of long-chain fatty acids produced by transesterification of oils (11-13). Due to excess consumption of biodiesel, the gap between production and demand is widening. To fulfill the gap, with the advent of science and technology many researchers adopted new methods to explore various alternative agro-industrial waste oils for the production of biodiesel, such as soybean, rapeseed, coconut, rice bran, barley, wheat, peanut, corn, olive, sunflower, palm, jatropha and neem oils (14, 15). Of these oils, non-edible fruit oils dominate the biodiesel sector. However, utilization of non-edible dried fruits of Lagerstroemia speciosa for the production of...
biodiesel has not been reported. The plant *Lagerstroemia speciosa* belongs to the family *Lythraceae* of dicotyledons. This plant is commonly called as “Pride of India” It is a small to medium-sized tree growing to 20 m (66 ft) tall, with smooth and flaky bark, deciduous oval to elliptic leaves that are 8-15 cm (3.1-5.9 in) long and 3-7 cm (1.2-2.8 in) broad with an acute apex. The flowers are produced in erect panicles of 20-40 cm (7.9-15.7 in) long, each flower has six white to purple petals of 2-3.5 cm (0.79-1.38 in) long (16,17). The present research will be focusing on extraction of oils from non-edible dried fruits of *Lagerstroemia speciosa* for the production of biodiesel.

**Materials and Methods**

**Experimental Chemicals**

All chemicals and reagents used in this research were of analytical grade and are mostly purchased from Sigma, USA and Hi-media, Mumbai.

**Plant Collection and Preparation of Powder for Lipid Extraction**

Dried fruits of *Lagerstroemia speciosa* used for this study was collected in the month of November, 2018, at the location of 13.043443 N, 77.591154 E, Bruhat Bengaluru Mahanagara Palike (BBMP) park, Hebbal, Bengalore, Karnataka, India and authenticated at Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore, by Dr. V. Rama Rao with an accession number, RRCBI-3933 (Fig-1). The sample was collected in a sterile plastic container and it was brought to the laboratory for further processing (11).

The dried fruits were washed for 2-3 times with tap water to remove the surface debris and then dried in an oven at 40°C for 24 h (18). The dried fruits were milled to a coarse powder and stored in air tight container for oil extraction.

**Extraction of Lipid from Powder**

The total lipid content was extracted by modified Bligh and Dyer method (19). Experiment was conducted in two sets (A and B). In a 250 ml conical flask 10 g of dried powder and two-fold of 2M HCL were added and the mixture was vortexed for 24 h. After the 24 h set A was subjected to sonication (GT Sonic-GT-1730QTS) at 33KHz for 20 min at 35±2°C. During the treatment process, the temperature was maintained at 35±2°C by adding ice cubes (20). The set B was considered as control. Both the sets were subjected to centrifugation at 5000rpm for 10 min (Remi C24 Plus). After centrifugation the supernatant from set A and pellet suspension from set B was transferred into a 100 ml conical flask containing 4ml of distilled water, 20ml methanol, and 10ml of chloroform and vortexed for 15 min at room temperature. The chloroform layer was separated by centrifuging at 5000rpm for 10 min. After centrifugation, the chloroform phase was evaporated with rotary vapor and the residue was preserved for transesterification.

**Biodiesel Generation by Transesterification of the Lipids**

Transesterification was carried out in two sets in a 250 ml glass beaker equipped with a magnetic stirrer. 10ml of the lipid was taken in separate beakers for set A and set B and heated...
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**Results and Discussion**

In biodiesel produced from sonicated dried non-edible fruits of *Lagerstroemia speciosa*, 2,4-di-tert-butylphenol, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid (Z, Z)-methyl ester, 9-octadecenoic acid methyl ester, methyl stearate, cis-11,14-eicosadienoic acid methyl ester, methyl 18-methylnonadecanoate were recognized as the main compounds in GC-MS analysis (Table-1 and Fig-2 and 3). In non-sonicated dried non-edible fruits of *Lagerstroemia speciosa*, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid (Z, Z)-methyl ester, 9-octadecenoic acid methyl ester and methyl stearate were recognized as main compounds (Table 2 and Fig 4 and 5). According to NIST17.lib library and previous reports of Dwivedi *et al.*, 9,12-octadecadienoic acid (Z, Z)-methyl ester was considered as one of the chief biodiesel compound and same was the major constituent with 83.23% of peak area (Fig-6), with retention time of 21.4, 85.18% of peak area with a retention time of 21.4 for both sonicated and non-sonicated samples (23).

**Table 1:** GC-MS results of sonicated dried non-edible fruits oils of *Lagerstroemia speciosa*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak Value</th>
<th>Compound name</th>
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<tbody>
<tr>
<td>1</td>
<td>13.481</td>
<td>2,4-di-tert-butylphenol</td>
</tr>
<tr>
<td>2</td>
<td>18.782</td>
<td>Hexadecanoic acid methyl ester</td>
</tr>
<tr>
<td>3</td>
<td>21.443</td>
<td>9,12-Octadecadienoic acid (Z, Z)-methyl ester</td>
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<td>4</td>
<td>21.527</td>
<td>9-Octadecenoic acid methyl ester</td>
</tr>
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<td>5</td>
<td>21.932</td>
<td>Methyl stearate</td>
</tr>
<tr>
<td>6</td>
<td>24.458</td>
<td>cis-11,14-Eicosadienoic acid methyl ester</td>
</tr>
<tr>
<td>7</td>
<td>24.924</td>
<td>Methyl 18-methylnonadecanoate</td>
</tr>
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</table>

**Analysis of Fatty Acid Methyl Esters**: Biodiesel produced from dried non-edible fruits of *Lagerstroemia speciosa* was analysed by using mass spectrometer (Shimadzu GCMS-QP2010SE) with two narrow-bore capillary columns, coupled to agas chromatograph (Shimadzu GC-QP2010). The GC column used was fused with silica capillary column (QP2010, 30m × 250 μm, film thickness 0.25 μm). The pressure of the carrier gas (helium) was 72.6 kPa at the initial oven temperature with flow rate of 6.6 ml/min. All standards and samples were injected in split mode (split/column flow rate 1.20 ml/min). The injector temperature was 250°C; the column oven temperature was 60°C, rose to 280°C and total run time was 40 min. The mass spectrometer was operated in the electron impact (EI) mode at 82 eV in the scan range of 35-500 m/z. The temperature of the transfer line and of the ion source was set to a value of 200 and 280°C respectively. The injection sample volume was 8.0 μl. Peak identification of an oil was performed by comparison with retention times of standards and the mass spectra obtained compared with those available in the Wiley and NIST libraries (Wiley Registry TM, 8th Edition Mass Spectral Library and the NIST 08 Mass Spectral Library (NIST/EPA/NIH) 2017 version) with an acceptance criterion of a match above a critical factor of 80% (11, 22).

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Up to 50°C on hotplate (DLAB MS-H280-Pro). For both the sets 60 ml of sodium methoxide was added and stirred vigorously for 1 h on a magnetic stirrer (21). The mixture was transferred to a separating funnel and glycerol was allowed to settle and separate for 24 h. After draining the glycerol, methyl esters present in the upper layer were collected and analyzed using Gas Chromatograph-Mass Spectrometer.

**Results and Discussion**

In biodiesel produced from sonicated dried non-edible fruits of *Lagerstroemia speciosa*, 2,4-di-tert-butylphenol, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid (Z, Z)-methyl ester, 9-octadecenoic acid methyl ester, methyl stearate, cis-11,14-eicosadienoic acid methyl ester, methyl 18-methylnonadecanoate were recognized as the main compounds in GC-MS analysis (Table-1 and Fig-2 and 3). In non-sonicated dried non-edible fruits of *Lagerstroemia speciosa*, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid (Z, Z)-methyl ester, 9-octadecenoic acid methyl ester and methyl stearate were recognized as main compounds (Table 2 and Fig 4 and 5). According to NIST17.lib library and previous reports of Dwivedi *et al.*, 9,12-octadecadienoic acid (Z, Z)-methyl ester was considered as one of the chief biodiesel compound and same was the major constituent with 83.23% of peak area (Fig-6), with retention time of 21.4, 85.18% of peak area with a retention time of 21.4 for both sonicated and non-sonicated samples (23).
Table 2: GC-MS results of Non-sonicated dried non-edible fruits oils of *Lagerstroemia speciosa*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak Value</th>
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<td>2</td>
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<td>9,12-Octadecadienoic acid (Z, Z)-methyl ester</td>
</tr>
<tr>
<td>3</td>
<td>21.526</td>
<td>9-Octadecenoic acid methyl ester</td>
</tr>
<tr>
<td>4</td>
<td>21.930</td>
<td>Methyl stearate</td>
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Fig-3: GC-MS image of Sonicated sample

Fig-4: Development of organic layer in non-sonicated sample
Conclusion

Biodiesel production has evoked tremendous attention due to the advantage of environmental compatibility, biodegradable and alternative to petroleum fossil fuel. In order to fulfil the gap between production and demand various techniques adopted for obtaining high yield of biodiesel from the agro-industrial residues, which are considered as environmental pollutants. Based on our research we are suggesting application of ultrasonication will also help in generating high yield of biofuel producing compounds from dried waste. This technique may be applicable for large scale production of eco-friendly biofuels by exploring other substrates.

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References


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