

REVIEW: EXTRACTION OF OLEIC ACID FROM NEEM LEAVES

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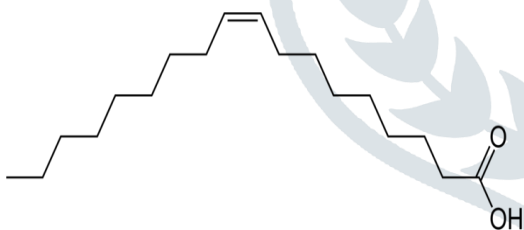
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Abstract:- Herbs play an important role in human's day-to-day life. They are used in mainly three sectors food, pharmaceutical and nutraceutical in industries. Today we fear to have fruit or vegetable brought from market due to the involvement of the chemical pesticides in the farming. Thus there arises a need of organic pesticides which will help both the farmer as well as customer on the same hand. There several herbs with active components which may prevent the pest impact on the crops which should be extracted from respective plants and has to be mixed in a fixed proportion. The different raw materials kokum, cardamom, neem (*Azadirachta indica*), fennel, etc. Thus our main objective is to extract out a pure organic pesticide from herbal extracts. Raw materials are selected based on the anti-microbial, anti-inflammatory, anti-tumour properties. Oleic acid was found to be 25-54% neem oils fatty acid content and has property to produce soap. By using UV- spectrometer, we found the concentration of oleic acid in the Neem leaves. From the reviews and experimentation, the concentration of oleic acid increases with increase in temperature as well as RPM.

Keywords: *Azadirachta indica*, oleic acid, pharmaceutical, spectrometer.

1. INTRODUCTION

Oleic acid (cis-9, 10-octadecenoic) has been said to be the most widely occurring of the fatty acids. Olive, tea seed, and almond oils.



Have served as the sources of these several preparations, but olive oil has been usually selected as the raw material, because of the relatively simple nature of its component fatty acids, and because of its high oleic acid content (75 to 80 per cent). The synthetic oleic acid was believed ^[1] to be identical with the natural acid. Spite of the fact that, theoretically, there can exist sixteen isomeric octadecenoic acids, depending on the position of the double bond, only two or three of these, in addition to oleic acid, have been found to be naturally occurring. Vaccenic acid (11, 12-octadecenoic) was identified in whale oil by means of its dihydroxy derivative to have isolated vaccenic acid from beef fat and to have identified it. This same acid has been reported in butter fat, mutton fat, and lard in very small amounts. The presence of a 10, 11-octadecenoic acid in pork liver lipids. Although several of the earlier investigators claimed high purity for their products, the preparation of pure oleic acid was almost impossible by the methods employed. This was due to. The occurrence of the acid along with saturated acids on one hand and more unsaturated acids such as; linoleic on other. The methods of separation

from both types of extraneous material were far from quantitative, many of the methods being based on differences in solubility of divalent and other metal salts. Furthermore, efficient stills were not available, so that the separations were complicated by the presence of C₁₆ and C₂₀ acids, both saturated and unsaturated ^[1].

Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is odorless, colorless oil, although commercial samples may be yellowish. In chemical terms, oleic acid is classified as a monounsaturated omega-9 fatty acid, abbreviated with a lipid number of 18:1 cis-9. It has the formula CH₃(CH₂)₇CH=CH(CH₂)₇COOH. The term "oleic" means related to, or derived from, oil of olive, the oil that is predominantly composed of oleic acid ^[2]. Oleic acid (cis-9, 10-octadecenoic) has been said to be the most widely occurring of the fatty acids. The best preparations of this acid have been made. Olive, tea seed, and Almond oils have served as the sources of these several preparations, but olive oil has been usually selected as the raw material, because of the relatively simple nature of its component fatty acids, and because of its high oleic acid content (75 to 80 per cent). The synthetic oleic acid was believed to be identical with the natural acid ^[2].

In spite of the fact that, theoretically, there can exist sixteen isomeric octadecenoic acids, depending on the position of the double bond, only two or three of these, in addition to oleic acid, have been found to be naturally occurring. Petroselinic acid (6, 7- octadecenoic) was found in parsley seed oil. Vaccenic acid (11, 12-octadecenoic) was identified in whale oil by means of its dihydroxy derivative have isolated vaccenic acid from beef fat and to have identified it. This same acid has been reported in butter fat, mutton fat, and lard in very small amounts. The presence of a 10, 11-octadecenoic acid in pork liver lipids ^[2].

Although several of the earlier investigators claimed high purity for their products, the preparation of pure oleic acid was almost impossible by the methods employed. This was due to the occurrence of the acid along with saturated acids on the one hand, and more unsaturated acids, such as linoleic, on the other. The methods of separation from. Both types of extraneous material were far from quantitative, many of the methods being based on differences in solubility of divalent and other metal salts. Furthermore, efficient stills were not available, so that the separations were complicated by the presence of C₁₆ and C₂₀ acids, both saturated and unsaturated ^[2].

2. LITERATURE REVIEW

In 1944, R. Carl Milligan and J. B. Brown et al; had explained in his research paper of

—The isolation and properties of some naturally occurring octadecenoic (oleic) acids that Oleic acid (cis-9, 10-octadecenoic) has been said to be the most widely occurring of the fatty acids. The best preparations of this acid have been made by Bertram, Raymond, and Foreman. Olive, tea seed, and almond oils have served as the sources of these several preparations, but olive oil has been usually selected as the raw material, because of the relatively simple nature of its component fatty acids, and because of its high oleic acid content (75 to 80 per cent). The synthetic oleic acid of Noller and Bannerot was believed to be identical with the natural acid. ^[3] In 2013, Dr. V. V. L. N Prasad, Dr. Prakash V Diwan et al; had explained in his Research article of —Studies on

extraction and HPLC

Analysis of Azadirachtin from Kernels of Neem Seeds about the neem tree has been known for its unique properties in improving the human health and it also acts as an antiseptic agent. Among the various limonoid components of the neem, Azadirachtin is the most important component which has many anti-infective and anti-microbial properties. Azadirachtin is a tri terpenoidlimonoid obtained from various parts of the neem. Azadirachtin is extracted from the dried neemkernel powder using Di-Chloro methane as solvent. The UV absorbance of Azadirachtin was found to be 220nm. The qualitative analysis of Azadirachtin was carried out by HPLC, on a C-18 column at a flow rate of 1ml/min, using Acetonitril: Methanol: 1% Triethyl amine pH 4 (60:40:1) as mobile phase at 210nm. The TLC, UV and HPLC reports indicate the isolation of Azadirachtin from the seed kernel powder of the neem.^[4]

In 2009, Amal Kumar Ghimeray, Cheng-Wu Jin, Bimal Kumar Ghimire and Dong Ha Cho et al: had explained in his research paper of—Antioxidant activity and quantitative estimation of azadirachtin and nimbin in AzadirachtaIndicaA. Juss grown in foothills of Nepall about the leaf and bark fraction extracts of AzadirachtaindicaA. Juss. (Neem) grown in the foothills (subtropical region) of Nepal were evaluated for their antioxidant activity, total phenolic (TP) and totalflavonid (TF) contents. HPLC method was employed to quantify the amount of azadirachtin and nimbin present in the seed, leaf and the bark extracts of neem. The result showed that the highest azadirachtin content was found in the methanolic extract of the seed (3300 µg/g DW). Similarly, the hexane fraction of bark showed the highest nimbin content (271 µg/g DW) followed by the methanolic extract (260 µg/g DW).

Antioxidant activity was determined by measuring 1,1-diphenyl-2- picrylhydrazyl free radical scavenging activity, hydroxyl radical scavenging activity, DNA protection assay, metal chelating and the inhibition of peroxidation using linoleic acid system and their results were found at different magnitudes of potency. The results of TP content expressed in tannic acid equivalents ranged from 66.63 to 629.04 µg/mg in the bark extracts and 23.85 to 237.00 µg/mg in the leaf extracts, the content of TF expressed in quercetine equivalents ranged from 12.87 to 17.07 µg/mg in the bark and 13.72 to 93.17 µg/mg in the leaf extracts.^[6]

Udaya N. Wanasundara, P. K. J. P. D. Wanasundara, and Fereidoon Shahidi had explained in his book of —Conjugated Linoleic Acid Research Novel Separation Techniques for Isolation and Purification of Fatty Acids and Oil By-Products about soap stock is a low-cost raw material used for obtaining fatty acids, generally found in the source oil. Short- and medium-chain fatty acids are obtained from coconut and palm kernel soap stock. Neem soap stock provides high-grade stearic acid when hydrogenated and linoleic and palmitic acids when fractionated. Multiple distillation steps may be required to obtain acceptable color, stability, and economical hydrogenation. Readers are advised to refer to Sonntag (214) for further details for the processing of soapstock^[5]. Dr. Mohammed Elmokhtar Abd Elaziz had explained in his research paper of—Extraction of Neem Oil from Neem Seeds about The objective of this work is extract of Neem oil from kernels of Neem seed. A soxhlet extraction was used to extract the oil. The results obtained showed that the

percentage of Neem oil in the seed was 17.601, the saponification value was 192mg/g, iodine value was 67.38g/100g and the acidic value 17.5mg/g. Azadirachtin, a major component of Neem oil, is rapidly broken down.

Microbes and light break down the pesticide in soil, water and on plants. The half-life of azadirachtin in soil ranges from 3- 44 days. In water, the half-life ranges from 48 minutes to 4 days. It also rapidly breaks down on plant leaves; the half-life is 1 - 2.5 days. The remaining components of Neem oil are broken down by microbes in most soil and water environments [7]. Maria Yuliana Liauw, F. A. Natan, P. Widiyanti, D. Ikasari, N. Indraswati and F. E. Soetaredjo had explained in his journal of —Extraction of neem oil (*Azadirachta indica* A. Juss) using N-hexane and ethanol: studies of oil quality, kinetic and thermodynamic about Neem oil extraction from Neem seeds (*Azadirachta indica* A. Juss) with n-hexane and ethanol are presented. Effects of particle size, temperature and type of solvent on the extraction kinetic and thermodynamic parameters were studied.

Results showed that the maximum oil yields were 41.11% for ethanol and 44.29% for n-hexane at 50C, and 0.425-0.71 mm particle size. Based on psycho-chemical characteristics analysis showed that increasing temperature decreased iodine value but caused saponification, acid, and peroxide value became higher, which means higher extraction temperature result on higher oil yield but lower oil quality. The kinetic of neem oil extraction was derived from mass transfer rate equation. It also found that ΔH is positive, ΔS is positive, and ΔG is negative

3. METHODS OF OLEIC ACID EXTRACTION

3.1 Soxhlet Extraction:

In a conventional Soxhlet system, plant material is placed in a thimble-holder, and filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask, solute is separated from the solvent using distillation. Solute is left in the flask and fresh solvent passes back into the plant solid bed. The operation is repeated until complete extraction is achieved.

In a conventional Soxhlet system as plant material is placed in a thimble-case, and filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask, solute is separated from the solvent using distillation. Solute is left in the flask and fresh solvent passes back into the plant solid bed. The operation is repeated until complete extraction.

Until complete extraction is achieved. During Soxhlet extraction, the solvent is usually recovered by evaporation. The extraction and evaporation temperatures have a significant effect on the quality of final products [8].

3.2 Hydrotropic Extraction:

Hydrotrophy is a collective molecular phenomenon similar to micellar Solubilization but with a much higher capacity. It is a consequence of the tendency of amphiphilic hydro trope molecules to aggregate among themselves and probably with other hydrophobic molecules.

These aggregates are supposedly much smaller than surfactant micelles and far less cooperative. Another distinguishing feature of hydro tropes, unlike surfactants, is their ability to differentiate among different organic constituents of a mixture, even closely related substances.

It is this ability of molecular recognition that should be useful for the preferential extraction of a compound from naturally occurring raw materials. The high solubilisation capacity of hydro trop

Solutions should lead to high extraction capacities for otherwise insoluble organic- active elements. We demonstrate here the ability of hydrotropic solutions, in an analogous manner, to disrupt plant cell structures and aid in the extraction of hydrophobic constituents from the complex bio matrix. The hydrotropic effect is significant above a minimum hydro trope concentration (MHC) that is a characteristic of a given hydro trope, analogous to the critical micelle concentration (CMC) of a surfactant. However, because hydro tropes have relatively short hydrocarbon chains or hydrophobic groups, their MHCs are usually in the molar range. The solubility of an organic compound in a hydro trope solution rises almost exponentially immediately above the MHC, but at higher concentrations of the hydro trope, it might level off to a plateau depending on the nature of the solute [6].

3.3 Ultra-Sonication-Assisted Extraction

Sound waves, which have frequencies higher than 20 kHz, are mechanical vibrations in a solid, liquid and gas. Unlike electromagnetic waves, sound waves must travel in a matter and they involve expansion and compression cycles during travel in the medium. Expansion pulls molecules apart And compression pushes them together. The expansion can create bubbles in a liquid and produce negative pressure. The bubbles form, grow and finally collapse. Close to a solid boundary, cavity collapse is asymmetric and produces high-speed jets of liquid. The liquid jets have strong impact on the solid surface [4].

3.4 Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) offers a rapid delivery of energy to a total volume of solvent and solid plant matrix with subsequent heating of the solvent and solid matrix, efficiently and homogeneously. Because water within the plant matrix absorbs microwave energy, cell disruption is promoted by internal superheating, which facilitates desorption of chemicals from the matrix. It is observed using scanning electron microscope

microwave pre-treatment of fresh orange peels led to destructive changes in the plant tissue. These changes in the plant tissue due to microwave heating gave a considerable increase in the yield of extractable pectin. Furthermore, the migration of dissolved ions increased solvent penetration into the matrix and thus facilitated the release of the chemicals. The selection of an organic solvent for Microwave-assisted extraction (MAE) is essential; the solvent must be able to absorb microwave radiation and thereby becomes hot. The ability of an organic solvent to be useful for MAE can be assessed in terms of its dielectric constant ^[9].

3.5 Supercritical Fluid Extraction (SFE)

Supercritical state is achieved when the temperature and the pressure of a substance is raised over its critical value. During SFE, raw plant material is loaded into an extraction vessel, which is equipped with temperature controllers and pressure valves at both inlet and outlet to keep desired extraction conditions. The extraction vessel is pressurized with the fluid by a pump. The fluid and the dissolved compounds are transported to separators, where the solvation power of the fluid is decreased by decreasing the pressure or increasing the temperature of the fluid. The product is then collected via a valve located in the lower part of the separators. The fluid is further regenerated and cycled. SFE can be used to extract certain compounds from plants at temperature near to ambient, thus preventing the substance from incurring in thermal denaturation. SFE is an old technique of solvent extraction but its commercial application happened slowly due to the sophisticated and expensive high Pressure equipment and technology required SFE is favorably applicable for the qualitative and quantitative identification of constituents of natural products, including heat-labile compounds ^[5].

4. PROCESS

4.1 Pre-Processing:

Fresh Neem leaves were collected from the collage premises. From this the small twigs, big particle, bad leaves and small branches of leaves were removed. They were washed well in order to remove the dust particles. Then kept in tray drier for 10 min in order to remove moisture content and then kept under sun for 5-6 days. Then the leaves are grinded/ powdered in mixer well.

4.2 Processing:

Water bath is switched ON. 20gms of powdered neem leaves (raw material) were measured using weighing balance. Weighed powder along with 200ml of ethanol (Solvent) was poured into the extractor. Then the baffle was inserted in to the extractor and it was properly tightened. The agitator was switched ON and the RPM was set (as 200, 400 & 600). Samples are collected from extractor at an interval of 15 mins up-till 2:30 hrs. Then the setup was Switched OFF completely. Each of the sample bottles are labeled and collected in a beaker containing water is either moved for refrigeration (in order to prevent contamination or prevent microbial activity) or directly taken for UV analysis in analytical lab. Before analysis the samples are properly filtered by primarily normal filter paper and then with whatman filter paper. The remaining sample in extractor is sent to Soxhlet for solvent recovery and to get neem oil separately. The filtrate, residue and remaining extract are weighed properly for mass balance. The result obtained are then formulated and tabulated.

4.3 Post-Processing:

The extra oleic acid purchased was converted to soap & soap liquid by the soap making process after proper calculations. Then soap liquid is mixed with the concentrated extract of neem oil collected after solvent removal and is mixed with fixed amount of water. Thus a complete 100% organic pesticide is prepared.

5. CONCLUSION

The new integrated approach consisting of a screening of extraction conditions and an in vitro measuring of functional activities together with an exhaustive chemical characterization will provide us with a new tool to discover new bioactive compounds and to help the further design of processes to obtain such products in the greenest, sustainable, and efficient way, complying with the rules of green chemistry and green engineering.

Some technologies are mature enough to be used at large scale; others require more study and development but, in any case, it is advisable that new steps be taken to help build a more rational use of our natural resources. The possibility, mentioned in this chapter as a future trend, to build new platforms able, in a sustainable way, to run integrated processes including pre- treatments, extractions, reactions, and transformations in a more integrated way is one of our main goals and might help all of us to build a better future.

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