



**Sudan University of Science and Technology**



**College of Science**

**Department of Chemistry**

# **Extraction of *Neem* Oil from *Neem* Seeds**

**Athesis for submitted for the partial fulfillment for B.S.E degree  
in chemistry**

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**(2013 - 2014)**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ تَعَالَى:

﴿أَلَمْ تَرَ أَنَّ اللَّهَ أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ ثَمَرَاتٍ مُخْتَلِفًا أَلْوَانُهَا

وَمِنَ الْجِبَالِ جُدَدٌ بَيْضٌ وَحُمْرٌ مُخْتَلِفٌ أَلْوَانُهَا وَغَرَابِيبُ سُودٌ ﴿٢٧﴾

وَمِنَ النَّاسِ وَالْدَّوَابِّ وَالْأَنْعَامِ مُخْتَلِفٌ أَلْوَانُهُ، كَذَلِكَ إِنَّمَا يَخْشَى

اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ ﴿٢٨﴾

صدق الله العظيم

فاطر: ٢٧ - ٢٨

## English abstract:-

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 .

## مستخلص البحث

الهدف من هذا البحث استخلاص زيت من نوى بذور النيم .

وقد تم استخلاص الزيت بطريقه سوكلية .

والنتائج المتحصل عليها وضحت ان النسبة المئوية لزيت النيم فى نوى  
بذور النيم هى 17.601%، و قيمة التصبن هى 192 ملجرام/جرام ، والرقم اليودي  
هو 67.38 جرام/100 جرام ، وقيمة الحمضية هى 17.5 ملجرام/جرام .

# Dedication

This dedication to my dearest family in general. And my dear mother in particular who never stop supporting us also my dedication to all teachers in different educational levels who were lighting our way moreover. I would like to dedicate this work to my family of department of chemistry and my colleagues .

# Acknowledgment

First and foremost, thank God, the Almighty , who , help us to complete this work and it helped us.

Then I would like to thanks **Dr.Muhammad Mukhtar Abdul Aziz**, who supervised this research was a good help to us in this process where provided us whit all the necessary information and did not spare us something from his knowledge profuse ask God that makes him the balance of advantage and that sustains him health and wellness.

We also extend our thanks also to the Research Institute of Medical and Aromatic Plants us for their assistance.

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# Chapter One

## (1) Introduction:-

### (1-1) Natural products:-

Plants produce an enormous variety of natural products with highly diverse structure . These products are commonly termed secondary metabolites in contrast to the primary metabolites which are essential for plant growth and development . secondary metabolites were formerly regarded as waste products without physiological function for the plants . With the emergence of the field of chemical ecology about 30 years ago , it became evident ,however, that these natural products full fill important function in the interaction between plants and their biotic environment .

They can serve , for example, as defense compounds against herbivores and pathogens, as flower pigment that attract pollinators , or as hormones or signal molecule . In addition to their physiological function in plants , natural products also have a strong impact on human culture and have been used throughout human history as condiments pigment and pharmaceuticals .<sup>"Anne"</sup>

The most important types of natural products are:-

- i. Terpene
- ii. Steroides
- iii. Alkaloids
- iv. Saponins
- v. Flavonids

### **(1-2) *Azadirachta India* :-**

*Azadirachta India*, also known as *Neem*, *Nimtree*, and *Indian lilac* is a tree also in the mahogany family *Meliaceae*. It is one of two species in the genus *Azadirachta* and is native to India, Pakistan and Bangladesh growing in tropical and semi tropical regions. *Neem* tree is official tree of the Sindh province and is very common in all cities of Sindh. *Neem* tree also grows in islands in the southern part of Iran. Its fruits and seeds are the source of *Neem* oil. "Grin taxonomy"

*Neem* trees start producing fruit when it reaches the age of three years where the tree is made up of flowers in the month of May and June and produce fruit in the month of July and August and falling fruits naturally at maturity where it can be collected from under the trees and gives the tree amounting to 20-40 kg of fruit per year and these are used in the manufacture of dry fruits extirminator including through simple and available.

### **(1-3) Chemical compounds:-**

Salimuzzaman Siddiqui was the first scientist to bring the anthelmintic, antibacterial antifungal and antiviral constituent of the *Neem* tree to the attention of natural products chemists. In 1942 he extracted three bitter compounds from *Neem* oil, which he named as Nimbin, Nimbinin and Nimbidin respectively. "Ganguli 2002" The process involved extracting the water insoluble components with ether, petrol ether, ethyl acetate and dilute alcohol. The provisional naming was Nimbin (sulphur free crystalline product with melting point at 205<sup>0</sup> c, empirical composition C<sub>7</sub>H<sub>10</sub>O<sub>2</sub>), Nimbinin (with similar principle, melting at 192<sup>0</sup>c) and Nimbidin (cream-colored containing amorphous sulphur melting at 90\_\_ 100<sup>0</sup>c) Siddiqui

identified Nimbidin as the main active antibacterial ingredient, and the highest yielding bitter component in the *Neem* oil. "Siddiqui 1942" These compound are stable and found in substantial quantities .They also serve as natural insecticides."Siddiqui 2004"

#### **(1-4) Uses of *Neem*:-**

*Neem* leaves are dried in India and Pakistan in cupboard to prevent insect eating the clothes and also while storing rice in tins . *Neem* leaves are dried and burnt in the tropical regions of Pakistan to keep away mosquitoes . These leaves are also use in many Indian festivals like Ugadi. "Anna 2006"

##### **☒ As vegetable:-**

The tender shoots and flower of the *Neem* tree are eaten as a vegetable in India . A soup likedish called veppampoo charu made of the flower of *Neem* is prepared in tamil nadu . In west Bengal , young *Neem* leaves are fried in oil with tiny pieces of egg plant (brinjal) . The dish is called nim begun and is the first item during Bengali meal that acts as appetizer it is eaten with rice . "neem Baigan"

##### **☒ Traditional medicinal use :-**

*Neem* products are believed by ayurvedic practitioners to be anthelmintic , antifungal , antibacterial , antiviral , contraceptive and sedative."DP . Agrawals" . It is considered a major components in Ayurvedic and unani medicine and is particularly prescribed for skin diseases."Tamilnadu .com" *Neem* oil is used for healthy hair , to improve liver function , detoxify the blood , and balance blood sugar levels."Zillur Rahman" . *Neem* leaves have also been used to treat skin diseases like aczema ,psoriasis ,etc."Anna 2006"

How ever insufficient research has been done to assess the purported benefits of *Neem*. In adults , short term use of *Neem* is safe while long term use may harm the kidneys or liver is small children , *Neem* oil is toxic and

can lead to death *Neem* may also cause miscarriages , infertility , and low blood sugar. "neem ,webMD"

☒ Other uses of *Neem*:-

- i. Toiletries: *Neem* oil is uses for prepared soup , shampoo, balms , and creams .
- ii. Tooth brush : effective in reducing plaque and gingival inflammation .
- iii. Tree : the *Neem* tree is of great importance for it is anti desertification and good carbon dioxide sink .
- iv. *Neem* gum : is used as a bulking agent and for the preparation of special purpose foods.
- v. *Neem* blossoms : are used in Andhra Pradesh , Tamil Nadu and Karnataka to prepare Ugadi pachhadi . A mixture of *Neem* flower and jiggery is prepared and offered to friend and relatives.
- vi. Cosmetics: *Neem* is precived in India as a beauty aid powder leaves are a major component of at least one widely used facial cream . purified *Neem* oil is also used in nail polish and other cosmetics .
- vii. Bird repellent : *Neem* leaf boiled in water can be used as a very cost effective bird repellent , espically for sparrows .
- viii. Lubricant : *Neem* oil is commonly used to grease cat wheels .
- ix. Fertilizer : *Neem* has demon start considerable potential as a fertilizer .
- x. Resin : An exudates can be tapped from the trunk wounding the bark .
- xi. Soap: 80T% of India' s supply of *Neem* oil is now used by *Neem* oil soap manufacturers."Ziller 1993"

## **(1-5) The stages of the preparation of the *Neem***

### **pesticide:-**

#### **i) combining stage:-**

Are gathering under deciduous trees *Neem* during the season to produce fruit and transported to the work site where they are cleaned of dirt

#### **ii) stage drying and storage:-**

The fruit are drying in the sun in concrete ponds with a dedicated drying stirred from time to time by means of a thermal drying at 60<sup>0</sup>c is then stored in a cool and dry store .

#### **iii) stage grinding fruits:-**

Fruits are grinding mill by the stomach for it and the resulting powder packaged in plastic container sealed and stored for use when demand is not mixed with water but when you use them and when mixed with water should be used within 48 hours with a maximum speed due to the decomposition of the pesticide .

#### **iv) phase extraction of pesticide active ingredients:-**

Soak powder *Neem* in water at a rate of 11.5 kg per hundred liter of water in the tank for a period ranging between 24-48 hours with the addition of a solvent or drops of liquid soap with moving the demodulator in a while and the solution is filtered using a refinery suitable then be pesticide ready for use and can be as powder precipitated from the solution earners in treatment of nematodes and as improved soil sprinkle the solution which was prepared

as an insecticide directly at rates of 200ml-800ml/100 liters of water or maximum of two liters by hectare . "Sharma 1994"

### **(1-6) *Neem* oil:-**

*Neem* oil is a vegetable oil pressed from the fruits and seed of the *Neem*.

*Neem* oil varies in color , it can be golden yellow , yellowish brown , reddish brown , dark brown , greenish brown or bright red . It has a rather strong odor that is said to combine the odors , of peanut and garlic . It composed mainly of triglycerides and contains many triterpenoid compounds , which are responsible for the bitter taste . It is hydrophobic in nature in order to emulsify . It in water for application purpose . It must be formulated with appropriate surfactants . "Wikipedia .org/wiki/Neem.oil"

Azadirachtin is the most well known and studied triterpenoid in *Neem* oil. The Azadirachtin content of *Neem* oil varies from 300ppm to over 250ppm depending on the extracting technology and quality of the *Neem* seeds crushed. *Neem* oil also contains steroids (campesterol , beta sosterol , stigmasterol ). "Wikipedia.org/wiki/Neem oil"

#### **(1-6-1)The active ingredients in *Neem* oil are:-**

- i. Azadirachtin
- ii. Clarified hydrophobic extract of *Neem* oil

Azadirachtin and clarified hydrophobic extract of *Neem* oil are derived from the natural oil found in seeds of the *Neem* tree .

When the natural *Neem* oil is removed from the seeds and treated with alcohol , virtually all the azadirachtin and related substances separate from the oil it self .

The remaining oil without the azadirachtin is called clarified hydrophobic extract of *Neem* oil. "EPA"

### **(1-6-2) *Neem* oil method:-**

The method of processing is likely to affect the composition of the oil, since the methods used, such as pressing (expelling) or solvent extraction are unlikely to remove exactly the same mix of components in the same proportions. The *Neem* oil yield that can be obtained from *Neem* kernels also varies widely in literature from 25% to 45%.

The oil can be obtained through pressing (crushing) of the seed kernel both through cold pressing or through a process incorporating temperature control. *Neem* seed oil can also be obtained by solvent extraction of the *Neem* seed, fruit, oil cake or kernels. A large industry in India extracts the oil remaining in the seed cake using hexane. This solvent extracted oil is a lower quality as compared to cold pressed oil and is mostly used for soap manufacturing. *Neem* cake is a product obtained in the solvent extraction process for *Neem* oil. "Mishra 1995"

### **(1-6-3) The affections of *Neem* oil:-**

#### **(1-6-3-A) Some signs and symptoms from a brief exposure to *Neem* oil:-**

*Neem* oil can be slightly irritating to the eyes and skin. Azadirachtin, a component of *Neem* oil, can be very irritating to the skin and stomach. The remaining portion of *Neem* oil is made of fatty acids, essential oils and other substances that are commonly eaten in a normal diet.

In other countries, *Neem* oil has been used on cats for flea control. Some adverse reactions have been reported. Symptoms include feeling sluggish, excessive salivation, impaired movement, trembling, twitching, and



convulsions . Some of the cat died. However, most of them recovered within 1 to 5 days "npic"

### **(1-6-3-B) The effect of *Neem* oil when it enters the body:-**

Clarified hydrophobic *Neem* oil (without azadirachtin) is made of fatty acids and glycerides. These substances are commonly found in food. When they enter the body, they are broken down, used for energy, and incorporated into Cells.

### **(1-6-3-C) The effect of the environment on the *Neem* oil:-**

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.

### **(1-6-3-D) The effect of *Neem* oil on birds, fish, or other wildlife:-**

*Neem* oil is practically non-toxic to birds, mammals, bees and plants. *Neem* oil is slightly toxic to fish and other aquatic organisms.

Azadirachtin, a component of Neem oil, is moderately toxic to fish and other aquatic animals. It is important to remember that insects must eat the treated plant to be killed. Therefore, bees and other pollinators are not likely to be harmed . "npic"

#### **(1-6-4) Uses of *Neem* oil :-**

The *Neem* oil is not used for cooking purposes , it is used for preparing cosmetic (soap , hair products , body hygiene creams , hand creams) and in Ayurvedic unani and folklore traditional medicine in the treatment of wide range of afflictions . The most frequently reported indications in ancient Ayurvedic writing are skin diseases . Inflammations and fevers and more recently rheumatic disorders , insect repellent and insecticide effects . "Mishra 1995"

Traditional ayurvedic uses of *Neem* include the treatment of acne fever leprosy , malaria , ophthalmia and tube culosis . various folk remedies for *Neem* include us as an anthelmintic , antifeedant , antiseptic diuretic emmenagogue, contraceptive, febrifuge, parasiticide, pediculocide and insecticide. It has been used in traditional medicine for the treatment of tetanus, urticaria, eczema, scrofula and erysipelas. Traditional routes of administration of neem extracts included oral, vaginal and topical use. . *Neem* oil has an extensive history of human use in India and surrounding regions for a variety of therapeutic purposes. "Puri 1999"

Formulations made of neem oil also find wide usage as a biopesticide for organic farming, as it repels a wide variety of pests including the mealy bug, beet armyworm, aphids, the cabbage worm, thrips, whiteflies, mites, fungus gnats, beetles, moth larvae, mushroom flies, leafminers, caterpillars, locust, nematodes and the Japanese beetle . *Neem* oil is not known to be harmful to mammals, birds, earthworms or some

beneficial insects such as butter flies, honey bees and lady bugs if it is not concentrated directly into their area of habitat or on their food source. It can be used as a house hold pesticide for ant, bedbug , cockroach , house fly, sand fly, snail, termite and mosquitoes both as repellent and larvicide."Puri 1999"

*Neem* seed oil has also been found to prevent implantation and may even have an abortifacient effect similar to pennyroyal, juniper berries, wild ginger, myrrh and angelica. The effects were seen as many as ten days after fertilization in rats though it was most effective at no more than three days. In a study on rats, neem oil was given orally eight to ten days after implantation of the fetus on the uterine wall. In all cases, by day 15, the embryos were all completely resorbed by the body. The animals regained fertility on the next cycle showing no physical problems.

*Neem* seed oil has also been used as a renewable source for the preparation of polymeric coatings. It has been converted into various polymeric resins, including polyesteramides and polyetheramides. These resins may be utilized further for preparation of polyurethane coatings."Wikipedia.org/wiki/neem oil"

### **(1-6-5) Toxicity:-**

Studies done when Azadirachtin (the primary active pesticidal ingredient in *Neem* oil) was approved as a pesticide showed that when *Neem* leaves were fed to male albino rats for 11 weeks, 100% (reversible) infertility resulted.

*Neem* oil and other neem products such as neem leaves and neem tea should not be consumed by pregnant women, women trying to conceive, or children.

There is some evidence that internal medicinal use may be associated with liver damage in children. "npic"

## **(1-7) *Neem* cake :-**

*Neem* cake organic manure is the by-product obtained in the process of cold pressing of *Neem* tree fruits and kernels, and the solvent extraction process for *Neem* oil cake. It is a potential source of organic manure under the Bureau of Indian Standards. *Neem* has demonstrated considerable potential as a fertilizer. For this purpose, *Neem* cake and *Neem* leaves are especially promising. *Neem* cake can be used for partial replacement of poultry and cattle feed. "npic"

### **(1-7-1) Components of *Neem* cake:-**

*Neem* Cake has an adequate quantity of NPK in organic form for plant growth. Being a totally botanical product it contains 100% natural NPK content and other essential micro nutrients as N(Nitrogen 2.0% to 5.0%), P(Phosphorus 0.5% to 1.0%), K(Potassium 1.0% to 2.0%), Ca(Calcium 0.5% to 3.0%), Mg(Magnesium 0.3% to 1.0%), S(Sulphur 0.2% to 3.0%), Zn(Zinc 15 ppm to 60 ppm), Cu(Copper 4 ppm to 20 ppm), Fe (Iron 500 ppm to 1200 ppm), Mn (Manganese 20 ppm to 60 ppm). It is rich in both sulphur compounds and bitter limonoids.

According to research calculations, *Neem* cake seems to make soil more fertile due to an ingredient that blocks soil bacteria from converting nitrogenous compounds into nitrogen gas. It is a nitrification inhibitor and prolongs the availability of nitrogen to both short duration and long duration crops."Neem Tamiladu.com"

### **(1-7-2) Uses as a fertilizer :-**

*Neem* cake organic manure protects plant roots from nematodes, soil grubs and white ants probably due to its residual limonoid content. It also acts as a natural fertilizer with pesticidal properties. *Neem* cake is widely used in India to fertilize paddy, cotton and sugarcane. Usage of *Neem* cake have shown an increase in the dry matter in *Tectona grandis* (Teak), *Acacia nilotica* (Gum Arabic), and other forest trees."Mishra1995"

*Neem* seed cake also reduce alkalinity in soil, as it produces organic acids on decomposition. Being totally natural, it is compatible with soil microbes, improves and rhizosphere microflora and hence ensures fertility of the soil. *Neem* Cake improves the organic matter content of the soil, helping improve soil texture, water holding capacity, and soil aeration for better root development."Puri 1999"

### **(1-7-3) Pest control:-**

*Neem* cake is effective in the management of insects and pests. The bitter principles of the soil and cake have been reported to have seven types of activities:-

- (a) antifeedant.
- (b) attractant.
- (c) repellent.
- (d) insecticide.
- (e) nematicide.
- (f) growth disruptor.
- (g) antimicrobial.

The cake contains salannin, nimbin, azadirachtin and azadiradione as the major components. Of these, azadirachtin and meliantriol are used as locust antifeedants while salannin is used as an antifeedant for the housefly."Mishra 1995"

## **(1-8) Azadirachtin**

### **(1-8-1) Introduction :-**

Azadirachtin is a chemical compound belonging to the limonid group , is a secondary metabolite present in neem seeds . it is a highly oxidized tetranortriterpenoid which boasts a plethora of oxygen functionality , comprising an enol ether , acetal , hemiacetal and tetra-substituted oxirane as well as a variety of carboxylic esters .

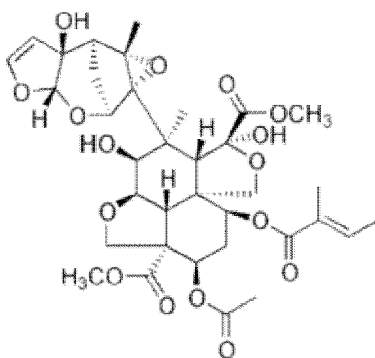
Various components of *Neem* consists of varies amount of azadirachtin . *Neem* compounds of a general class of natural products called Limonids . Almost each and every part of the *Neem* tree have medicinal peoperties about 60% of *Neem* fruit goes uncollected ."[www.mastlyherbs.com](http://www.mastlyherbs.com)"

Fruit and seeds are used for oil extraction where as neem oil is widely used in soap industry . Neem extracts are used as technical material for formulation these formulation are used in crop protection . Seeds contain kernel which in turn contains Azadirachtin and other better compounds ." <http://en.wikipedia.org> "

Azadirachtin is the most important and active component the other component present are Nimbin , Nimbidin , Nimbinin , oleic acid , Stearic acid , Palmitic acid , quercitin and other limonids .

Azadirachtin is currently considered as neem's main agent for controlling insects . " [www.terawet.ocm](http://www.terawet.ocm) "

Molecular weight of Azadirachtin is 720 and its melting point is 160<sup>0</sup> c.



Structure of azadirachtin [www.neemfoundation.com](http://www.neemfoundation.com)

### (1-8-2) Chemistry of azadirachtin:-

Azadirachtin has a complex molecular structure, and as a result the first synthesis was not published for over 22 years after the compound discovery. The first total synthesis by Stevenley in 2007. <sup>Veitch 2007</sup>

Both secondary and tertiary hydroxyl groups and tetra-hydro-furan ether are present and the molecular structure reveals 16 stereogenic centers, 7 of which are tetra-substituted. These characteristics explain the great difficulty encountered when trying to produce it by a synthetic approach. The described synthesis was actually a relay approach, with the heavily functionalized decalin being made total synthesis in a small scale but also being derived from the natural products itself for the purpose of obtaining gram amounts of the material to complete the synthesis. <sup>Veitch 2007</sup>

### (1-8-3) Occurrence and uses of azadirachtin :-

It was initially found to be active as a feeding inhibitor towards the desert locust. <sup>Butter 1968</sup> It is now known to affect over 200 species of insect by acting mainly as an anti-feedant and growth disruptor and as such it possesses considerable toxicity towards insects. It fulfills many of the

criteria needed for a natural insecticide if it is to replace synthetic compounds .

Azadirachtin is biodegradable and shows very low toxicity to mammals . This compound is found in seeds of the neem tree . Many more compounds related to Azadirachtin are present in the seeds as well as in the bark and leaves of the *neem* tree which also show strong biological activities among various pest insects .<sup>"senthil 2005"</sup>

#### **(1-8-4)Studies on extraction and HPLC analysis of azadirachtin from kernels of *Neem* seeds :-**

Chemicals:-

The chemical used like di-chloro methane , ethyl acetate hexane acetonitrile (HPLC grade) , methanol (HPLC grade) , triethyl amine (GR grade) ortho-phosphoric acid (GR grade) <sup>"sethi 1997"</sup>

Materials:-

ripe *neem* fruit , Hyderabad

Instruments:-

Soxhlet set up , uv-visible spectrophotometer ,HPLC <sup>"willard1988"</sup>

Method:-

Preparation of *neem* extract :

The whole neem tree contains bitterness in varied extent but higher concentration of it found in the neem kernel . *Neem* kernel is a valuable source of major limnoid . It is there for essential to understand the scientific method of fruit collection and depulping to get kernels .



### **(1-8-5) Collection of *Neem* fruit :-**

The *Neem* yields fruits during May to August every year . being rich in carbohydrates neem fruit gets attacked by fungi when come in contact with soil . such fruits may damage the quality of the final products prepared from these fruits . hence it is strongly recommended to avoid contact of *Neem* fruits with soil . as the fruit ripens they must be depulped as early as possible.

### **(1-8-6) Depulping of *Neem* fruits:-**

Depulping is a process to remove seed coat and pulp from the *Neem* seed . It is done by hand and using mechanical depulper .Rub the ripe *Neem* fruits between palms in the bucket of water and wash the seed . by use clean water for depulping . *Neem* Research and Technology Development Center (NRTDC) developed a mechanical depulper to handle large quantity of *Neem* fruits . After depulping and cleaning the *Neem* seeds in a cool and dry place . if process properly these neem seeds can be stored for about 6-12 months

### **(1-8-7) Separation of the kernels:-**

The dried *Neem* seeds are grand slightly by the hand and the outer shell of the seed is removed , kernels are present inside the shell which are separated and then made into powder using a grinder . it should be powdered such this no oil comes out of it . This coarse powdered is used for further studies for the extraction of azadirachtin ."willard1988"

### **(1-8-8) Extraction of azadirachtin from *Neem* kernel powder:-**

For extraction of azadirachtin by solvent process weight about 500 g of fine tea powder sized clean neem kernel powder . it should be pounded in

such away that no oil comes out of it . make a thimble and fill it with kernel powder , place it in a soxhlet apparatus and add about 600 ml of di-chloro-methane . keep it in a heating mantle and heat the reflux for about 12 hours, when the powder kernel is extracted with solvents like di-chloro-methane or ethyl acetate liminoides and other constituents get dissolved in it . leaving the seed cake without any active components . The solvent from this mixture is recovered by distillation . the distillation or concentrated solution is kept a side for cooling hexane is added to the concentrate and then filtered using vaccum pump . the above residue is dried which gives a pale greenish colored powder . this powder consist of azadirachtin and a very small quantity of nimbin . 100 g of neem kernel powder on extraction gives about 1 g of azadirachtin . "Patrizia"

#### **(1-8-9) TLC confirmation :-**

Take a small amount of azadirachtin and dissolve it in methanol , spot the sample on a TLC plate and place it in a beaker consisting iso-propanol : hexane(2.5 : 17.5) as mobile phase . Run till 3/4<sup>th</sup> of the plate , then place it in iodine chamber . it gives two pale yellow colored spots . the upper spot indicates azadirachtin and the below spot indicates nimbin . "Hui-Pring Huang"

#### **(1-8-10) UV-Spectrophotometer:-**

Different concentrations of Azadirachtin were prepared with different solvents and scanned in the UV region . the wave length 220nm was selected for Azadirachtin where it show maximum absorbance . lower concentrations does not show any absorbance hence concentrations (above 10mcg) were taken for calibration . the calibration curves were linear over the concentration range of 30-70 Mg/ml of Azadirachtin . absorbance were plotted versus respective concentrations . "Otmer"

**(1-8-11) High performance liquid chromatography (HPLC) :-**

The retention time of Azadirachtin is determined using HPLC . The column used for chromatographic separations was reverse phase phenomena . a hence the analytical wave length of 210nm was set and the sample was injected . The chromatographic separation were accomplished using mobile phase consisting of acetonitril : methanol : 1% triethyl amine adjusted to PH 4 (60:40:1) , filtered through a filter using value stage vaccum pump . mobile phase was pumped at a flow rate of 1ml/min at room temperature . "Hui Ping

## Chapter Two

### **(2-1) The Materials and apparatus :-**

#### **(2-1-1) The materials :-**

For Extraction of Neem oil :-

Neem seeds – n-hexane .

For saponification value :-

Alcoholic potassium hydroxide (0.5M) – hydrochloric acid solution (0.5M) –

Neem oil – phenolphthalein indicator .

for Iodine value :-

Neem oil – chloroform – wij's solution –potassium iodide 10% - sodium thiosulphate (0.05M) – starch – distilled water .

For acidic value:-

Neem oil - phenolphthalein indicator – diethylether –potassium hydroxide (0.1M) .

#### **(2-1-2) Apparatus:-**

Soxhlet chamber – conical flasks – 100 ml volumetric flask – burette – beakers – pipette – droppers- sensitive balance – water bath.

## **(2-2) procedure :-**

For extraction of Neem oil :-

100 g of Neem seeds was placed into the thimble and placed in the soxhlet chamber .

500 ml of n- hexane were placed in a round bottem flask , and assemble for soxhlet extractor . and the distillation process was begun .

after the completing of the extraction process , the solvent and extractor were placed on water bath to evaporate the solvent and the extracted oil was weighted .

For saponification :-

2.00 g of Neem oil was weighed in a conical flask , 25 ml of alcoholic potassium hydroxide (0.5M) were added from burette and then refluxed on water bath for 45 minutes.

It was cooled and titrated against hydrochloric acid solution (0.5M) until the end point by using phenolphthalein indicator , and recorded the volume ( $V_1$ ).

the titration was repeated without the sample and recorded the volume ( $V_2$ ) .

For iodine value :-

0.21 g of Neem oil was weighed in a conical flask , 10 ml of chloroform were added and 25 ml of wijk's solution were added The conical flask was covered and left in dark for 30 minutes (the solution was stirred several times during this period ). 10ml of potassium iodide 10% and 100 ml of distilled water were added then the librated iodine was titrated against

(0.05M) sodium thiosulphate solution by using starch as indicator . and the volume ( $V_1$ ) was recorded .

The titration was repeated without the oil and the volume ( $V_2$ ) was recorded .

For acidic value :-

5.01 g of Neem oil were weighed in a conical flask , 20% of ethanol and ether were added by measuring cylinder, and shaken well and titrated against potassium hydroxide solution and the volume ( $V_1$ ) was recorded .

The titration was repeated without the oil and the volume ( $V_2$ ) was recorded .

## Chapter Three

(3) Results and discussion :-

(3-1) Results :-

For Neem oil :-

Weight of essential oil/g	17.601
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For saponification :-

V <sub>1</sub>	V <sub>2</sub>
2.3 ml	16.0 ml

For iodine value :-

V <sub>1</sub>	V <sub>2</sub>
5.3 ml	27.6 ml

For acidic value :-

V <sub>1</sub>	V <sub>2</sub>
6.7 ml	22.3 ml

### **(3-1-1) Calculation :-**

Percentage of Neem oil :-

$$\% \text{ oil} = (17.601 / 100) \times 100 = 17.601\% .$$

Saponification Value :-

The volume of acid that reacted with the sample ( $V_3$ ) =  $V_2 - V_1$

$$V_3 = 16.0 - 2.3 = 13.7 \text{ ml.}$$

$$\text{Number of moles of HCl} = M.V.10^{-3} = 0.5 \times 13.7 \times 10^{-3} = 6.85 \times 10^{-3}$$

$$\text{Number of moles of KOH} = 6.85 \times 10^{-3}$$

$$\text{Weight of KOH (for 2g of oil)} = 6.85 \times 10^{-3} \times 56.1 = 0.384 \text{ g}$$

$$\text{weight of KOH (for 2g of oil)} = 0.384 \times 1000 = 385 \text{ mg}$$

$$\text{weight of KOH in 1 g of oil} = 385/2 = 192 \text{ mg/g}$$

$$SV = (V_2 - V_1) \times 28.5 / \text{weight}$$

$$SV = (16.0 - 2.3) \times 28.5 / 2 = 192 \text{ mg/g}$$

Iodine Value :-

$$\text{Iodine Value} = (V_2 - V_1) \times M \times 12.69 / \text{weight}$$

$$= (27.6 - 5.3) \times 0.05 \times 12069 / 0.21 = 67.38 \text{ g/100g}$$

Acidic Value :-

The volume of the acid that reacted with the sample ( $V_3$ ) =  $V_2 - V_1$

$$V_3 = 22.3 - 6.7 = 15.6 \text{ ml}$$



$$\text{Number of moles of HCl} = M.V/1000 = 0.1 \times 15.6 /1000 \\ = 1.56 \times 10^{-3}$$

$$\text{Number of moles of KOH} = 1.56 \times 10^{-3}$$

$$\text{Weight of KOH (for 5g of oil)} = \text{Number of moles} \times M.wt \\ = 1.56 \times 10^{-3} \times 56.1 = 0.0875g$$

$$\text{Weight of KOH (for 5mg of oil)} = 87.5 \text{ mg}$$

$$\text{Weight of KOH ( for 1 mg of oil)} = 17.5 \text{ mg/g}$$

Or from the relation :-

$$AV = (V_2 - V_1) \times 5.61 / \text{weight} = (22.3 - 6.7) \times 5.61 / 5.01 = 17.5 \text{ mg/g}$$

### **(3-2) Discussion and suggestions :-**

#### **(3-2-1) Discussion :-**

The contain of Neem oil from Neem seeds kernels was found it represented about 17.6 % .

And the tested which calculated to extracted Neem oil were found the saponification value equal 192 mg/g , Iodine value equal 67.38 g/100g and acidic value equal 17.5mg/g .

**(3-2-2) suggestions :-**

1- separate the Azadirachtin from Neem oil and determine the percentage .

2- make the qualitative and quantitative analysis for specific part of Neem trees from different places and compare between them .

3- separate the component of Neem kernel powder by using HPLC.

4- study the effects of Neem oil on the human , animals , and environment and certain which is more affected .

5- study the structure of Azadirachtin and find method to the synthesis .

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