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Extraction, characterization and kinetics of ultrasonic assisted extraction of biooil from *Annona squamosa* seeds

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The current research work investigated the extraction of biooil from custard apple seed (*Annona squamosa)*. The Biooil extraction process has been carried out using the method of ultrasonic-assisted solvent extraction. The process conditions for biooil extraction have been optimized for attaining the maximum yield of biooil. The presence of functional groups and fatty acids in the extracted *Annona squamosa* seed oil has been analyzed by FT-IR and GC-MS analysis. The predominant compound present in the extracted oil was oleic acid, which is confirmed by GC-MS. The kinetics study results indicated that the biooil extraction process fits very well into first order reaction kinetics. The physicochemical properties of biooil have also been analyzed. The maximum biooil yield of 23% (w/w) from *Annona squamosa* seed has been achieved under the optimal process conditions with the extraction time of 20 min, temperature of 50°C and hexane to biomass ratio of 3:1 v/w.

Keywords: *Annona squamosa* seed oil, Characterization, Optimization, Reaction kinetics, Ultrasonic-assisted extraction

The demand for global energy continues to grow due to its increase in living standards and the prosperity of the emerging world leading to significant inequality in energy consumption. To decarbonize the aviation sector, the biofuels play a major role since the required energy demands are not met by hydrogen or batteries for energy density in aviation sector. In 2019, the demand and supply for liquid fuels (biodiesel, bioethanol etc.) in the global market has increased at its peak. In a report by the International Energy Agency (IEA) the consumption of global energy around the world could exceed75% in 2050 which could lead to increased CO_2 emission[1].

Global liquid fuels are subjected to heightened levels of uncertainty. From the March-Short-Term Energy Outlook (STEO) an assumption was made stating that there could be an in increase in 3.6% by 2022 and by 2.7% in 2023 after growing to 5.7% in 2021. The primary energy that dominates the outlook is renewable energy, where the world shifts toward lower-carbon energy sources. The renewable energy led by biomass, biofuel, bio-methane, wind, geothermal, solar energy accounts for entire growth in the global power generation. The depletion of the primary energy sources led to alternative energy source known as Biodiesel which was given in 1992 by the National -Soy- Diesel Development Board, USA[2].

Plant and animal sources can be used for the biodiesel production from their oils. Edible oils, non-edible oils, algae, waste cooking oils could be a major biomass source for the biodiesel production. Furthermore, a report states that nearly 350 crops that produce biodiesel could be used for worldwide production. Sunflower oil, Soy bean oil, Rapeseed oil, coconut oil are the edible oils used for biodiesel production and they account for 95% of the biodiesel being produced currently[3,4]. The production of biodiesel from these sources could elicit global imbalance for food supply and further instigate food insecurity[5,6].

The second generation biodiesel production is effectuated using non edible oil which can be obtained from different plant sources such as jojoba, tobacco seed, jatropha, neem, mahua, candlenut, karanja, sea mango, yellow oleander[7]. Although edible oils and non-edible oils have similar saturated and unsaturated fatty acids, non-edible oils obtained from these sources are incompatible for human consumption because of the presence of toxic substances. In order to diminish the food supply issue non edible oil producing plants can be grown amidst waste lands extensively[8].

The sugar apple, sweet sop or custard apple is commonly called as Sitaphal in traditional language.

This belongs to the Annonaceae family with botanical name *Annona squamosa*, commonly found in deciduous forests, grows wild in various parts of India[9,10]. Custard apple (*Annona Squamosa*), is a tropical tree or shrub, native to the Amazon rainforest. The cone-shaped fruit, with a purple skin can be used to prepare milkshakes, ice cream and even sorbets[11]. It is grown ubiquitously in low land tropical climates that include Southern Mexico, Tropical Africa, Australia, Central and South America, Hawaii, USA, Polynesia, Florida and the Antilles. The height of the sugar apple tree is from 3 to 6m along with irregular branches. The fruit is 6 to10 cm long ovoid or rather conical, made up of knobby segments that are pale green or grey green externally. The inner fruit is creamy white, conically segmented, palatable flesh. The segments enclose a barrel- shaped black or dark brown seed which is about 1.25 cm long. Overall totally 20 to 38 or possibly more seeds are present in an average fruit[12]. Research work indicates custard apple seed contains 26% of oil content. Custard apple seed oil is used in cosmetic industries to produce shampoos and soap for black hairs and it gives shine and strength to hair. Studies convey that the seed powder of custard apple causes severe redness to the skin and it might also cause extreme eye injury leading to blindness.

To obtain higher yield of biooil from *Annona squamosa* seed, the method of ultrasonic assisted solvent extraction method was found to be a facile and highly effective method. The methods work in low thermal conditions such that the thermal damage to the extracted biooil could be reduced and along with the preservation of molecular structure of the functional groups present in the extracted oil[13]. The ultrasonic-assisted solvent extraction was done by influencing various parameters such as ultrasonic power, solvent of volume to solid used, ultrasonication time, and temperature. This method of ultrasonic-assisted biooil extraction was found to produce the higher yield when compared to conventional methods[14].

In the present work, the biooil extraction from *Annona squamosa* seed was done using ultrasonic-assisted solvent extraction method and thus the process conditions for biooil extraction was optimized to obtain highest yield of biooil. The obtained biooil was characterized using FT-IR and GC-MS to study the presence of functional groups and fatty acids in the extracted biooil. The physicochemical properties of the biooil such as pour point, cloud point, its kinematic viscosity, moisture content, density and calorific value was also analyzed.

Experimental Section

Materials

The Custard apple (*AnnonaSquamosa*) fruit seeds were collected from seed shop located in Coimbatore district. The solvents required for the extraction and optimization process were Methanol, hexane, Isopropanol, chloroform and diethyl ether. All the solvents were analytical grade (99.9 % extra pure) from Merck laboratories, Mumbai, India which was used in further processes as such.

Preparation of the sample

The A*nnona squamosa* fruit seeds were collected, weighed and dried under the sun for seven days. The moisture content was removed by further drying the seeds in an oven at 60°C for 3 days. The seeds dried were ground to fine powder and stored at room temperature for further processing.

Biooil extraction from *Annona squamosa* seed by ultrasonic-assisted extraction

The ultrasonic assisted extraction method was an important objective carried out for the extraction of biooil from *Annona squamosa* seeds. Different solvents such as methanol, hexane, isopropanol, chloroform and diethyl ether were used for the extraction process and the yield was noted.Since hexane shows high yield of biooil, it was further used for the extraction process[15]. Ultrasonic assisted method of extraction shows better improvement in the biooil yield. For the extraction of biooil 10 g of the finely ground powder of *Annona Squamosa* seed was taken and was dissolved well with hexane.

Annona Squamosa seed powder (10 g) was taken and mixed well with 100 mL of the homogeneous solvent hexane in a 250 mL beaker. This was sealed with an aluminium foil for the prevention of solvent evaporation. The extraction was done for 20 min in an ultrasonicator equipment. The sample was kept inside the ultrasonicator equipment where the probe touches the seed-solvent mixture and the setup was switched on. When the probe touches the sample the sample breaks into smaller particle which releases oil. The processed sample was taken for further process[16]. The filtration of the ultrasonicated sample was done using Grade 1 whatmann filter paper and the filtrate collected was kept inside a hot air oven at 60°C for

8 h for solvent evaporation. After evaporation the bio-oil was obtained. The bio-oilwas stored for further studies. The biooil yield extracted using ultrasonic-assisted method was calculated using the Equation[17] 1.

$$Oil\,Yield\,(\%) = \frac{Weight\ of\ extracted\ oil}{Weight\ of\ seed} * 100 \quad \ldots (1)$$

Kinetics of biooil extraction from *Annona squamosa* seeds

The kinetics for the process of oil extraction was observed at different temperature (30, 40, 50, 60 °C) and time (5, 10, 15, 20 min). It is represented by Equation 2,

$$(dY/dt) = KY^n \quad \ldots (2)$$

Where, K denoted as the rate of the reaction (min^{-1}), Y was the biooil yield in %, n denoted as the order of kinetics and t as the time for extraction. The graph was plotted between ln[Y] versus ln(dY/dt) at the studied extraction time and temperature. The intercept and slope determine the rate constant[18].

The activation energy for the oil extraction process can be obtained from Arrhenius Equation 3.

$$K = Ae^{-Ea/RT} \quad \ldots (3)$$

The graph was plotted between the values of log K and 1/T, where T in kelvin. The slope was determined from the line and the activation energy was calculated for biooil extraction from *Annona squamosa* seeds.

Characterization of extracted biooil

The Fourier Transform- Infra Red spectroscopy for the extracted biooil indicates the functional group and the molecular geometry which were noted. The Gas- Chromatography with Mass Spectroscopy (GC-MS) was used to identify the fatty acids present and their peaks were noted. The calorific value, pour and cloud point, density, viscosity and moisture content and free fatty acid (FFA) content were analyzed.

Results and Discussion

Optimization of biooil extraction

The parameters that were studied for optimization are different solvents, solvent to biomass ratio, various temperature and time.

Effect of different solvents on biooil extraction

For effective biooil extraction from the *Annona squamosa* seeds, selection of solvent plays a major role. The biomass was subjected to the following solvents such as Chloroform, hexane Methanol, Isopropanol and Diethyl ether. From these, the highest yield of oil extraction 14% was achieved using Hexane when compared to the oil yields such as 7.0, 5.4, 5.8 and 3.6% using the solvents as Chloroform, Methanol, Isopropanol and Diethyl alcohol respectively. Figure 1(a) depicts the solvents that showed low yields when compared to Hexane. Therefore, the solvent hexane was used for further processes.

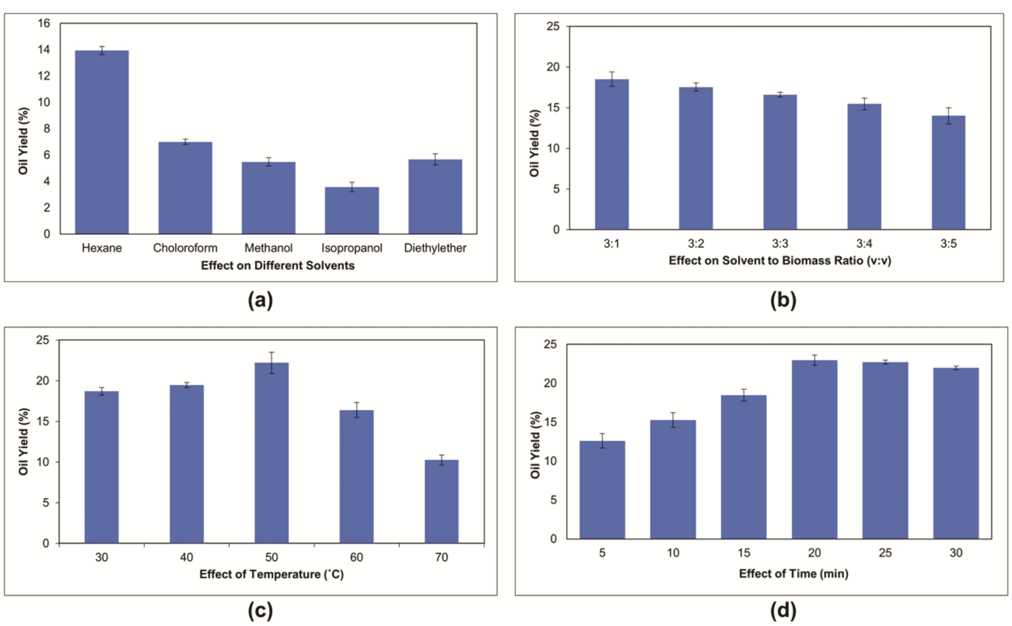

Fig. 1 — (a) Effect of different solvents; (b) Effect of solvent to biomass ratio; (c) Effect of temperature and (d) Effect of time on ultrasonic assisted extraction of *Annona squamosa* seed oil

Effect of solvent to biomass ratio on biooil extraction

A homogeneous solvent was used for the extraction process which enhances the oil yield from *Annona squamosa* seeds. The effect of the solvent to biomass ratio for the extraction of oil was studied. The solvent to biomass ratio was investigated for various ranges[19]. In Fig. 1(b), the maximum biooil yield of 18% was obtained at 3:1 solvent to biomass ratio. The other solvent to biomass ratio shows low yield. Therefore 3:1 ratio was found to be the optimum ratio for extraction of oil. On further increasing the solvent to biomass ratio had no increase in oil yield[20]. Therefore, it is observed that the biooil yield was directly proportional to the solvent ratio until reaching the equilibrium.

Effect of temperature on biooil extraction

The effect of extraction temperature plays a significant effect in the process reaction rate and intensification of biooil. The *Annona squamosa* seed oil was subjected under different temperatures such as 30, 40, 50, 60 and 70°C with a reaction time of 30 min to identify optimized temperature. It was found that at 50 °C there was a maximum yield of 22% biooil Fig. 1(c) and further on increasing the temperature, a decrease in biooil yield was noted. As the temperature increased due to the loss of solvent a decrease in biooil was noted[16].

Effect of time on biooil extraction

The time for extraction plays a major role in biooil yield. The yield of bio oil increases in proportion to the reaction time. Longer exposure of sample with solvents leads to improper separation of oil which affects the process purification. The *Annona squamosa* seed oil was subjected to various extraction time such as 5, 10, 15, 20, 25, 30 min and the effect of extraction process was studied[16]. A maximum yield of about 23% biooil at 20 min which was maintained for future processes Fig. 1(d).

Characterization of extracted *Annona squamosa* seed oil using FTIR analysis

The functional groups present in the seed oil of *Annona squamosa* were analyzed using FT-IR spectrum as shown in Fig. 2. The peaks between 4000 cm^{-1}and 500 cm^{-1} were recorded. The asymmetric stretching vibration of O-H groups and symmetric stretching vibration of C-H groups corresponds to 3006.50 cm^{-1} which represents the presence of carboxylic acids, alcohol and alkene. The peaks at 1235.73, 1159.98, 1116.77 and 1096.62 cm^{-1} represent C-O and C-N stretching vibration. The peaks at 1461.05, 1374.36 and 721.44 cm^{-1}denotes O-H, C-H and C=C bending vibration respectively. The lowest peak at 589.46 cm^{-1} indicates the presence of halo compounds[21].

Characterization of extracted *Annona squamosa*seed oil using GC-MS analysis

The occurrence of fatty acids in *Annona squamosa* seed oil was determined using GC-MS instrument (Agilent 8890 GC system). The obtained chromatogram was depictedin Fig. 3. The free fatty acids in the biooil were found to plays a significant role in transesterification process for methyl ester production. The highest peak represents the presence of Oleic Acid with the area percentage of 58.12% and retention time of 29.9 min. The peaks with the area percentage of 9.93%, 5.32% and 4.87% denotes the presence of γ-Sitosterol, Stigmasterol and Campesterol with the retention time of 40.521, 39.817, 39.464 mins respectively. The abundant presence of Oleic acid confirms that the biooil is highly suitable for the production of methyl esters[21].

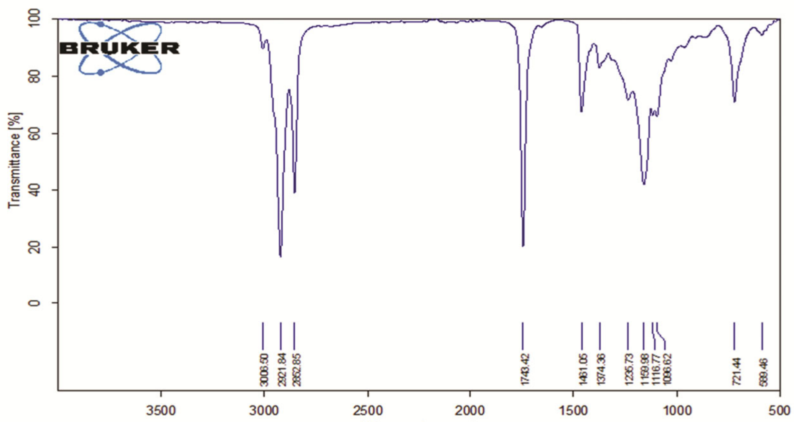

Fig. 2 — FT-IR spectrum of extracted *Annona squamosa*seed oil

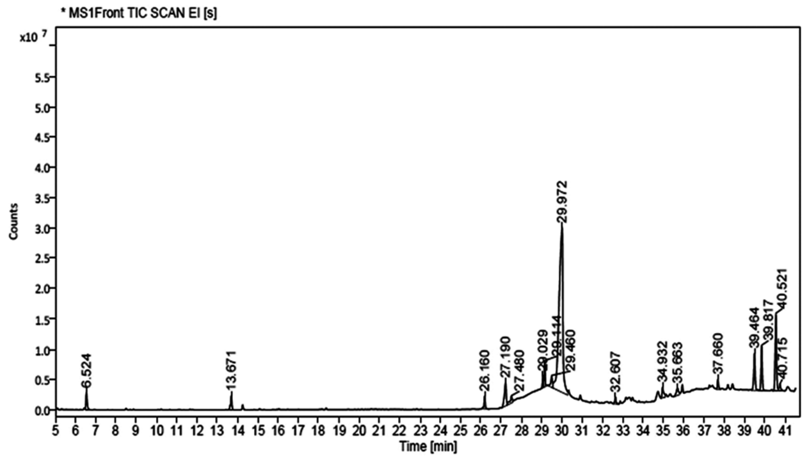

Fig. 3 — GC-MS spectrum of extracted *Annona squamosa* seed oil

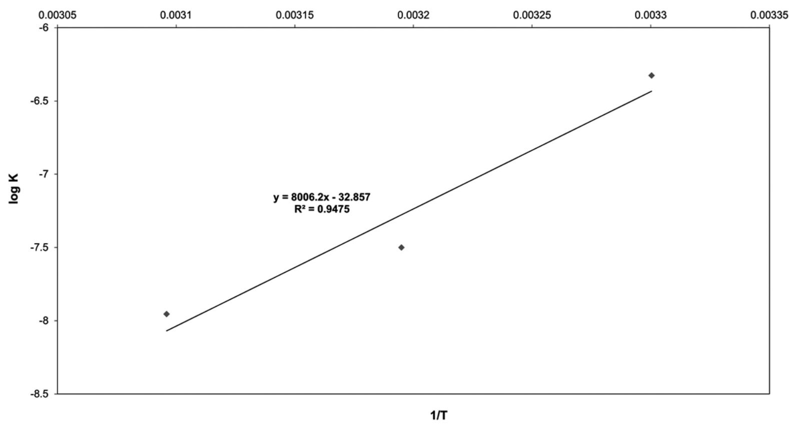

Fig. 4 — Arrhenius plot of log(K) versus 1/T for determination of activation energy

Kinetics study and determination of activation energy

The kinetics for oil extraction were studied under various time (5, 10, 15, 20 min) and intervals of temperature (30, 40, 50, 60°C). The graph drawn between log (Y)vs log(dY/dt) at different time and temperature was noted. The biooil extraction process from *Annona squamosa* seed found to be fitted suitably under first-order reaction kinetics model. The process of biooil extraction was subjected to different extraction time intervals and temperature to study the kinetics. The minimum energy required for the biooil extraction process was determined by the graph plotted between log(K) and 1/T shown in Fig. 4. The activation energy for the biooil extraction process was determined and found to be Ea = 66.56 kJ/mol[22].

Physicochemical properties of extracted *Annona squamosa* seed oil

The physicochemical properties of the extracted *Annona squamosa* seed oil was analyzed and thus the results were shown in Table 1. The free fatty acid of *Annona squamosa* seed oil was 9.02 NaOH in mL/ g of oil[23]. The oil density is 0.928 g/cc and 6.42 cSt is the kinematic viscosity which was analyzed at 40°C for the sample. Cloud and pour points are 6 and -8°C respectively. The moisture content is 1.00%. Higher moisture content affects the process of transesterification and decreases yield of the methyl ester. The determined calorific value of

Table 1 — Physicochemical properties of extracted *Annona squamosa* seed oil

S. No.	Parameters	Results
1	FFA	9.02 NaOH in mL/ g of oil
2	Density	0.928 g/cc
3	Kinematic viscosity @ 40ºC	6.42 cSt
4	Cloud point	6°C
5	Pour point	-8°C
6	Moisture content	1.00 %
7	Calorific value	6315.808 Cal/g

Annona squamosa seed oil was found to be 6315.808 Cal/g[22].

Conclusion

The homogeneous solvent based ultrasonic assisted solvent extraction method has been used of extraction of biooil from the *Annona squamosa* seeds. The extraction time of 20 min, temperature of 50 °C and hexane to biomass ratio of 3:1 v/w are found as optimum for maximum biooil yield of 23% (w/w) from *Annona squamosa* seeds. The kinetics of the ultrasonic-assisted biooil extraction process is found to fit to first order reaction kinetics. The estimated activation energy of the extraction process is found to be E_a= 66.56 KJ/mol. The peaks obtained using FT-IR confirm the presence of functional groups and the types of vibrations were noted. The GC-MS results confirm the presence of oleic acid which is the desired fatty acid in the extracted biooil.

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