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Extraction of Neem (Azadirachta Indica) oil using blends of hexane, ethyl acetate and acetone by sonication

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ABSTRACT

Neem oil is a very beneficial oil for human utility in various fields and is commonly extracted by solvents like hexane and ethanol. This work stresses on investigating the usability of blends of pure solvents while adopting a new technology of sono assisted extraction for the extraction of oil from neem seeds. The prepared blends have been analyzed on the basis of total pressure, bubble point temperature, molecular weight for the different compositions solvents that is to be mixed to form the blends. In this work, it is proposed to use ethyl acetate + acetone, hexane + acetone and hexane + ethyl acetate binary blends with a comparison to unary pure solvents and also recording the solvent recovery in each case after distillation. The reusability of the solvents recovered after the first sets of extraction is also a topic of major concern.

Keywords*— Neem Oil, Blends, Sono Assisted Extraction, Hexane, Ethyl Acetate, Acetone*

1. INTRODUCTION

Chemical engineering has always aimed at improvising various processes in every way possible by application of various methodologies and then sorting out the best in terms of economy and productivity. Among all unit operations, extraction is one of the most illustrious methods which has been used for drawing out many products from natural sources which include oils[1], dyes[2], aromatics[3] and other valuable products.

In our day to day life, a wide variety of oils are consumed which are of both vegetable (soybean, corn, rapeseed, sunflower, peanut etc.) and animal (oil from fish and marine mammals). The vegetable parts like leaves, fruits, seeds and fat reserves inside animals contain an adequate amount of oils which can be extracted commercially by various methods, two of the prominent methods being mechanical pressing and solvent extraction[4]. Whereas mechanical pressing, as the name suggests, squeezes out oil from the target by application of pressure, solvent extraction uses a solvent to extract oil out of the natural sources by taking the advantage of preferential solubility in the solvent chosen[5].

Neem(*Azadirachta indica*) is well known for its medicinal values[6] and has been well accredited for so since the ancient times for its role in Ayurveda[7]. It's every part including leaves, seeds, roots, fruits, flowers and even the bark is used for medicinal purposes because of the high pharmacological properties. Neem tree is found in several parts of India, is very common in Maharashtra, Rajasthan, Madhya Pradesh, Telangana, Andhra Pradesh and Tamil Nadu.

There are many methods to extract neem oil from the seeds like mechanical pressing, supercritical extraction and solvent extraction. The oil produced with mechanical pressing may be contaminated with minute metal contents and is the process is time and labor intensive[8] operating and investment costs are high in supercritical fluid extraction along with the requirement of high pressure[9]. Solvent extraction gives higher yield, less turbid oil as compared to that of mechanical pressing and relatively low operating cost than supercritical fluid extraction.

During the past few decades, the use of ultrasonic waves in different fields of chemical engineering had been surfaced. In commercial solvent extraction, the ultrasonic agitation is employed substituting the mechanical agitation which promises desirable effects like increasing conversion, improvement in yield and better selectivity[10]. Moreover, sonication had been proved to increase the mass transfer rate at the molecular level by local cavitation[11].

This work is about testing the applicability of solvent blends of hexane, ethyl acetate and acetone for extraction of neem oil from the seeds using sonication and investigating the results regarding the yield and rate using the new blends. The solvents used are

three combinations of blend viz. n-hexane and ethyl ester, ethyl ester and acetone, and acetone and n-hexane. All these solvents are of low vapor pressure as compared to that of neem oil. This enables the oil to be recovered very easily since the solvents get separated by heating at the temperature at which they form vapor.

The solvent recovery is of real significance in the operation. Laboratory scale distillation is an efficient and safe method in separating the oil from the solvents as direct heating can cause fire hazard due to the flammability of the vapors produced from the blended solvents.

2. LITERATURE REVIEW

There had been lots of experimental work done in the field of oil extraction especially solvent extraction. The extraction of different vegetable oils from herbs, fruits, seeds etc. had been done using solvents such as hexane, butanol, ethanol, methanol, ethyl acetate, sodium hydroxide solution, iso-propanol, butanone etc.

The real mechanism of ultrasonic extraction was explained in the work of Maricela Toma, Mircea Vinatoru et al.[12]. They performed ultrasonic experiments to investigate the effects of ultrasound on vegetal tissues of various plant species. They reached a defined mechanism in which ultrasonic energy causes enhanced hydration of cell wall while the vegetal fragmentation takes place simultaneously. This facilitates the process of diffusion i.e. increased rate of mass transfer.

In a review article by Mircea Vinatoru[13] on ultrasonically assisted extraction of bioactive principles of herbs, he mentioned about the various conventional techniques of extraction, laying emphasis on the use of ultrasound. He explained how the ultrasonic energy can cause more swelling and hydration of the cell walls of plant matter causing increased pore size and enhanced mass transfer by diffusion.

Kinetics of ultrasonic extraction was studied on garden and glutinous species of sage by D.T. Velickovic, D.M. Milenovic et al.[14]. They used petroleum ether, 70% ethanol and water as solvents for extraction. They concluded that ultrasonic extraction can successfully be described mathematically using the unsteady state diffusion through plant material, the film theory and the empirical equation of Ponomaryov, which have most often been used to describe the kinetics of classical maceration.

In the field of food technology, the ultrasonic –assisted extraction has received much popularity. Another review paper by Kamaljit Vilkhu et al.[15] mentioned the use of ultrasound in the extraction of many plant materials, this includes, oil, protein and bioactive from plant (e.g. polyphenolics, anthocyanins, aromatic compounds, polysaccharides and functional compounds) which increased yield of extracted components, increased rate of extraction, achieving reduction in extraction time and higher processing throughput. It was stated that ultrasound can enhance existing extraction processes and enable new commercial extraction opportunities and processes.

Neem oil extraction had been found in the work of Maria Y.L. et al.[16] where they extracted the oil using n-hexane and ethanol and investigated the kinetic and thermodynamic aspects of the extraction. The yield of oil was found to be more with hexane than ethanol for similar operating conditions and the increase in temperature decreased the oil quality. The extraction was reported to follow the first order kinetics.

The use of blends had not received much attention in solvent extraction over pure solvent. However, in a paper by Haizhou Li et al.[17] extraction of soybean oil using hexane, isopropanol and a 3:2 hexane-isopropanol mixture using ultrasonication had been mentioned. The findings state that the oil can be extracted with higher yields by the use of (3:2) hexane-isopropanol mixture/blend as compared to the pure solvents. It was also stated that there was a decrease in the processing time of extraction by using ultrasonication.
Linseed oil/ flaxseed oil was extracted by n-hexane using UAE (ultrasonic assisted extraction) technique by Zhen-Shan Zhang, Li-Jun Wang et al.[18]. They recorded a shorter extraction duration using ultrasound assistance. They recorded that 50 W ultrasonic power, 30˚C extraction temperature, 30 min extraction time and 6:1 (v/w) ratio of liquid to solid are very good conditions for ultrasonic assisted extraction.

Qing-An Zhang, Zhi-Qui Zhang et al.[19] extracted almond oil from autoclaved almond powder by the ultrasonic-assisted method. Their research was aimed to determine optimum process conditions of ultrasound-assisted extraction of phycocyanin compound from microalgae *Spirulina platensis.* They optimized by using microalgae and the obtained optimal conditions can be potentially scaled up to isolate phycocyanin for large production in the food and pharmaceutical industries.

Tobacco oil was extracted using UAE with n-hexane and petroleum ether by Ivana T. Stanisavljevic et al.[20]. Their work laid stress on the kinetic model of the UAE by simple two-step mechanism and found that ultrasound influenced the washing of oil from the seeds and the mass transfer of oil through both the seeds and the seed particles. In recovering the tobacco seed oil from the seeds the UAE was found more efficient than chemical batch extraction. Also, the tobacco seed oil recovery was found to be dependent on the extent of comminution of the seeds.

Shweta Shah et al.[21] worked on Jatropa oil extraction from jatropa seeds by using ultrasonic energy. They reported that use of ultrasonication as a pretreatment before aqueous oil extraction and aqueous enzymatic oil extraction was found to be useful in the case of extraction of oil from the seeds of *Jatropha curcas L.* The use of ultrasonication for 10 minutes at pH 9.0 followed by aqueous oil extraction gave a yield of 67%. However, the maximum yield of 74% was obtained by ultrasonication for 5 minutes followed by aqueous enzymatic oil extraction using an alkaline protease at pH 9.0. Furthermore, they even found that use of ultrasonication also resulted in reducing the process time from 18 to 6 hours.

3. MATERIALS AND METHODS

3.1. Neem oil

Neem oil is a non-edible fixed oil obtained from fully matured seeds of the tree. Apart from all other parts of the tree, the seed has a high concentration of its oil containing about 20% oil. It is yellow in colour with specific odor and bitter taste. It is soluble in ether and chloroform. Its specific gravity ranges between .913-.918. It contains glycerides and unsaturated fatty acids. The main fatty acids are oleic (50%) and stearic acids (20%). The oil contains 2% of bitters, which are sulfur containing compounds such as nimbin, nimbidin, nimbinin and nimbidol. The unsaponifable part contains nimbosterol (.03%). The boiling point is about 230˚C

Uses of neem oil:

- Nimbin, nimbidin components possess anti-viral activity
- Oleic and stearic acids for soap manufacturing
- As medicine in rheumatism
- As a pesticide, insecticide
- As a medicated soap for skin disease
- In drugs for a variety of diseases such as diabetes and tuberculosis

3.2. Solvents

Various solvents can be used for extraction. However extensive researches and consideration of various factors such as commercial economics, edibility of the various products obtained from extraction, physical properties of the solvent especially its low boiling point etc. reveal hexane to be the best of the many solvents tried over the years. Ester solvents like ethyl acetate are also reported as a potential solvent for many vegetable oil extraction[22]. Methyl ethyl ketone is also used for solvent extraction at the commercial level [23], hence being a member of the homologous series of ketone, its properties being similar to acetone. Solvents used are all volatile at room temperature and hence will vaporize leaving the oil behind which generally don't vaporize at ambient temperature. The solvents are handled very carefully and must be kept away from fire sources keeping in mind their extensive flammability.

3.3. Laboratory batch distillation apparatus

Laboratory scale distillation is almost exclusively run as batch distillations. The device used in distillation sometimes referred to as a still consists at a minimum of a re-boiler or pot in which the source material is heated, a condenser in which the heated vapor is cooled back to the liquid state, and a receiver in which the concentrated or purified liquid called distillate is collected. In simple distillation, the vapor is immediately channelled into a condenser. Consequently, the distillate is not pure but rather its composition is identical to the composition of the vapors at the given temperature and pressure.

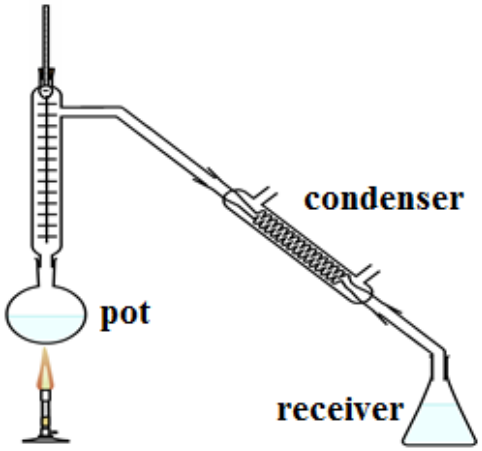

Fig. 1: Laboratory distillation apparatus

Simple distillation is effective only when the liquid boiling points differ greatly (rule of thumb is 25˚C)[18] or when separating liquids from non-volatile solids or oils. For these cases, the vapor pressures of the components are usually different enough that the distillate may be sufficiently pure for its intended purpose.

3.4. Ultrasonication

Ultrasound ranging from 20 to 100kHz is used in chemical systems in which both chemical and physical alterations are desired as it has the capacity to cause cavitation of bubbles[24, 25]. When applied on liquid, ultrasound waves consist of a cyclic succession of rarefaction and compression phases imparted by mechanical vibration. Compression cycles exert positive pressure and pull the molecules apart[18]. When pressure amplitude exceeds the tensile strength of liquid in the rarefaction regions, small vapor filled voids called capitations are formed which served as a medium to concentrate the diffused sound energy. Once the cavity experience overgrowth and is not able to sustain the available energy that is absorbed, the liquid will rush in the cavity causing it to implode[26]. Now each bubble will act as a hotspot and generate energy to increase the temperature and pressure up to 5000K and 500atm, respectively, and cooling rate as fast as 109K/s[26]. The available enormous local temperature and pressure make way for an unusual mechanism for high energy chemical reactions.

3.5. Material preparation

- Neem seeds were first washed to remove mud and other sticky substance.
- These are subsequently dried in sunlight for about 3 hours.
- The seeds were then grinded in a mixer grinder to get particle size that passes through 12 B.S.S i.e. 150 I.S.S mesh size.

3.6. Extraction procedure

- The solvent samples (pure solvents - hexane, ethyl acetate and acetone; blends – hexane and ethyl acetate, ethyl acetate and acetone, acetone and hexane (of different ratios)) of 30ml were first prepared in 100ml conical flasks and covered by rubber corks to prevent their vaporization at room temperature.
- 5gm of crushed powder neem seed was weighed for every solvent sample and added to the solvents in the conical flasks.
- Neem oil was extracted using solvents by subjecting the mixture of seed powder and solvent in conical flasks to sonication in the bath sonicator for 20 minutes at a frequency of 50 Hz.
- The samples were then filtered using Whatman filter paper Grade 4.

3.7. Solvent recovery (Batch distillation)

- Solvent recovery is done using a lab scale distillation apparatus in which an extracted sample is taken in a pot (500ml round bottom distillation flask).
- The samples were heated until the solvent is collected by condensation in the receiver. The operational temperature needs to be estimated by judging the boiling points of the individual blends which are discussed in the results section.
- The distillation operation occurs over a period of 20-30 minutes for a single sample. The condensate is then collected and the recovered solvent is measured by a measuring cylinder. The quantity of oil left in the pot is separately measured.
- Solvent recovered is calculated by the following formula

% Solvent recovery = Amount of condensate recovered / Amount of solvent taken initially for extraction

4. RESULTS AND DISCUSSIONS

4.1. Solvent blend characteristics

In this work three commercially used solvents for vegetable oil extraction viz. n-hexane, ethyl acetate and acetone are used to create the following blends: n-hexane & ethyl acetate, ethyl acetate & acetone, acetone & n-hexane, each having different ratios of the pure components. In order to substantiate the distillation process for the oil separation and solvent recovery at the very outset it is necessary to estimate the vapor pressures and the bubble (boiling points) of all the blends with different ratios of pure components which demands the standard properties such as molecular mass, vapor pressure and boiling points of the individual pure components which are tabulated in table 1.

Table 1: Properties of pure solvents

Name of the solvent	Molecular mass(g/mol)	Vapor pressure@ 25˚C (mm Hg)	Boiling point at 1 atm (˚C)
n-Hexane	86	149.86	68
Ethyl acetate	88	94.24	77
Acetone	54	232.41	56

All the blends mentioned forming homogeneous mixtures. The ratios of the binary blends studied are 1:1, 2:3, 3:2, 3:7 and 7:3. The calculations of the total vapor pressure of the blends are done using Dalton's law of partial pressure and the boiling points of each blend were determined according to a method described in "Principles of Mass Transfer and separation Processes" by Binay K. Dutta pg. 336-337 and using standard Antoine equations of pure solvents. The calculations are mentioned in tables **2, 3&4**.

Table 2: Blend A: n-Hexane and Ethyl acetate (30 ml)

Molar composition	Avg. Molecular weight(g/mol)	Vapor pressure@ 25˚C (mm Hg)	Boiling point at 1 atm (˚C)
1:1	87	122.05	73
2:3	87.2	116.48	73.8
3:2	86.8	127.61	72.2
3:7	87.4	110.92	74.6
7:3	86.8	133.17	71.4

Table 3: Blend B: Ethyl acetate and Acetone (30 ml)

Molar composition	Avg. Molecular weight(g/mol)	Vapor pressure@ 25˚C (mm Hg)	Boiling point at 1 atm (˚C)
1:1	71	163.32	65
2:3	67.6	177.14	62.8
3:2	74.4	149.50	67
3:7	64.2	190.96	61
7:3	77.8	135.69	69.8

Table 4: Blend C: n-Hexane and Acetone (30 ml)

Molar composition	Avg.Molecular weight(g/mol)	Vapor pressure@ 25˚C (mm Hg)	Boiling point at 1 atm (˚C)
1:1	70	191.13	61.6
2:3	73.2	182.88	63
3:2	66.8	199.39	60.4
3:7	76.4	174.62	64.3
7:3	63.6	207.64	59.2

The values from the tables 1,2,3 and 4 depict that the total vapor pressure of all the solvent blends of different compositions is well below the atmospheric pressure viz. 760 mm Hg and the total pressure of any blend is within the range of vapor pressures of pure solvents at ambient temperature. The less total vapor pressure is a desirable characteristic of any solvent mixture i.e. blend to be used for extraction due to its ease of storage and operation as it will not boil at ambient temperature.

The calculated values of boiling points of all the fifteen blends range between 59.2-77˚C demarcating the operational temperature of the distillation unit as 80˚C which is a very important corollary in order to carry out the experiment. The boiling point of neem oil is known to be close to 230˚C and thus an operating temperature of 80˚C seems healthy for retention of oil and recovering the solvent by distillation.

4.2. Amount of oil collected and solvent recovery

Basic observation during the extraction process was that before extraction the solvent is colorless and after extraction, the oil presence can be confirmed by the brownish yellow color of the homogenous solvent. The amount of oil left in the pot was measured after each distillation process and was measured as about 1.5-2 ml from 5gm of crushed seeds by 30 ml of solvent. Figure 3 is the graphical representation of the calculated values of the % solvent recovery according to the formula mentioned in section 3.6. The values showed a range between 30-60% and the average solvent recovery was calculated at 41.5%. The low recovery of the solvents can be attributed to the loss of solvent during various activities like preparation of samples, filtration and the loading of solvent blends. The maximum losses occurred during the filtration procedure when the solvents vaporize from the solvents over the filter bed from the wet filter paper.

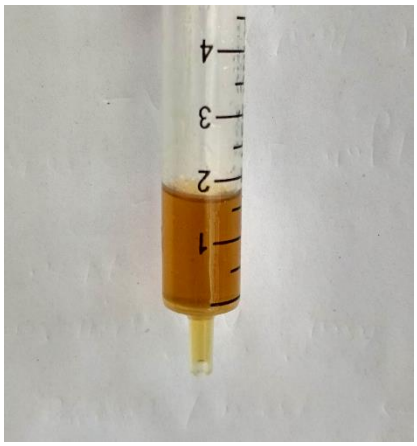

Fig. 2: A sample of extracted neem oil collected after distillation

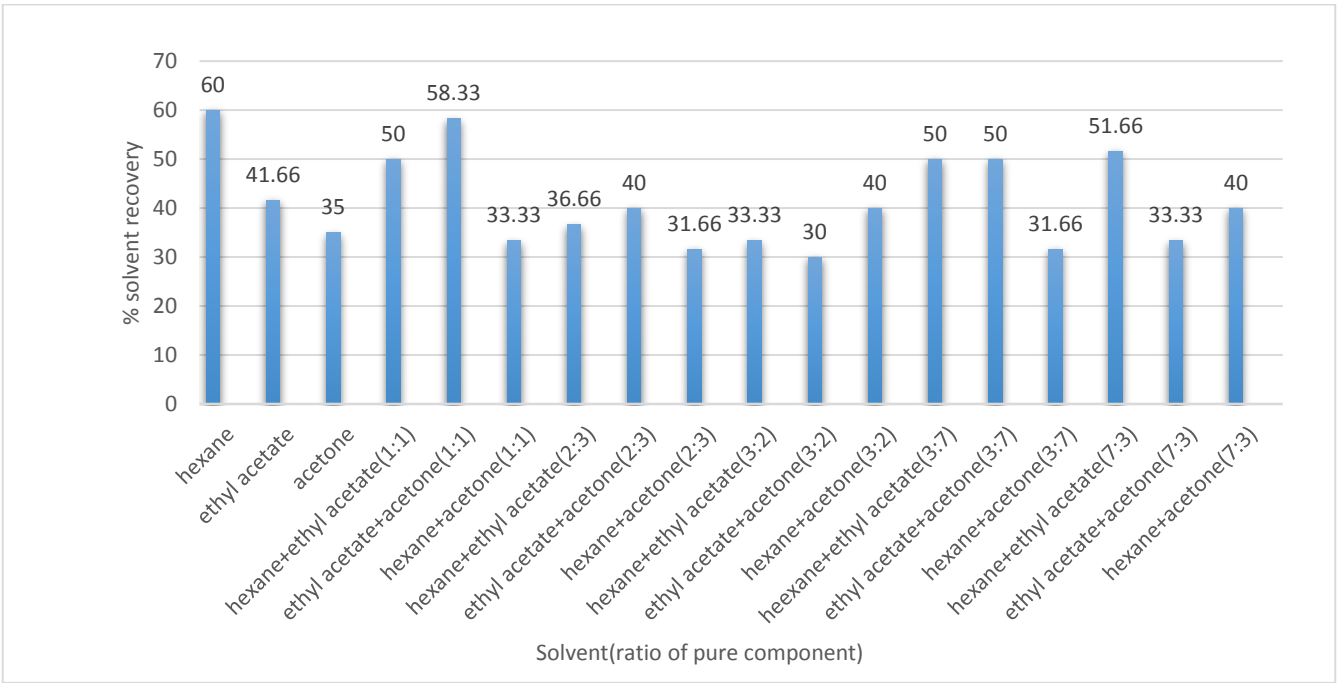

Fig. 3: Plot showing the percentage of the different solvent amount recovered as condensate

4.3. Reusability of the recovered solvents

As of total 66.5/150 ml of Blend A (hexane & ethyl acetate of different ratios), 63.5/150 ml of Blend B (ethyl acetate and acetone of different ratios) and 53/150 ml of Blend C (hexane & acetone of different ratios) were recovered. The reusability of these three solvents was further checked by undergoing the same process of extraction and batch distillation as described in sections 3.5 & 3.6. The calculated data for the extraction of oil using the recovered solvents are tabulated in table 5. The new ratio is calculated based on the previously considered ratios and the amount of solvent recovered for each ratio, the total pressures and boiling points are calculated using Dalton's law and Antoine equations respectively.

Table 5: Data of recovered solvent blends

Recovered Solvent	New calculated ratio	Avg. Molecular weight(g/mol)	Vapor pressure@ 25˚C (mm Hg)	Boiling point at 1 atm (˚C)	Extracted amount of oil using a recovered solvent (ml)
Hexane +Ethyl acetate	1:1	87	122.05	73	1.5
Ethyl Acetate + Acetone	.92:1	70.3	166.20	63.9	2
Hexane + Acetone	1.06:1	70.4	190	62.3	1

The calculated vapor pressures are well below the atmospheric pressure value of 760mm Hg and thus show the ability of the recovered solvents to be used in the extraction process and their calculated boiling points show their ability to be used for distillation at 80˚C. Upon distillation the oil extracted was measured and was found to be in the range 1-2 ml which is equivalent to the range of extracted oil from the initial solvent blends, thereby stating the potential of the recovered solvents in further extraction uses.

5. CONCLUSION

The neem oil was successfully extracted using both pure solvents and their binary blends. However, from the experimental studies, it is difficult to ascertain the superiority of blends over pure solvents in extraction process as the amount of oil recovered for each of the solvents (both pure solvents and blends) ranged between 1.5-2ml. The average percentage solvent recovery was calculated as 41.5% which can be further improved by preventing the loss of solvents especially during the filtration of the sonicated samples. The reusability of the recovered solvents is also fairly possible in the same manner as during the first hand extraction since the extracted amount obtained after distillation is in the same range value of what was observed during the first extraction.

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