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Optimization of oil extraction and characterization from *tamarindus indica* Linn seed oil

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Abstract: The oil yield, fatty acid composition and the physicochemical and quality characteristics of *Tamarindus indica* Linn seed oils obtained by solvent extraction were determined. Effect of various solvent and solvent combinations on the extraction of *T. indica* seed oil showed that ethanol as an alternative solvent to have better yield with 8%. Optimized process conditions were solid to ethanol ratio of 1:6w/v, under reflux for 6h and agitation speed 100rpm. Extracted oil analyzed by Gas Chromatography-Mass spectroscopy. *T. indica* oil was tested for its physical and chemical properties including percentage of fatty acid, kinematic viscosity, saponification value, unsaponifiable matter. The stability of *T. indica* oil during storage at room temperature and during heat treatment was studied. Result of the present research indicates that ethanol can be a better alternative to other solvents. Also indicate that the *T. indica* seed oil can be used as a potential alternative to nutritional food.

Keywords: *Tamarindus Indica* Seeds, *Tarmindus Indica* Seed Oil, Free Fatty Acid, Gas Chromatography Mass Spectroscopy

1. Introduction

Tamarindus indica Linn belongs to the dicotyledonous family Leguminosae which is the third largest family of flowering plants with a total of 727 genera recognized [1-3]. *T. indica* Linn is a tree type plant, which is indigenous to tropical Africa, North and South America, Florida, Brazil, and is also cultivated in subtropical China, Pakistan, Indochina, Philippines, Java and Spain [4, 5]. The major production areas are in the Asian countries India and Thailand [6].

It is a multipurpose tree of which almost every part finds at least some use [7]. *T. indica* fruit pulp, a dessert fruit, is often eaten directly from the pod [3, 8]. *T. indica* seed, a by product of the tamarind pulp industry, is an under utilized or waste material [9]. *T. indica* seed size is very variable [10, 11]. *T. indica* seed contain a crude lipid 6.94-11.43% [12]. A large quantity of oil and fats, whether for human consumption or for industrial purposes are presently derived from plant sources [13]. Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical significance [13].

There are three major steps in solvent extraction i.e., oil seed preparation, oil seed extraction and desolventizing of the oil and meal [14]. The quality characteristics of crude oil obtained by solvent extraction methods are primarily dependent on extraction solvents, extraction temperature, and pretreatment of oil seeds [15].

In this study, the effect of conditioning on the yield of ethanol extracted oil was investigated. The effects of parameter process such as solid to solvent ratio, reaction time, temperature and agitation speed were studied. Physical and chemical properties of the ethanol extracted oil were also characterized.

2. Material and Methods

2.1. Plant Material

T. indica seeds were collected freshly from Aurangabad district in the month of May-June, 2013. The material was authenticated by department of Botany, Dr. Babasaheb Amebedkar Marathawada Univesrsity, Aurangabad (MS) India.

2.2. Chemical and Reagent

All chemicals used in the experiments, such as potassium hydroxide, iodine, methanol, chloroform, n-hexane, petroleum ether, iso-propanol, ethanol and phenolphthalein indicator were of analytical reagent (AR) grade obtained from S.D. Fine-chem limited, India.

2.3. Preparation of Powder from T. Indica Seeds

Fresh seeds of *T. indica* were dried in an incubator for 2 days at 40 ºC, crushed in an electrical grinder to have powdered. These *T. indica* powder was used for all experiments.

2.4. Optimization of Oil Extraction from T. Indica Seeds

The effect of five main factors which are type of solvent, temperature, solvent to solid ratio, agitation speed and reaction time were investigated to optimize the extraction operating conditions for achieving maximum oil yield. 100g of grinded powder was extracted with polar and non polar solvents namely n-hexane, petroleum ether, chloroform, methanol, chloroform: methanol (2:1v/v) [16], iso-propanol and ethanol. The extraction temperature was reflux of the solvent while the reaction time 6h, agitation speed 100rpm and the solid to solvent ratio 1:6w/v. At the end of the extraction, the micelle was filtered using a vacuum filtration (Millipore glass base and funnel) to remove suspended solids. Subsequently, the solvent was separated from the oil using rotary vacuum evaporator and collected in the receiving flask. The oil which was remained in the sample flask, weighed after the process completed. The percentage of extracted oil was calculated. All experiments were repeated at least twice.

2.5. Gas Chromatography- Mass Spectrometry (Gc-Ms)

The fatty acid analysis was performed on JEOL JMS 600H Agilest 68g ON, equipped with 30m×0.32 mmHP-5 column, stationary phase coating 0.50µm. The column temperature was kept at 250 °C for 2min, with increase at 5 °C per min up to injector temperature 250 °C, split ratio 1:35, the carrier gas (Helium) flow rate 1.8ml/min. The compounds were identified by the Gc-Ms intensity of retention time (RT) and by comparison with those present in the National Institute for Standard Technology Computer Data Bank library of 2010. The results were expressed as the relative percentage of each individual fatty acid (FA) present in each sample given by the corresponding RT.

3. Results and Discussions

3.1. Effect of Polar and Non Polar Solvents on T. Indica Seed Oil Yield

Various polar and non-polar solvents were tested for their efficiency to extract oil from *T. indica* seeds. The oil extraction capabilities of chloroform: methanol (2:1v/v), methanol, chloroform, n-hexane, iso-propanol, ethanol and petroleum ether shown in Fig. 1. The extraction yield with ethanol was found to be about 8%, more than that of chloroform: methanol (2:1v/v), oil yield with hexane was also 8%, chloroform, petroleum ether, iso-propanol and methanol (7%, 5%, 4%, 3% and 2%, respectively). Under similar conditions, solid to solvent ratio (1:6w/v), time (6h), temperature reflux and agitation speed (100rpm). This could be attributed to a general understanding that more percentage of non-polar lipids in comparison to polar lipids (glycol and phospo) in plant seeds [17, 18], since seeds contain both polar and non-polar lipids combination of polar and non-polar solvents were studied. Ethanol has a worthy candidate to investigate as an alternative solvent, since it has low cost and it may be produced from a large variety of biological materials using simple technology [19]. Other studies shown that drying methods have significant effects on oil extraction efficiency, physical properties and chemical compositions of aromatic plants [20, 21]. Several studies have been carried out, both at laboratory and pilot scales, aimed to replace hexane with other hydrocarbons [22, 23], or alcohols [24], as solvents for oil extraction. Among hydrocarbon solvents, heptane and iso-hexane were recommended as potential substitutes for hexane to extract oil from cotton seeds [22, 23]. With respect to the use of alcohol, iso-propanol and ethanol were the most promising solvents for the oil extraction from cotton seeds [25].

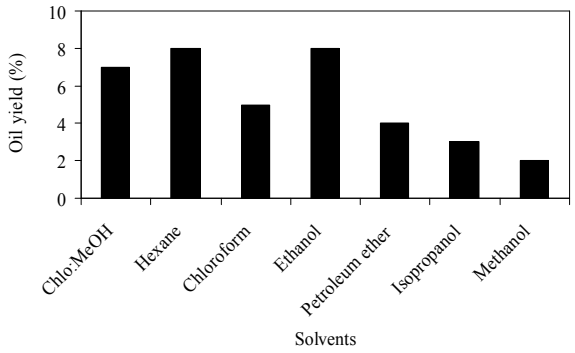

Figure 1. *Effect of different solvent on the extraction of oil with 1:6w/v ratio of Tamarindus indica seed powder to solvent.*

4. Optimization of Oil Extraction

4.1. Effect of Solid to Solvent Ratio on Oil Yield

The ratio of solid to solvent is important parameter for oil extraction process. In the present study experiments were conducted with solid to solvent ratios of 1:2, 1:4, 1:6 and 1:8w/v. Reaction temperature was constant at 80 ºC (reflux), reaction time 6h with agitation speed 100rpm Fig. 2, shows that oil extraction increases with increase in solid to solvent ratio from 1:2, 1:4, 1:6 and 1:8w/v. In case of ethanol the maximum extraction were found to be 4, 6, 8 and 8%, respectively. Based on the results, the solid to solvent ratio of 1:6w/v would be sufficient to extract the maximum yield of oil. Thus, *T. indica* powder to ethanol

ratio of 1:6w/v was selected as being the optimum. Similar results have been obtained in related research works [26-29].

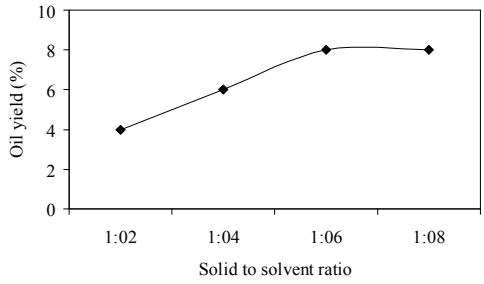

Figure 2. *Effect of solid to solvent ratio on oil extraction yield. Reaction conditions: agitation speed 100rpm. Reflux temperature, reaction time 6h and various solid to solvent ratio.*

4.2. Effect of Reaction Temperature on Oil Yield

The experiments were conducted with varying temperature, constant solid to solvent ratio for 6h and agitation speed 100rpm. The effect of extraction temperature on the amount of extracted oil shown in Fig. 3. When using ethanol as the solvent, the amount of extracted oil increased around 3% by increasing the extraction temperature from 70-80 °C. Extraction at boiling point (around 80 °C) gives about 8% of oil, 3% higher than at 70 °C. However extraction at 90 °C temperature gives 6% of oil yield. For the effect of the temperature it was observed that oil yield increases to 3% at reflux (80 °C). These results were in accordance with those reported by Tchiegang et al. [30] having explained that the reduction of the oil yield would be at high temperature. An increase of temperature reduces the oil kinematic viscosity with an increasing mobility of biopolymers in cellular walls. Based on these findings reflux temperature (around 80 °C) was taken as optimum temperature for better yield.

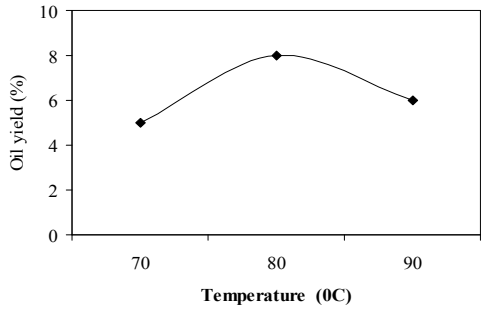

Figure 3. *Effect of temperature on oil extraction yield. Reaction conditions: 1:6w/v solid to solvent ratio, 100rpm agitation speed, reaction time 6h and various reaction temperatures in ºC.*

4.3. Effect of Reaction Time on Oil Yield

The extraction time is important parameter for oil extraction. Extraction of oil from *T. indica* seeds at different reaction times i.e. 2, 4, 6 and 8h were carried out. *T. indica* seed powder to ethanol ratio was constant as 1:6w/v; reaction temperature reflux (around 80 ºC), with agitation speed 100rpm Fig. 4, shows the total amount of oil extracted from *T. indica* seeds at different reaction times. The amount of extracted oil by ethanol did not change after 6h. Maximum extracted oil yield was achieved after 6h with 8%. It was observed that the oil yield increased with increased reaction time. In case of reaction for 6h oil yield increased by 5% compared to 4h reaction sample. And in reaction time 8h oil yield same as 6h reaction sample. Hence, 6h was chosen as optimum time for *T. indica* seed oil yield.

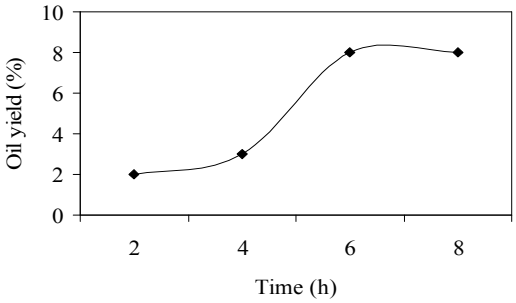

Figure 4. *Effect of reaction time on oil extraction yield. Reaction conditions: 1:6w/v solid to solvent ratio, agitation speed 100rpm and various reaction times.*

4.4. Effect of Agitation Speed on Oil Yield

The agitation speed is another important parameter for oil extraction process. Effect of different agitation speed (rpm) i.e. 50, 100 and 150rpm were carried out, keeping all other same conditions, *T. indica* seed powder to ethanol ratio was constant as 1:6w/v, reaction temperature reflux (around 80 ºC), reaction time 6h Fig. 5. It was found that the percentage extraction increased with agitation speed 50 to100rpm. However, no further increase in percentage of oil extraction was observed at 100rpm. The extraction of oil yield was 6% at 50rpm, 8% at 100rpm and 8% at 150rpm. It may be stated that 100rpm was found to be sufficient enough to overcome the mass transfer limitation. Thus, the optimum speed of agitation for oil extraction was found to be 100rpm.

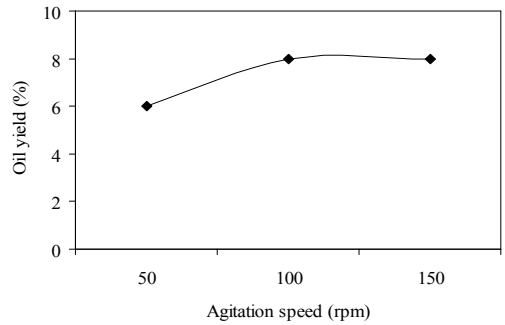

Figure 5. *Effect of agitation speed on oil extraction yield. Reaction conditions: 1:6w/v solid to solvent ratio, Reflux temperature, reaction time 6h and various agitation speed (rpm).*

4.5. Free Fatty Acid Analysis of T. Indica Seed OilS

The FA analyses of the *T. indica* seed oils are presented

in the chromatograms on Fig. 6. The fatty acid composition data presented in Table 1.The free fatty acid composition of *T. indica* oil was consist mainly of myristic acid (C14:1) was the major fatty acid of the *T. indica* seed oil followed by linoleic acid (C18:2), stearic acid (C18:0), luric acid (C12:0), octanoic acid C8:0), oleic acid (C18:1), lignoceric acid (C24:0), palmitic acid (C16:0), arachidic acid (C20:0) and behenic acid (C22:0). The detected levels of anti-nutritional fatty acid, behenic acid in *T. indica* (0.03%) is in agreement with earlier reports in the same species [31]. Linoleic and linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance. The values of some of the fatty acids in *T. indica* were found to be different from that earlier reported by [32]. This could be due to the variation in environmental conditions in which the plants were grown. The levels of fatty acids were known to vary largely with season and geographical location [33, 34]. The variation in the fatty acid composition could be due to the fact that the plant seeds are from different ecological origin. The variation in the composition and oil yield observed in this study could be related to several factors for example changes in temperature, extraction and environmental effect [35]. The composition of the fatty acids (FA) in the plant fruit seed oils studied showed presence of various components which may be of nutritive value since they contain appreciable quantity of essential FA (EFA) that play important role in human life. Hence, must be obtained from diet [36]. The EFA are long-chain polyunsaturated FA (PFA) derived from linolenic, linoleic and oleic acids [37]. The omega-3 FA is derived from linolenic acid, omega-6 from linoleic acid and omega-9 from oleic acid [38]. There were many papers focusing the analysis of fatty acid in plant seeds such as flaxseed (*Linum usitatissimum* L.) [39]. Grape seed oil [40]. Thai durian aril (*Durio zibetbinus Murr*.) [41], Australian purlane (*Portulaca oleracea*).

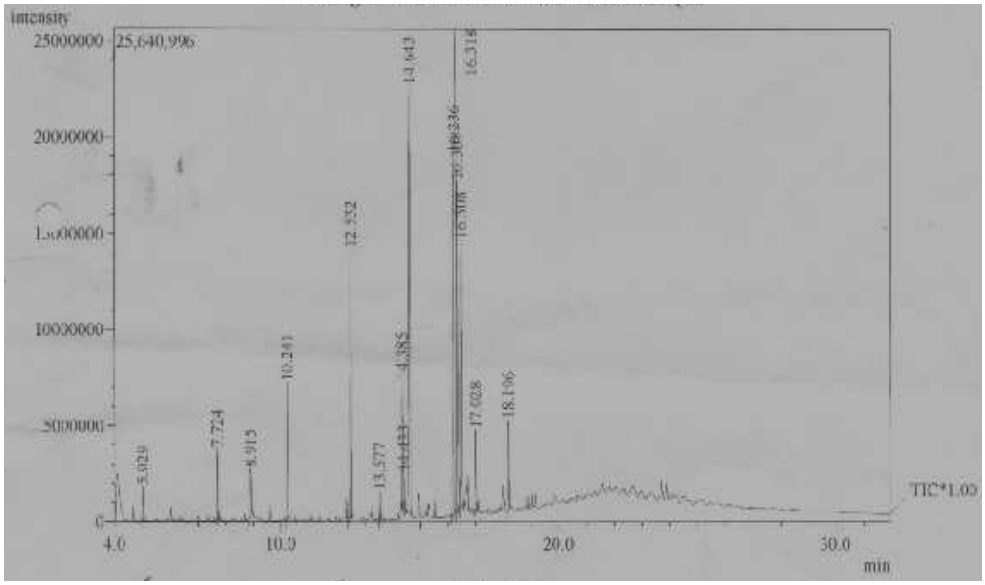

Figure 6. *Gas Chromatography-Mass spectrometry (GC-MS) analysis of Tarmindus indica seed oil.*

Table 1. *Fatty acid composition (%) of T.indica seed oil*

Fatty acid	Determined values (%)
Oleic acid (C18:1)	0.19
Linoleic acid (C18:2)	0.41
Myristic acid (C14:1)	1
Luric acid (C12:0)	0.32
Octanoic acid (C8:0)	0.3
Palmitic acid (C16:0)	0.13
Stearic acid (C18:0)	0.4
Lignoceric acid (C24:0)	0.14
Arachidic acid (C20:0)	0.06
Behenic acid (C22:0)	0.02

4.6. Physical and Chemical Properties of T. Indica Seed Oil

Table 2, present the results of the physicochemical analysis of the oil of *T. indica* was greenish in colour. The free fatty acid content was 2.92±0.30% in *T. indica* seed oil. The iodine value was found as 95±0.40g iodine/100g. High iodine values for vegetable oil may be due to the high content of unsaturated fatty acids in these oils, as observed by Abdullah and Salimon, [42].186.10±0.30 mgKOH/g had the highest saponification value of *T. indica* oil. The saponification value indicates the average molecular weight of triglycerides in the oil. The mean value obtained in this study was similar to sunflower and corn oils. *T. indica* oil contains 1.22% of unsaponifiable matter. The minor substances of the oil contained in unsaponifiable matter have anti-oxidant and other health benefits in animals, human subjects and useful in softening the skin. *Terminalia*

catapa seed oil injection has been used in clinical trials for the treatment of rectal prolapse in children [43]. The peroxide value was 4.61±0.30 mgO2/g oil in *T. indica*. The oil was liquid at room temperature (25 ºC). Kinematic viscosity at 40 ºC was 38.00±0.10mm2/sec; specific gravity at 25 ºC was 0.911±0.30g/cm3.

Table 2. *Physical and chemical properties of ethanol extracted oil from T. indica seeds*

Properties	Unit	Results
Colour		Greenish
Free fatty acid	%	2.92±0.30
Kinematic viscosity (40 ºC)	mm2/sec	38.00±0.10
Specific gravity (25 ºC)	g/cm3	0.911±0.40
Acid value	mgKOH/g	0.5±0.02
Iodine value	gI/100g	95.00±0.40
Flash point	ºC	110
Cloud point	ºC	2
Pour point	ºC	-4
Saponification value	mgKOH/g	186.10±0.30
Unsaponifiable matter	%	1.22±0.20
Peroxide value	mgO2/g	4.61±0.30
State at room temp.		Liquid

5. Conclusion

The present study deals with extraction of oil by use of solvent extraction. Four main operating parameters affecting the solid liquid extraction of *T. indica* seeds were optimized based on the maximum oil yield. The optimum conditions for the lab scale solid liquid extraction was obtained at temperature reflux (around 80 °C), extraction time 6h, solid to solvent ratio of 1:6w/v, agitation speed 100rpm and ethanol as a solvent. Ethanol gives better oil yield compared to hexane, chloroform, methanol, iso-propanol and petroleum ether. Based on the observations made above, it can be concluded that ethanol, a green and safe solvent can be a better alternative to other solvents. The physicochemical properties of the oils were also analyzed. The *T. indica* seeds contain crude oil and fatty acids, i.e. 8% and 2.92% respectively. Research results indicate that the *T. indica* seed oil can be used as a potential alternative to nutritional food. Further research to evaluate effects of *T. indica* treatments on the biological activities and anti-oxidant principles of extracted oil is recommended to explore their potential uses for pharmaceutical applications.

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