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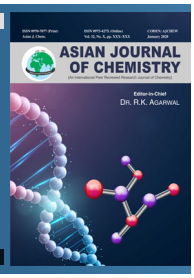

Oil Extraction from *Trichosanthes tricuspidata* Seed using Conventional Soxhlet Apparatus

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Soxhlet set up is used for the extraction of bio-oil from the seeds of *Trichosanthes tricuspidata* using various solvents (methanol, hexane, isopropyl alcohol). In the present study, the oil from solvent extraction method is investigated for the presence of fatty acid groups and also tested for suitability of this oil being used as a cheap source for producing bio-oil. The bio-oil extraction is influenced by various factors like time of extraction, types of solvent used for extraction and the production rate is also predominately influenced by the particle size of the sample (seed) for extraction. The analysis of the constituents in bio-oil produced is done through a GC-MS and the functional group is determined by FT-IR. This work is carried out in a lab scale and hence the extraction process is initiated with the particle size ranging from 1 mm, 0.55 mm and 3 mm with a variable time period of extraction such as 1, 2, 3 and 7 h. Particle size of 3 mm was selected in order to study the effect of solvent type. The experimental results have indicated the optimal condition for the highest yield of oil extraction of 20 % (w/w). The optimum values of extraction time and particle size are 180 min and 0.55 mm, respectively and also the experimental results have revealed isopropyl alcohol as the suitable extraction solvent.

Keywords: *Trichosanthes tricuspidata*, Soxhlet extraction, Bio-oil, Fatty acids.

INTRODUCTION

Globalization has led to increase in consumption of non-renewable resources due to a mass production of vehicles for transportation [1]. The main source of fuel for many vehicles is the fossil fuel which is non-renewable. Hence in future, the world will be in need of an alternative fuel produced from bio-oil [2]. This bio-fuel which are derived from bio-oil have many advantages over the petroleum based fuel. They have less sulfur and harmful greenhouse gas emission. The bio-fuel produced from bio-oil is renewable, sustainable, cost-effective and efficient source of energy [3]. Among many alternatives, hydrogen, bio-fuels, natural gas and syn-gas are likely to emerge as the four strategically important sustainable fuel sources in the near future. Thus, among all these four sources, bio-fuel is the most environmentally friendly energy resource [4].

Currently, many industries are finding the solution for this energy crisis and have an estimation that oil from the crude (fossil) can be replaced by the alternative sources like bio-oil (extracted from plant fruits, seeds, *etc*.) and biogas produced from the biomass. In this work, we are investigating a rare indigenous species called *Trichosanthes tricuspidata* seed oil. This species belongs to the genus *Tricuspidata* and not much of previous work has been done on this particular species. *Trichosanthes tricuspidata* (family: Cucurbitaceae) commonly known as Lal indrayani is found at an elevation of 1200 to 2300 m. It grows as a large climber, often attaining a height of 9-10 m. *Trichosanthes tricuspidata* has been widely used for curing many diseases like asthma, migraine, fever, diabetic carbuncles and other maladies [5]. *Trichosanthes tricuspidata* (family: Cucurbitaceae) is found in the eastern Himalayas in India and in southern China, southern Japan, Malaysia and tropical Australia. The methanolic extract of fruit of *Trichosanthes tricuspidata* is used as a larvicide for killing *Culex quinquefasciatus* which causes malaria [6].

Presently, only a few studies have been done on this species because it is highly vulnerable and found only in some specific regions. The extract of fruit of this plant found to be cytotoxic

in KB cells, and two new cucurbitacins were reported: *tricuspidatin* and 2-O-glucocucurbitacin and also the presence of a protease (an enzyme which break down protein and peptides) from the sarcocarp of the fruits of this plant [7]. Cucurbitane, hexanorcucurbitane and octanorcucurbitane glycosides are obtained from fruits of *Trichosanthes tricuspidata* [8]. From the fruits of *T. tricuspidata*, 14 cucurbitane glycosides were isolated such as cucurbitacin K, 2-O-β-glucopyranoside, a hexanorcucurbitane glucoside and octanorcucurbitane glucosides along with two known cucurbitane glucosides [9-11].

In this experiment, extraction of oil from the solid matrix containing phytoconstituents (seed) of *T. tricuspidata* fruit is kept in contact with a suitable solvent without any impurities. There are many types of extraction methods like soxhlet, maceration, microwave assisted extraction, ultrasonic assisted extraction, *etc*. [12,13]. Conventional soxhlet method of extraction is the easiest and cheapest method to test and evaluate the sample at lab scale [12].

The main objective of this study is to estimate the chemical constituents of bio-oil extracted from the seeds of *T. tricuspidata* fruit. Apart from evaluating the chemical constituents, the yield of bio-oil is also optimized to get the maximum quantity of oil by adjusting various parameters *viz*. time, type of solvents, particle size, *etc*. The physico-chemical and GC-MS analysis performed for the identification of the fatty acids. The bio-oil extracted is also tested for the basic fuel properties like iodine value, kinematic viscosity and saponification value, which will determine the fuel quality of bio-oil [14].

EXPERIMENTAL

The fruit of *Trichosanthes tricuspidata* was collected from Thanjavur city, situated in the southern part of India during the mid summer of year 2018. The fruit sample was washed with water to remove any dirt on the scarp. After that, the scarp is peeled off and the pulp is dissected into two halves. The seeds are separated manually and then dried under the sun for few days. Then, *Trichosanthes tricuspidata* seeds were grinded using mechanical grinder. After this step, the seeds were separated using sieve-shaker into three different particle sizes *viz*. 0.55 mm, 1.00 mm and 3.00 mm. These particles are tested to determine the optimum size for obtaining the greater yield of oil.

Selection of solvents: Choice of solvent is made based on the maximum extraction yield of oil at a particular time interval by the solvent chosen. In this test, the extraction procedure has been carried out for about 3 h using different types of solvents (methanol, ethanol, isopropanol, hexane, *etc*.) and with a fixed amount of *Trichosanthes tricuspidata* seed (75 g). The above leaching process is carried out with a fixed particle size of 3 mm in conventional Soxhlet setup.

Experimental setup: Solvent extraction is done in a Soxhlet apparatus to extract *Trichosanthes tricuspidata* oil from its seeds. The apparatus consists of a glass extractor, fitted in between a round bottom flask at the bottom and a water cooling condenser at the top. Inside the glass thimble holder, solid matrix of seeds is placed within thimble by a cotton bag suspended with a thread in the glass extractor. The round-bottom distillation flask initially contained an extracting solvent and it is heated up by electrothermal heating mantle having a maximum capacity of 1 L and 300 W power up to a maximum temperature of 450 ºC. The solvent inside the flask is heated till it attains the boiling point and the vapours start moving up in the condenser by contacting through the sample matrix (seed powder). After a thorough contact and extraction of the required bio-active compounds, the solvent condenses and accumulate again into the flask (extractor). When the solvent contact takes place, the bio-active compounds are leached out from the seeds. The process of leaching thus take place and also the mass transfer occurs between the seed and the solvent particles until most of the desired oil is extracted from the seed. The main force which is driving the above process is hydrostatic pressure head, the sample surface area of contact and also the time of contact. The above factors mainly influence the yield of oil extraction.

Extraction of *Trichosanthes tricuspidata* seed oil: Soxhlet apparatus is used for extraction of oil by employing various solvents. This process is continuously repeated several times until the extraction of oil is complete. The extraction was carried out for a time period 1, 2, 3 and 7 h with different solvents with a stable weight of seed (75 g) in 25 mm × 300 mm cellulose thimble. The sample is placed in the extraction chamber of a 250 mL soxhlet apparatus fitted with the condenser, which is placed on a 1000 mL distillation flask, a constant volume of solvent (400 mL) in the distillation flask in our process. Seed oil was then extracted under reflux with isopropanol for 1, 2, 3 and 7 h (15-20 cycles/h). After that, the solvent is removed using a rotary evaporator under vacuum. The experimentation was performed in triplicate and the mean value was reported. The yield of oil extracted was expressed as a percentage of the weight of oil extracted obtained relative to the weight of date seeds used for extraction.

$$\text{Yield of oil extraction} = \frac{\text{Weight of oil extraction}}{\text{Weight of } T.\ tricuspidata} \times 100$$

Analysis of extracted seed oil: The GC-MS analysis was carried out using Shimadzu GC-MS QP 2010 Plus consist of automatic operation controller (AOC-20i interfaced auto-injector) a gas chromatogram and mass spectrometer. Restek Rtx-5MS capillary column (Diameter 0.32 mm, length 30 m, thickness 0.50 m) has been employed for separation of components. Helium with a purity of 98 % was used as carrier gas at a constant flow rate of 1.73 mL/min and an injection volume of 0.5 L was employed. The temperature of injector was maintained at 270 ºC. The oven temperature was programmed from 50 ºC for 2 min, inversed to 150 ºC at 8 ºC /min and to 240 ºC at 8 ºC/min and held at 250 ºC for 20 min. Mass spectra were obtained at 80 eV. 1 µL of the sample was then manually injected into the GC/MS using a 5 µL micro syringe (SGE, Australia). Data analysis is performed using GC/MSD CHEMSTATION software that is used to handle mass spectra and chromatograms.

RESULTS AND DISCUSSION

Characterization of *Trichosanthes tricuspidata* seed: The seed constitutes about 65 % of the fruit weight. The oil contents in seed were estimated to be about 12 % . The moisture content and volatile matter content of *Trichosanthus tricuspidata* was also determined by following the British Standards Institution procedure. The moisture content was found to be 11 %

which seems to be higher because the seeds used were fresh and not dried enough.

Effect of different solvents: Many studies have confirmed that in plant species, polar solvents produce a higher yields of phenolic concentration as compared to non-polar solvents [15]. Similarly, in the present work different types of solvents were used to extract the bio-oil from the seed of the sample. The highly polar solvents like water can also be used for extraction but separation of oil would be difficult (Fig. 1). On the other side, absolute alcoholic solvents decreases the yield. So, applications of combined mixture of water with other organic solvents makes it a moderately polar medium, which ensure the optimal conditions for extraction and may result in increase yield. The extraction by swelling of seed materials and the contact surface area between the solid seed matrix and solvent finally improves the extraction yield.

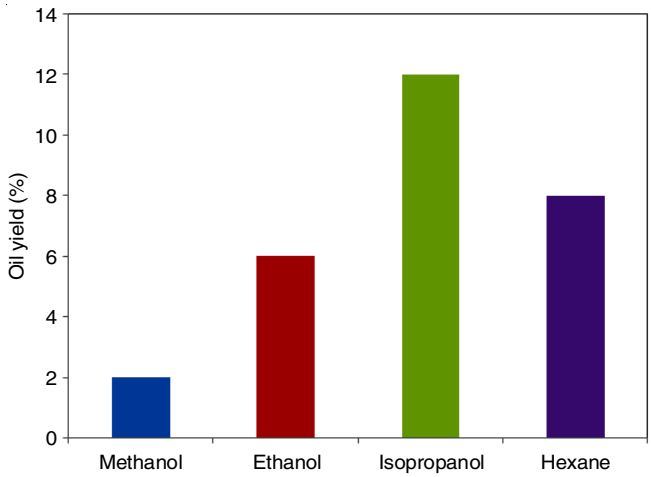

Fig. 1. Effect of solvent on oil extraction yield

Effect of contact time: It is well known that the rate of extraction will increase as the contact time increases. Thus, it has been observed that the oil extraction rate was quick at the starting of extraction process before reaching the steady state (Fig. 2). This is because the driving force for transfer of oil from the solid phase to liquid phase is higher at the start of the process. In other words, the difference in oil concentration between the solid phase and solvent phase is greater in the initial extraction process. Therefore, the oil diffuses rapidly from date seed to the solvent and the maximum amount of extractable oil was transferred. The oil yields unchanged even after prolonging the time of extraction. The optimum extraction time is about 3 h for all particle sizes ranging from 0.55 to 3 mm.

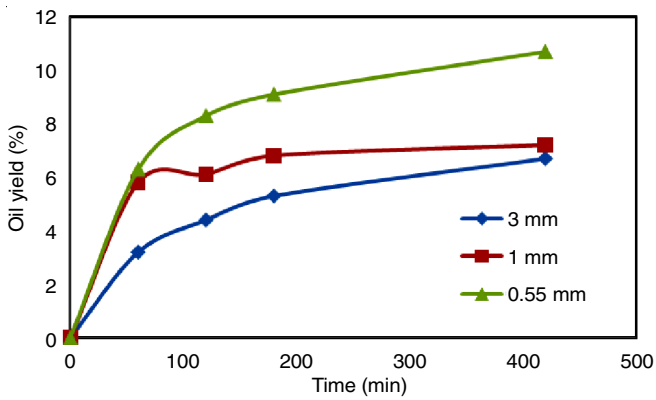

Fig. 2. Effect of time on oil extraction

Effect of particle size of grounded seeds: In this, the optimum particle size of grounded seed is selected and finalized after many trials. In the present work, it is concluded that the highest yield corresponds to the particle size 0.55 mm and due to the larger surface area available thereby making a particle solvent contact better (Fig. 3). This results in more mass transfer rate between the particle surface and the solvent (*i.e.* solid phase (seed) to liquid phase (solvent)). Also, the time taken for the solvent to diffuse inside the small particle seed is lower than large particle.

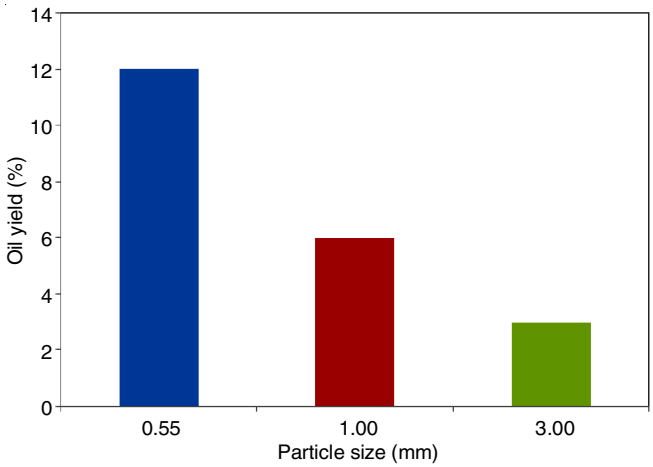

Fig. 3. Effect of particle size on oil extraction yield

FTIR analysis: The functional groups identified in the oil extracted using isopropyl alcohol solvent were characterized. The peak at 462.72 cm^{-1} represents the presence of various inorganic compounds. The small peak at 719.23 cm^{-1} represents the presence of aromatic compound. The peaks in 1114-852 cm^{-1} region corresponds to the stretching vibration of C-O ester groups and the CH_2 wag. The wide peak around 1159.53 cm^{-1} is attributed to the C-O stretching alcohol group. The peak at 1726.75 cm^{-1} is assigned to C=O stretching vibration of carboxylic acids of the esters. The peaks centered at 2923.23 and 2857.42 cm^{-1} are assigned to the stretching vibrations of aliphatic C-H in CH_2 and terminal CH_3 groups, respectively. The two small peaks at 3648 and 1652 cm^{-1} corresponds to the stretching bending vibration of O-H bonds of H_2O molecule present in the oil (Fig. 4).

GC-MS: In the present work, the extraction of oil using isopropyl alcohol as the extracting solvent from the seeds of *Trichosanthes tricuspidata* has been tested for the presence of various saturated and unsaturated fatty acids. The presence of compounds are given in Table-1 using GC-MS spectrum (Fig. 5).

Conclusion

The bio-oil extracted from *Trichosanthes tricuspidata* seed is very much similar to other bio-oils in chemical composition. As a result, it can be inferred that *Trichosanthes tricuspidata* is rich in linoleic acid. FTIR analysis showed that the seed oil is highly dominant with oxygenated species. GC-MS analysis

TABLE-1
FATTY ACID COMPOSITION OF TRICHOSANTHES TRICUSPIDATA SEED OIL
EXTRACTED USING ISOPROPYL ALCOHOL AS THE EXTRACTING SOLVENT

Peak	Retention time (min)	Area (%)	Name
1	18.97	7.27	*n*-Hexadecanoic acid
2	20.662	24.12	9,12-Octadecadienoic acid
3	20.726	42.53	9-Octadecenoic acid, (E) *cis*-vaccenic acid *cis*-10-Nonadecenoic acid
4	20.901	9.64	Octadecanoic acid, Oleic acid
5	22.048	2.12	*n*-hexylamine, N-acetyl-1-cyno-[1,2,3]triazole-4-carboxylic acid, methyl ester ethanone
6	23.521	6.32	Butyl 9,12-octadecadienoate, Methyl 9,12-heptadecadienoate, 9,12-octadecadienoic acid, methyl ester
7	23.573	7.99	2-Methyl-z,z3,13-octadecadienol 9-Octadecenal, (z)-cyclopropaneoctanal

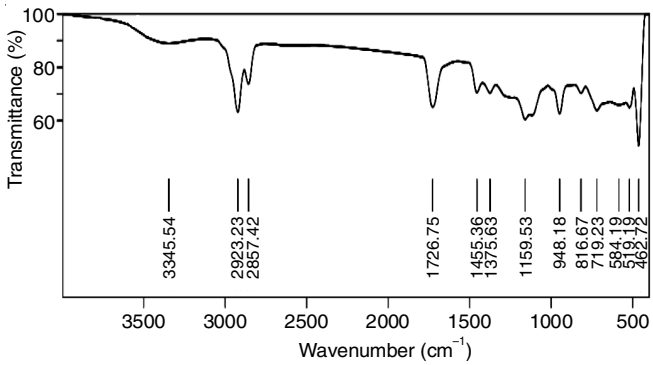

Fig. 4. FTIR result of the seed oil of *Trichosanthes tricuspidata*

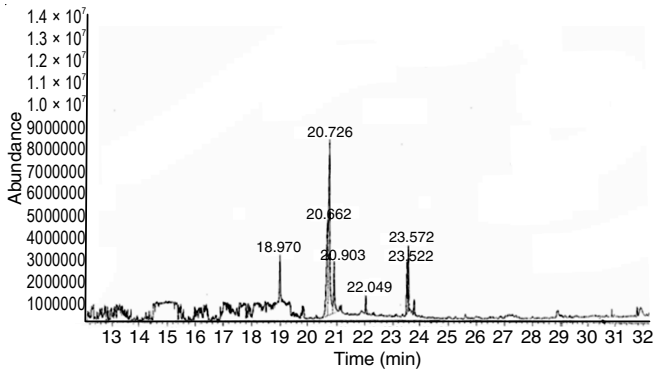

Fig. 5. GC/MS scan report of *Trichosanthes tricuspidata* seed oil using isopropanol solvent

of the oil indicates the presence of low molecular weight fatty acids with no unsaturation. The best oil yield was obtained for a extraction time of 3 h corresponding to a particle size of 0.55 mm using Soxhlet extractor and isopropyl alcohol as solvent.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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