ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
ADDIS ABABA INSITUTE OF TECHNOLOGY
DEPARTMENT OF CHEMICAL ENGINEERING

EXTRACTION AND CHARACTERIZATION OF ESSENTIAL OIL FROM MARGOSA SEED

By

Wondesen Workneh

JUNE 2011
ADDIS ABABA, ETHIOPIA

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A thesis Submitted to the Research and Graduate School of Addis Ababa University, Addis Ababa Institute of Technology, Department of Chemical Engineering in partial fulfillment of the requirements for the attainment of the Degree of Masters of Science in Chemical Engineering under Process Engineering Stream.

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Advisor: Dr. Ing Zebene Kiflie

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List of acronyms

AIDS	Acquire Immune Deficiency Syndrome
AOAC	Associates of Analytical Chemistry
ASTM	American Society of Testing and Material
EHNRI	Ethiopian Health and Nutrition Research Institute
GC	Gas chromatography
Icipe	International Center of Insect Physiology and Ecology
MS	Mass-spectrometer
SCFE	Supercritical fluid extraction

Abstract

The objective of this study was to develop a process for the extraction of essential oil from Neem seed. The extraction was carried out using soxhlet and agitated mixing vessel extraction units. In the soxhlet extraction a n-hexane was chosen to determine the effect of time and particle size on yield of the extraction oil. In the agitated mixing vessel n-hexane and ethanol were used to investigate the effects of solvents, particle sizes, and temperatures (30, 40 and 50^{o}C) on the quality and yield. A general factorial design was applied to both extraction processes using DESIGN EXPERT software and linear regression model was obtained growing the individual effect of time, particle size, temperature and solvent type as parameter and their interaction in the entire extraction process. For the soxhlet extraction, the minimum oil yield has been determined as 37.625% after the extraction time of 2hours with particle size ranges from 0.85-1.4mm and maximum oil yield 46.545% was found at the extraction time of 6 and 8hours in a particle size ranges from 0.425-0.75mm. Therefore, increasing extraction time and decreasing particle size will increase the amount of oil extracted. In the batch experiment, results showed that the maximum oil yields were 33.19% for ethanol and 47.32% for n-hexane at 50^{o}C, and 0.425-0.71 mm particle size. The Neem oil was found to have a colour of golden yellow due to the presence of Nimbidin. Based on psycho-chemical characteristics, analysis showed that increasing temperature decrease iodine value, but increase saponification, and acid value, which means that higher extraction temperature results in higher oil yield but lower oil quality. The rough profitability evaluation indicates us this project is beneficial as it is clearly observed from the cost estimation, the rate of return on investment was calculated and has 79.45% and the payback period tells us the plant return its total investment cost in one year.

1. INTRODUCTION

1.1 Background

Margosa (Neem) tree, which is also known as Azadrichta indica, is one of the best known trees in India, which is known for its medicinal properties. The main reason behind the popularity of the Neem oil is that it is used to treat few of the most common problems that the people face.

The Neem tree (Azadrichta indica) is among the fastest-growing trees, it attains a height of about 12-13 feet and is a drought resistant tree, found mainly in Amhara region. Neem can grow in tropical and subtropical regions with semi-arid to humid climates. Neem tree will adapt to a mean annual rainfall of 450-1200 mm, mean temperatures of 25-35ºC and grow at altitudes of up to 800 meters above sea level (m.a.s.l.)

The tree is so much resourceful that almost all of its parts are used in some form or another. From toothpastes to oils, from cosmetics to medicines, Neem oil is used as an important ingredient. Its healing properties are simply awesome. It flourishes very well in the tropical area conditions where it provides luxurious shade, firewood and also used for afforestation. It produces large quantities of seeds that are hardly used.

Neem is a very versatile tree, which is mainly found in India. This tree is said to possess magical healing, medicinal as well as pesticide properties. Neem has well emerged as a perfect solution to some of the major problems like locust attack, diabetes, population growth, etc.

Today, we will find neem being used in almost every household, industry or institution in some form or another. The relationship with this useful tree is age old. The tree is known for its effective as well as versatile applications. Taking bath in water soaked with Neem leaves, using Neem twigs as brush are some common ways that helps in boosting up your immunity.

Modern science is also studying Neem for its potential use in the field of medicine. Preliminary results and clinical evidence tend to support the extraordinary benefits of Neem oil, Neem leaf and Neem barks. Other studies indicate that Neem supports the structure and function of the immune system. Again, it is interesting to note the wide-ranging benefit of Neem and how it is now being recognized throughout the world, and confirmed by scientific studies. Those

interested in learning more about Neem and its uses might start with some of the current literature on the subject. Worldwide, scientists are busy experimenting on potential of Neem and its products to unveil their potential.

1.2 Statement of the problem

Neem (Margosa) tree is fast growing and easy to cultivate, can survive drought and poor soil and keeps its leaves all year round. Since Ethiopia is located around the tropical region, thus, the weather makes a suitable environment for the growth of Neem tree. Mostly, Margosa (Neem) tree is available in large amount in Amhara, Afar, Gambella, Jijiga, Tigray region (Icipe-Ethiopia). Most Ethiopian people plant this tree without having a hardly known about its use. For example, merely, in Amhara region, the rural people use Neem leaves for village medicine, especially to cure malaria and diabetes disease.

From the literature, it is observed, world science approves the application of Neem trees for medicinal purpose for internal as well as external treatment problems. Unlike its medicinal purpose Neem (Azadrichta indica) is also used for production of cosmetics, soap, biodiesel, etc from the Neem oil. Its waste also used for fertilizer. Neem oil has no any side effects and it is environmentally friendly. (Neem: A Tree for Solving Global Problems, 1992)

The Ethiopian people prepare their village medicine traditionally from Neem leaves but, the concentration of the active constituent's chemical is very week. Disparate Neem leaves, Neem seed has a more concentrated azadirachtin chemical, so, extracting Neem oil from Neem seed manually requires more effort. Extraction of Neem oil from Margosa tree is a very simple process even every one can prepare it traditionally at home. Processing Neem seed in small industrial scale requires not complicated physical equipment and it is cost effective in regarding energy and raw materials saving. Thus, we can extract more concentrated Neem oil from the seed in small scale amount rather than preparing it manually and make it commercially available.

A Neem tree normally starts fruiting after 3-5 years. In about 10 years it becomes fully productive. Under favorable conditions fresh fruit yield per fully grown tree is about 50 kg per year. There is a potential of producing Neem oil since, about 75% of the weather in Ethiopia is suitable for growth of Neem tree and currently, there are about 25 places that the plant is mostly available (icipe-Ethiopia); as a result, we can plant the tree and produce the oil in small scale.

1.3 Objectives

1.3.1. General objective

The general objective of the study was to develop the process of the extraction of essential oil from Neem (Margosa) seed.

1.3.2. Specific objectives

The specific objectives of the study were:

- To investigate the effect of particle size on the extraction process.
- To study the effects of operating conditions and solvent type on the quantity and quality of the oil.
- To characterize Neem oil.

1.4 Significance of the study

- This study will contribute to a significant improvement from traditional methods to technological manufacturing of the oil for the use of Neem extracts.
- This work will show the possibility of getting the oil and this can be used us an additive in soap and cosmetic industries to improve the performance quality of the product.
- The experimental work showed that the possibility of getting the appropriate operating conditions for extraction of Neem oil.

2. LITERATURE REVIEW

2.1. History of Neem

On the Indian sub-continent, the neem tree has been used for more than 4,500 years. The earliest documentation of Neem mentions the fruit, seeds, oil, leaves, roots and bark for their advantageous medicinal properties. In the first millennium BC the Neem tree was called the "Sarva Roga Nivarini" (= one that could cure all ailments and ills). The Indian physicians CHARAKA (2nd century AD) and SUSRUTA (4th century AD), whose books provided the foundation of the Indian system of natural treatment, the Ayurveda, also mention the tree and its medical use. [Neem Foundation, 1997-2]

With the advent of Europeans on the Indian subcontinent, the religious practices around the neem tree were stigmatized as heathen practice and over time most practical uses were abandoned. However, at the beginning of this century the neem tree was still highly esteemed by Indian emigrants and they took it along to the places where they settled. Thus, the neem tree was introduced in places like Australia, Eastand sub-Sahelian Africa, South East Asia, and South America. In Indian agriculture, neem cake (the remains from the oil production out of neem seeds) was in use as a fertilizer and pesticide in sugar cane fields up to the 1930s.

With the end of the colonials era, interest in the neem tree was on the rise again. Pioneering work in the possible commercial use of Neem oil and cake had been done by the Indian Institute of Science in Bangalore as early as the 1920’s. Recalling the insecticidal properties of Neem, researchers began programs in the early sixties to identify the active principles and screen them against major crop pests.

2.2. Description of Neem Tree

Neem is truly a tree with roots firmly embedded in the cultures of its people. For 2000 years in India, Neem twigs have been chewed on to clean teeth, Neem leaf juice applied on skin to treat disorders, Neem tea drunken as a tonic, and Neem leaves placed in the home to ward away bugs (Muñoz-Valenzuela 2007).

Its fruit (about the size of an olive) are eaten raw or cooked, and young twigs and flowers are sometimes eaten as vegetables. The fruit is also a major food source for birds and bats (they eat

the pulp, not the seed), among others. The gum (resin), which is colorless, sticky, and malodorous, is also a high-protein food additive used in Southeast Asia, known as “neem glue”. Even the leaves are a source of food; they are used as fodder in the dry season (AgroForestry-Tree Database). In Gambella also, children eat the pulp when it becomes yellowish.

Neem also has important fuel uses: the wood is used as firewood and to produce excellent-grade charcoal, and the oil is used as lamp oil throughout India. The timber, although it has a rough grain and does not polish well, is used locally to make furniture. Its popularity in being used to make furniture is partly due to its insect repellent properties, for insects are deterred from coming near the furniture or the items inside. The wood is also popular for fencing and construction. In addition, the tree bark has 12% to 14% tannins, which makes it a good source for tannin chemicals (Agro Forestry Tree Database).

Neem has a well-developed root system that can extract nutrients from lower soil levels, making it an important agent in erosion control because it is virtually drought-resistant. As such it is useful as a dune fixation tree (Agro Forestry Tree Database). Farmers in India use neem cake (the residue left after extracting oil from the seeds) as an organic manure and soil amendment; it enhances the efficiency of nitrogen fertilizers by reducing the rate of nitrification and hampering pests such as nematodes, fungi, and insects (Agro Forestry Tree Database).

2.3. Geographic Distribution

2.3.1. Overview of neem in the world

The neem tree is native to India, Indonesia, Malaysia, Myanmar, Pakistan, Senegal, Sri Lanka, and Thailand. It has since been transplanted to many parts of the world, including several countries in Africa, South America, Latin America, the Caribbean, Middle East, and others (Agro Forestry Tree Database). In the United States, neem is grown in Florida and California.

2.3.2. Overview of neem in Ethiopia

Ethiopia is located in the tropical region as a result the weather condition makes a suitable environment for the growth of neem tree and about 65-75% of the conditions in Ethiopia are appropriate for plantation of the tree. There are about 25 places that neem tree has observed in

Ethiopia (IRLI, department of ICIPE-Ethiopia). Partially, it is found in the following regions, Gambella, Jijiga, and Umera etc. The people in Gambella do not have information about the high importance of the tree but some children eat the yellow fruit otherwise, they use to make the city green throughout the year. In this year (2010/11) ICIPE is doing a project on some parts of neem tree (leaves and bark) for treatment of malaria and it is expecting to implement in April of the year.

2.4. **Botanical Description**

The Neem tree (*Azadirachta indica)*, a member of the Meliaceae family (Mahogany family) (Agro Forestry Tree Database), is also called the Indian neem tree, Indian lilac, and Margosa tree. Neem populations are heterogeneous in all respects, owing greatly to differences in soil and climate. The trees themselves are known to have genetic variation in height, branching type, leaf form, and color (Muñoz-Valenzuela 2007).

Height generally ranges from 15 to 25 m, or even 30 m (Agro Forestry Tree Database) with limbs of 15 m in length. Neem has a large, round crown of about 10 m (maximum 20 m) in diameter. These foliage proportions provide for shade nearly year-round (Agro Forestry Tree Database).

Figure2.1: Neem Tree (Agro Forestry Tree Database)

Shiny dark green leaves are innately compound (leaflets attached in two rows to the main vein, as in Figure 2). The 10 to 12 serrated leaflets on each leaf are 7 cm long by 2.5 cm wide (Muñoz-Valenzuela 2007) and the leaf blade is glabrous. When damaged, the leaves emit a garlic odor.

Figure2.2: Neem leaves (Mansha Enterprises).

White flowers are found as inflorescences with joined sepals. The fruit is yellow, fleshy, about 1 to 2 cm long, and has one (infrequently, two) seeds.

Figure2.3: Neem flower and Fruit (Mansha Enterprises)

Like other characteristics of the tree population, the fruit form, size, weight, kernel proportion, composition, and oil content and quality are also varied (Muñoz-Valenzuela 2007).

Apart from Azadirchata indica there are two other know species of neem: Azadirachta siamensis and Azadirachta excelsa. (MotherNature, 1999)

Azadirachta Siamensis

Azadirachta siamensis grows in Thailand, where the seeds and young leaves of the so-called "sweet" neem are used as additions to many spices. The leaves are about twice as large as in Azadirachta india and less bitter. The seeds are also considerably larger and the kernels are rather of an emerald green than white. The medical uses of Azadirachta siamensis in Thailand are similar to those of Azadirachta indica in India (Mattias Giger 2011).

Azadirachta excelsa

Azadirachta excelsa grows in remote areas of Malaysia and the Philippine islands. It grows up to 160 feet (50 m) tall and is found deep in the mostly inaccessible rainforests. Because of its scarcity Azadirchata excelsa, like Azadirachta siamensis, is not used extensively for commercial products but plays a role in some indigenous medicines such as anti-malarials. (Mattias Giger 2011).

Table2.1: Taxonomical description of Neem

Kingdom	Plantae
Division	Magnoliophyta
Order	Sapindales
Family	Meliaceae
Genus	Azadirachta

Natural Habitat: Except for waterlogged soil, neem can grow in almost any climate in the lowland tropics, ranging from mixed forest in India, lowland monsoon forest in Indonesia, evergreen forest and dry deciduous forest in Africa (Agro Forestry Tree Database), to more recently the Caribbean, California, and Florida.

Neem can grow within an altitude range of 0 to 1500 m, and tolerate conditions to 40°C with a mean annual rainfall of 450 to 1200 mm (Agro Forestry Tree Database).

The adult neem is able to tolerate some frost, but requires a significant amount of sunlight. The young neem, on the other hand, can grow at least for the first few years in areas that are predominantly shaded, but are not able to tolerate frost (Agro Forestry Tree Database).

Neem can grow in soils from neutral to alkaline, but the optimum pH is 6.2 to 7. It can also live well on shallow, stony, sandy soils, or where there is a hard calcareous or clay pan near the surface (Agro- Forestry Tree Database). In an ideal habitat, neem can live 150 to 200 years (Neem Foundation).

Reproductive Biology: Neem first produces white fragrant blossoms, then develops hard green fruits which are bitter to the taste. Subsequently, the flesh (pericarp) softens and the fruit changes color to yellow. The ripe yellow fruit is sweet. From the beginning of the flowering stage to the seed falling (after the fruit shrivels away), is 27 weeks.

At four or five years old, neem can produce flowers and fruit, but only after 10 to 12 years will it produce economically viable seed quantities (Agro-Forestry Tree Database). A mature tree produces 30 to 50 kg fruit annually (Neem Foundation), or even as much as 50 to 100 kg of fruit per year. It is pollinated by insects such as honeybees. Neem may be self-incompatible, as some isolated trees do not set fruit (Agro-Forestry Tree Database).

The flowering and fruiting seasons vary, depending on the region and time of year. For example, in Thailand neem produces flowers and fruits year-round, whereas in East Africa, with its defined dry and wet seasons, it does not (Agro-Forestry Tree Database).

2.5. **Importance of Neem Tree**

Over thousands of years, Neem has been used by hundreds of millions of people and no hazards have been documented for normal dosages. (Klaus Ferlow1926)

Every part of this fascinating tree has been used, from ancient to modern times, to treat hundreds of different maladies. While it is still revered in India for its superior healing properties, recent investigation has dramatically increased worldwide interest in Neem and many products are now manufactured using this miraculous herb. More than any other Indian herb, Neem proved useful in helping the body resist diseases and restore the proper balance to the body's systems.

Neem Powder: Neem and its parts are available in powdered form which is put to many uses in industries ranging from cosmetics to oral care, from agriculture to medicine. Neem powder is used in agriculture to protect plants from insects and pest; it can also be applied as organic manure. It is also used in veterinary medicine to cure worms, intestinal problems and other internal as well as external infections.

Table2.2: The use of Neem tree and its products

Parts of neem tree and its product	**Uses in**			
	Pharmaceutical industry	**Cosmetic industry**	**Agriculture industry**	**Oral care**
Neem leaf	Inflammation, skin related diseases like acne, rashes…	Face and body cream	Natural pesticide, insecticide	Manufacturing of tooth paste, mouse washes
Neem kernel	-	Manufacturing of skin products	pesticide, insecticide, crop and plant protection	-
Neem seed cake	-	-	Pest repellant, organic fertilizer	-
Neem flower	Manufacturing of drugs	As an astringent, facial cream	Honey	-
Neem oil	-	Skin product, body lotion, beauty facial care	Pesticide, insecticide, fungicide	-
Neem seed	Drug and medicine: pimples, blemishes, skin infection, birth control	Skin creams, moisturizers, face packs	Pesticide, insecticide	-
Neem bark	Birth control, skin disease, ulcer, gastrointestinal problems	-	-	Dental product, ayurvedic skin

Neem is one of the most powerful blood purifier, detoxifiers and immune system boosters known. Hundreds of disease has been shown to respond favorably to neem.Neem leaf can be

taken as tea or incapsules. Neem oil can be applied externally or a few drops can be put in an empty capsul and taken internally. Some of the main importances of Neem oil are:

Skin conditions: Neem has remarkable effect on chronic skin conditions that often fail to respond to medical drugs, acne, psoriasis, eczema, ringworm and even stubborn warts are among the conditions that can clear up easily when high quality, organic neem oil used. Medical drugs can produce harmful side effects such as rashes, allergic reactions and redness. In addition neem oil can be used as an excellent component of cosmetics to help clear, beautify and rejuvenate the skin.

Stomach Ulcers: Neem has proven successful in treating stomach ulcers. Its antihistamine and antibacterial compounds can reduce inflammation and destroy ulcer causing bacteria.

Injuries :minor skin abrasions, sprains and bruises are easily treated by applying neem oil locally. The anti-inflammatory and anti bacterial compounds of neem are delightfully soothing and help to heal the injured areas quickly.

Cancer: Neem has been tested externally on many types of cancers including skin cancer, and internally againest lymphocytic cancer. Its polysaccharides and limonoids have reduced cancerous tumors in numerous scientific studies.

Diabetes: Neem has been found to reduce insulin requirements for diabetics by up to 50% for nonkeytonic, insulin fast and insulin-sensitive diabetes without altering blood glucose levels. This success has resulted in the Indian government approving the sale of Neem capsules and tablets through pharmacies and clinics. These preparations are essentially pure, powered Neem leaves.

Hearts Disease:Neem has beeen scientifically tested for its ability to reduce blood pressure, blood clots, heart irregularities and cholestrol levels. Since the antihistamine effects of nimbidin found in neem leaves has been found to cause blood vessel to dilate, it may be why neem can reduce high blood pressure. A recent study showed that neem lowered high cholestrol levels inonly one month.

AIDS: Neem contains potent immune modulating polysaccharide compounds which may be responsible for increasing antibody production, while other components in neem appear to

stimulate immune function by enhancing cellular mediated response. This dual action helps the body ward off the multiple infections so commonly see with AIDS. The National Institutes of Health reports encouraging results from in vitro tests for Neem as an antiviral agent against the AIDS virus.

Fungi, Parasites and Viruses: Neem has been proven under strict laboratory conditions to successfuly kill harmful fungi, parasites and viruses. Even though its mode of action is not yet known, neem does kill beneficial intestinal flora or produce side effect.

2.6. Extraction of Essential Oil

2.6.1. Essential oils

Essential oils from neem trees are the volatile, organic constituents of plant matter and contribute to both medicinal and agricultural usage. These oils were termed essential because they were thought to represent the very essence in pharmaceutical, cosmetic as well as agricultural purposes.

Volatile oils are chemically complex mixtures, often containing in excess of hundreds of individual components. Unlike Neem oil, most Essential oils have one to several major components which impart the characteristic odour and taste such as sweet and spicy, but Neem oil has bitter taste. However, there are also many minor constituents which also play their part in producing the final product.(Abdullah,2009).

Chemically, the essential oils are a complex and highly variable mixture of constituents that belong to two groups: terpenoids and aromatic compounds. The name terpene is derived from the English word “Turpentine” (Guenther, 1952; Guenther, 1985). The terpenes are the unsaturated hydrocarbons which have a distinct architectural and chemical relation to the simple isoprene molecule (CH_2==C (CH_3)—CH==CH_2).

Chemical analysis of essential oils is generally performed using gas chromatography (GC) (qualitative analysis) and gas chromatography –mass spectrometry (GC/MS) (quantitative analysis). Identification of the main components is carried out by the comparison of both the GC retention times and the MS data against those of the reference standards, Kovats retention indices (KI) and comparison with previous literature (Adams, 2001).

2.6.2. Sources and Isolation of essential oils

Essential oils are isolated from different aromatic plants across the world where they are esteemed as an imperative component of the native medicine systems. These essential oils can be produced in almost all plant organs such as flowers, buds, stems, leaves, fruits, seeds and roots etc. These are accumulated in secretary cells, cavities, channels, and epidermic cells (Burt, 2004). Almost all odoriferous plants contain essential oils. The raw material from which essential oils are manufactured may be fresh, partially dehydrated or dried (Ozcan, 2003).

The extraction of the essential oil depends mainly on the rate of diffusion of the oil through the plant tissues to an exposed surface from where the oil can be removed by a number of processes. There are different methods, depending upon the stability of the oil, for the extraction of the oil from the plant materials. The essential oils obtained by steam distillation or by cold-pressed are generally preferred for food and pharmacological applications.

Due to the bactericidal and fungicidal properties of essential oils, their pharmaceutical and food uses are becoming increasing important as alternatives to synthetic chemical products to protect the ecological equilibrium (Burt, 2004). The extracted oils can vary in quality, quantity and in the chemical composition depending upon the agro climate, plant organ, age and vegetative cycle stage (Masotti *et al*., 2003).

The complexity of the essential oils is a real challenge for determining their reliable and accurate compositional data. The rapid advances in spectroscopic and chromatographic techniques have totally changed the picture of chemical study of essential oils. Many techniques have been used for studying the chemical profiles of essential oil e.g. IR-spectroscopy, UV-spectroscopy, NMR spectroscopy and gas chromatography. The increasing importance of essential oils in various domains of human activities including pharmacy, cosmetics, aromatherapy, and food and beverages industry has prompted an extensive need of reliable methods for analyses of essential oils. The combination of gas chromatography and mass spectrometry (GC-MS) allows rapid and relaibale identification of essential oils components.

The yield and the quality of the essential oil are considerably affected by processing methods used for their handling and storage. The essential oils are enclosed in oil glands present in the cellular structure of the plant materials. Although essential oils may be produced from an

endemic population, there can be several reasons why the composition and thus, the essential oil quality from aromatic plants might differ greatly. Genetic, physiological and environmental factors as well as processing conditions may play an important role while defining the chemistry and chemical composition of essential oils.

2.6.3. Methods of essential oil extraction

Quality of oil depends on the type of extraction. Manufacturing of neem oil includes the collection of raw materials for the extraction and selection of extraction method.

2.6.3.1. Traditional methods of extracting Neem oil

Neem oil can be extracted traditionally at home using cold pressed extraction by hand and around 100 to 150 mgs of oil for every 1 kilogram of neem seed.

To press neem oil by hand, the kernels of the neem seed should be crushed in a mill or pound in a mortar. Add a small amount of water until the mixture forms a firm paste that can be kneaded. Knead the paste until oil drops form on the surface and press firmly to extract the oil. The kneading and pressing should be continued in turn until the maximum amount of oil is removed. The oil content of the seed kernel is about 45%, even though preparation of the oil at home possible, but this traditional method of processing Neem oil was not effective on percent yield. (The neem tree, HDRA - the organic organization)

Figure2.4: Neem oil cold pressed extraction pilot demonstration (Neem Tree Assessment for Socioeconomic Empowerment in Rural Burkina Faso)

2.6.3.2. Recent methods of Extraction

Extraction of oil from Neem seeds can be performed using three different methods: mechanical extraction, solvent extraction, and supercritical fluid extraction.

A) Mechanical extraction

Common method used to extract the Neem oil from the seed, since this method is effective for seed contain 30-70% oil. The mechanical extraction has several advantages compared to the other methods, such as simple equipment and low investment, low operating cost, and the oil does not undergo solvent separation process, etc.

Usually the quality and quantity of the oil obtained by mechanical extraction process are affected by various operating conditions such as pretreatment of the Neem seeds, extraction pressure, and storage condition. Effect of extraction condition on quality of oil has been investigated in several studies for wide variations of material, including conophor nut, olive, jojoba, and groundnut, and peanut kernel oil. The changes of oil quality during storage also have been investigated for numerous materials, such as soybean, peanut kernel, sunflower, olive, and fish oil.

Mechanical extraction of Neem seeds was performed using hydraulic pressing equipment (ENERPAC RC-256 and P-39). Untreated seed particles were pressed with various pressures to determine the optimum pressure. Pressure was started at 2000 psi as the oil started to flow out of the seedbed, and stopped at 6000 psi since the oil yield relatively constant at the pressure above 6000 psi. Mechanical extraction was performed for 25 minutes when the oil has stopped flowing out. Oil yield measurement was conducted using mass balance.

Mechanical extraction is the most widely used method to extract Neem oil from Neem seed. However, the oil produced with this method usually has a low price, since it’s turbid and contains a significant amount of water and metals contents.

B) Solvent Extraction

Solvent Extraction is a process which involves extracting oil from oil-bearing materials by treating it with a low boiler solvent as opposed to extracting the oils by mechanical pressing methods (such as expellers, hydraulic presses, etc.) The solvent extraction method recovers

almost all the oils and leaves behind only 0.5% to 0.7% residual oil in the raw material. In the case of mechanical pressing the residual oil left in the oil cake may be anywhere from 6% to 14%. The solvent extraction method can be applied directly to any low oil content raw materials. It can also be used to extract pre-pressed oil cakes obtained from high oil content materials. Because of the high percentage of recovered oil, solvent extraction has become the most popular method of extraction of oils and fats.

The process solvent extraction is basically a process of diffusion of a solvent into oil-bearing cells of the raw material resulting in a solution of the oil in solvent. Various solvents can be used for extraction. However, after extensive research 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. food grade n-hexane is considered to be the best and it is exclusively used for the purpose. In a nutshell, the extraction process consists of treating the raw material with hexane and recovering the oil by distillation of the resulting solution of oil in n-hexane called miscella. Evaporation and condensation from the distillation of miscella recovers the n-hexane absorbed in the material. The n-hexane thus recovered is reused for extraction. The low boiling point of hexane (67°C / 152°F) and the high solubility of oils and fats in it are the properties exploited in the solvent extraction process.

C) Two stage extraction process

This two stage extraction process is a mechanical extraction followed by solvent extraction. As raw material for neem-based extract processing, Neem seeds (*Azadirachta indica)* is used. Figure 2.5 shows the two stage schematic diagram of Neem-based extract processing plant resulting with equipment. A certain kilogram of dried Neem seeds was used in the process. The seeds firstly decorticated to obtain the seed kernel, then crushed and finally pressed to separate neem oil using mechanical extraction. By moving-bed contacting extraction technique, defatted neem cake will be extracted with solvent in an agitated-extraction vessel. After decantation of crude cake in mixing-settling tank, the neem solution is drained out, then filtered and evaporated until a specific volume; the so-called concentrated solvent-neem-based extract of the oil attains its quality. After quality measurement, the concentrate could be formulated for specific purpose as different commercial grade. Eventually, the product will be bottled and shipped to the consumer.

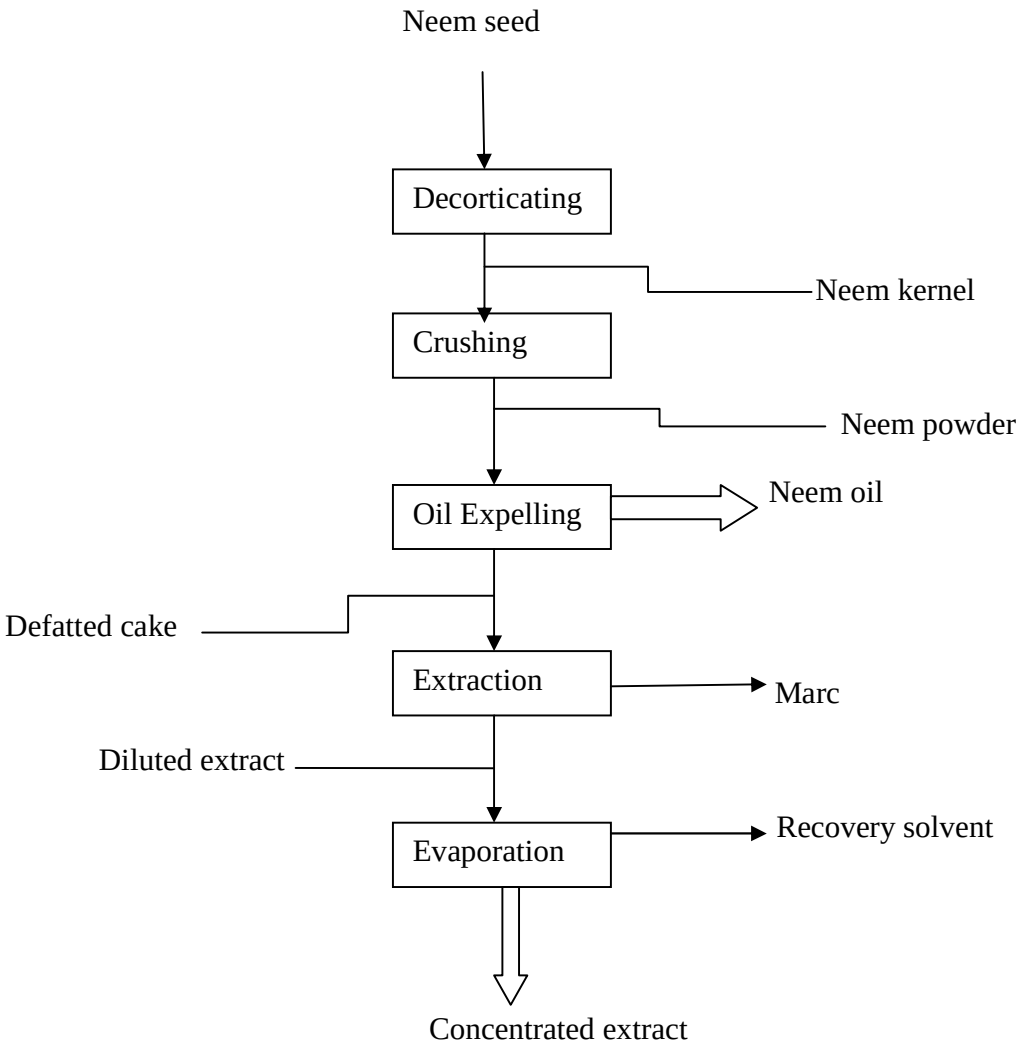


Figure2.5: Schematic diagram of two stages Neem-based extract processing plant resulting with equipment

D) Supercritical fluid extraction

The state of a substance is called supercritical fluid (SCF) when both temperature and pressure exceed the critical point values, see figure 2.6

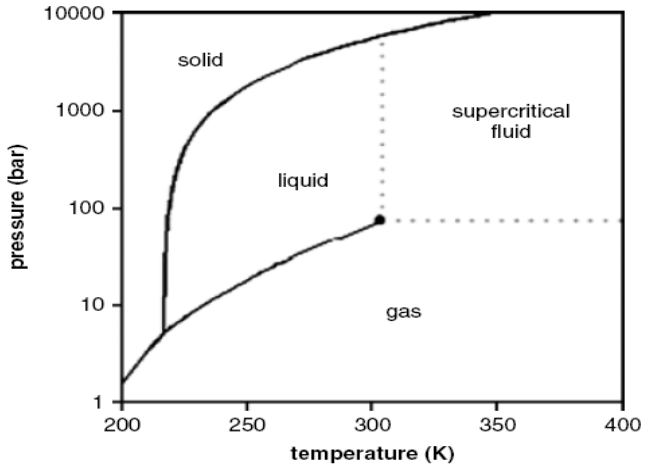


Figure2.6: Carbon dioxide pressure–temperature phase diagrams.

Extractions with supercritical fluid (SCF) solvents have emerged in recent years as highly promising environmentally benign technologies for the production of natural extracts with high potency of active ingredients – such as flavours, fragrances, spice oils and oleoresins, natural colours, nutraceuticals or herbal medicines – for the food, cosmetics, and pharmaceutical industries. Supercritical carbon dioxide (SC CO2) at near-ambient temperatures is the most desirable SCF solvent for extraction of natural products today as it is non-toxic, inexpensive, non-flammable, and non-polluting. Its near-ambient critical temperature (31.1 ◦C) makes it ideally suitable for thermally labile natural products. It is generally regarded as safe (GRAS) and yields contamination-free, tailor-made extracts having superior organoleptic profile and enhanced shelf life. The supercritical fluid extraction (SCFE) technique ensures high consistency and reliability in the quality and safety of the bioactive heat-sensitive botanical products because it does not alter the delicate balance of bioactivity of natural molecules. (Mamata Mukhopadhyay, 2008)

Extraction using supercritical fluid, the oil produced has very high purity; however the operating and investment cost is high.

In this study I used solvent extraction to extract the oil from Neem seed. The effect of parameters process such as time, temperature and particle size and solvents such ethanol and hexane were studied.

2.6.4. Factors affecting essential oil accumulation

Factors that determine the composition and yield of the essential oil obtained are numerous. In some instances it is difficult to segregate these factors from each other, since many are interdependent and influence one another (Terblanche, 2000). These variables may include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and postharvest drying and storage (Marotti 1994).

2.6.4.1. Seasonal and maturity variations

These two factors are interlinked with each other, because the specific ontogenic growth stage will differ as the season progresses. There are variations in the chemical profile of essential oils from various plants collected during different seasons. The essential oils yields varied considerably from month-to-month and was also influenced by the micro-environment (sun or shade) in which the plant was growing. Results obtained by Badi (2004) also indicated that timing of harvest is critical to both yield and oil composition.

2.6.4.2. Geographical variation

There are many reports in the literature showing the variation in the yield and chemical composition of the essential oil with respect to geographical regions (Uribe- Hernandez 1992). Chalchat et al. 1995 reported variations in the yield and chemical profile of essential oils, collected from different geographical locations, respectively. Such differences could be linked to the varied soil textures and possible adaption response of different populations, resulting in different chemical products being formed, without morphological differences being observed in the plants. (Hussain *et al*.,2008).

Altitude seems to be another important environmental factor influencing the essential oil content and chemical composition. Climatic factors such as heat and drought were also related to the essential oil profiles obtained (Uribe-Hermandez *et al*., 1992). Moreover, the preference of the plant for these conditions suggest that genetic make-up of the plant, rather than the soil-type in which it is growing, should have a greater influence on the chemical profile of the oil produced (Abdullah 2009).

2.6.4.3. Genetic variation

Genotype is typically defined as “the genetic make-up of an organism, as characterized by its physical appearance or phenotype”, while chemotype is generally defined as “a group of organisms that produce the same chemical profile for a particular class of secondary metabolites”. Variations in chemical profiles were observed from oils produced from specimens from the same population and location, demonstrating the presence of different chemotypes within this species. Genetic makeup of the plant is one of the most important contributors to their essential oil composition.

2.6.4.4. Other factors affecting yield and composition of essential oil

Other factors which affect the growing plants thus leading to variations in oil yield and composition, include part of plant used; post harvest drying; length of exposure to sunlight; availability of water, height above sea level, plant density, time of sowing and the presence of fungal diseases and insects. The oil composition and yield may also change as a result of the harvesting methods used, the isolation techniques employed, the moisture content of the plants at the time of harvest and the prevailing extraction conditions (Abdullah 2009).

Postharvest drying of material is an accepted practice in the production of essential oils. Drying methods include exposure to natural air in the shade, sun-drying, as well as drying by blowing warm air over the material. Postharvest drying is thought to improve oil yield and accelerate distillation, by improving heat transfer, in addition to providing increased loading capacity, due to loss of plant moisture. Further advantages include the reduction of microbial growth and the inhibition of some biochemical reactions in dried material. However, some amount of the oil may be lost during such post harvest treatment due to volatilization and mechanical damage to oil glands. Essential oil components (including terpenoids) are usually present in the free form, but may also be bound to sugar moieties, usually mono- or disaccharides (Abdullah 2009).

2.6.1. Chemical composition of Margosa oil

Neem elaborates a vast array of biologically active compounds which are chemically diverse and structurally complex. Neem chemistry dates back to 1880-90 when influenced by its folk-lore medicinal values, the chemist took up the isolation of active principle from its seed and other

parts. Siddiqui was the first to report the isolation of three products viz. nimbin, nimbidin and nimbinin from its oil.

The neem constituent belonging to chemically diverse classes have been divided into two major sections: (a) Isoprenoids & (b) Non-Isoprinoids. The later category comprises glycerides, polysaccharides, sulphurones compounds, flavonoids and their glycosides, amino acids, aliphatic compounds etc.

a) ISOPRENOIDS:

I. **Diterpenoids:** 24 compounds of this class have been isolated from root and stem bark of Neem. These chiefly belong to two groups' podocarpanoids and abietanoids. In early 60's, **sugiol** and **nimbiol** were reported first time.

II. **Triterpenoids:** The bitterness of neem is due to the occurrence of **limonoids** which are the **tetranortripenoids** based on apo-euphal skeleton. The term limonoid is derived from limonin, the first tetranortripenoid obtained from citrus bitter principle in 1841; the structure of which could be established only 1960. Out of 300 limonoids known today about 1/3 is accounted by Neem(Azadirachta indica) and chinaberry (Melia azedarach) alone.

Neem bitter principle can be conveniently classified under 8 groups: Protomeliacins , limonoids with a modified side chain, azadirone and its derivative , gedunin and its derivatives , vilasinin type compounds, C -secomeliacins – nimbin , salanin and azadirachtin.

III. **Protomeliacins:** The triterpenes containing C8 side chains C- 17 are supposed to be bio-genetic precursors of limonoids and hence known as protolimonoids or protomeliacins.

Meliantriol was the first triterpenyl alcohol, isolated from both neem oil and fresh fruits of Melia azedarach and shown to exhibit marked feeding inhibition against Desert locusts.

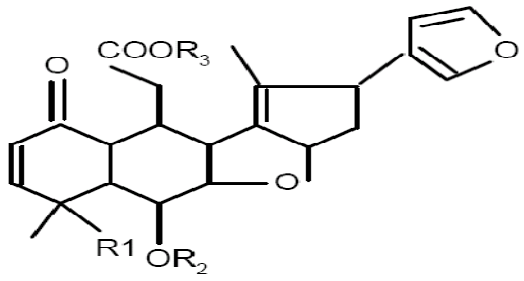
Siddiqui and his co-workers have added other protomeliacins **nimbocinone, nimolinone,** and **kulactone,** etc. **Nimbocinone** has been isolated from Neem leaves while most of the other constituents from fruit coats and whole fruits.

IV. **Limonoids with intact four rings and γ -hydroxybutenolide side chain:** The presence of a γ-hydroxybutenolide side chain in place of the furan ring is the characteristics of this group of compound. Two isomeric constituents, nimocinolides, isonimocinolide have been isolated

from Neem leaves where as isonimolicinolide from fresh fruits. Nimocinolides showed mild insect growth regulating properties.

V. **Gedunin and its derivatives:** This group consists of compounds wherein the D- ring has undergone oxidative expansion. Gedunin and its deacetyl derivatives have been found in Neem bark also in addition to their co-occurrence in seed oil. Gedunin was shown to posses both anti fungal and anti malarial property.

VI. **Azadirone and its natural analogues:** This group consists of limonoids in which all rings of the triterpenoid skeleton remain intact characteristics features of this group are presence of oxygen function at C3 and C7.



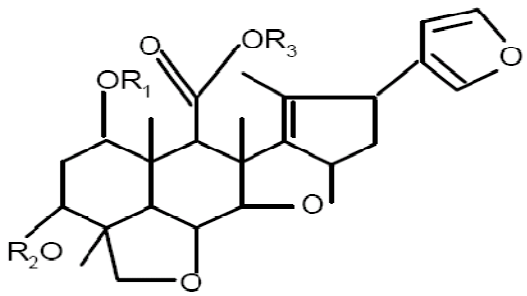
Azadirachtin A

R1 =COOMe **R2** = Ac **R3** = Me

Butterworth and Morgan (1968) isolated Azadirachtin A. Nahanishi first identified the group.

Source: every part of Neem but seed kernel is good source and in Neem oil 0.03% is present. In seed kernel maximum conc. of Azadirachtin A is 50000 ppm [Grace co (USA) formulation].

VII. **C-Secomeliacins:** This is a large and important group containing the most complex compound and it is specifically containing to neem. The 3 important sub-groups in this form are, nimbin, salanin, amd azadirachtin.There are 22 members of nimbin and salanin group have been isolated from Neem. Salanin has anti-feedant and detorant properties.



Salanin

R1= Tg(Tiglica C = 4), **R2**=Ac, **R3** =Me

The isolation of nimbin in 1942 marked the beginning of the chemistry of neem meliacins. The structure elucidation of nimbin was done very critically, carefully and cumbersome procedure. No technology was known at that time. This particular chemistry was done by 4 chlrol of chemistry.

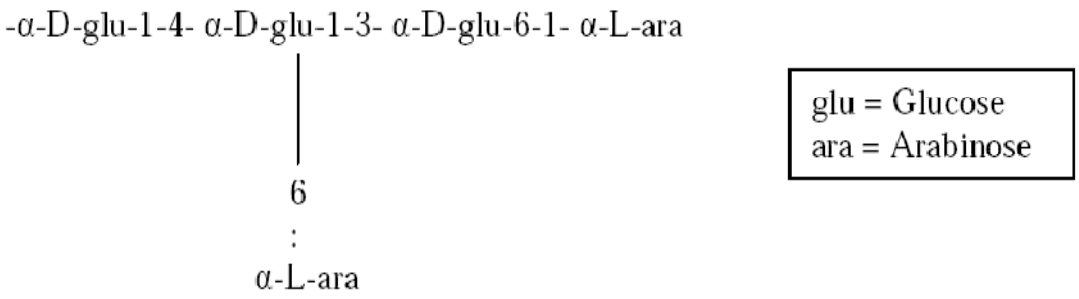
b) NON-ISOPRENOID constituents:

(Poly) phenolics: Flavonoids: The Neem leaves were reported to contain two flavonoids, quercetin and Isorhamnetin. The flowers were to contain kaempferol, myricetin and quercetin. The occurrence of a new isoprenylated flavanone, nimbaflavanone (8, 3'-di- isoprenyl-5,7-dihydroxy-4'-methoxyflavanone) in leaves is also reported.

Carbohydrates and Proteins: The gum exudates from the stem were found to be a very complex condensate proteins and heteropolysaccharides. The proteins are linked very tightly to the polysaccharides, which constitute the major components. A variety of smaller gum components have been identified after drastic degradation of the complex, e.g., D-glucose, D-glucuronic acid, Larabinose, L-fucose mannose, xylose etc were reported. The amino acid composition of the gum was also invested and it has been found the most abundant was aspartic acid. Among others serine and threonine were also found.

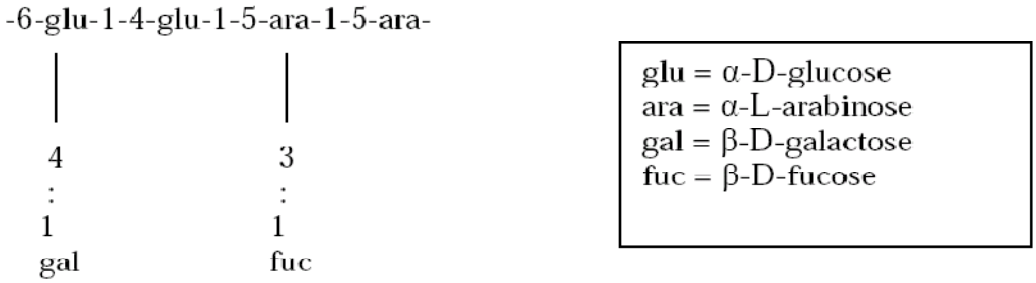
Polysaccharides Gla and Glb: Gla is composed of a repeating unit consisting of one molecule of α-L-arabinose and five molecule of α-D-glucose. The arabinose is linked (1-6) to one of the glucose molecules which are mutually linked (1-4). Glb is a branched arabinofucoglucan containing a main chain of (1-4) α-D-glucose molecules substituted in the 6 position with side chains of α-L-arabinose molecules and in the 4 position with 3-O-substituted fucose molecules.

Polysaccharides GIIa and GIIIa: GIIa is composed of the following repeating unit:



GIIIa is a branched arabinofucoglucan containg a main chain of (1-4)-linked α-D-glucose molecules substituted in the 6 position with side chain of α- arabinose and β-L-fucose.

Polysaccharides GIIIDO'2 Ia and GIIIDO'2IIa: GIIIDO'2 Ia is a branched fucogalactoglucoarabian containing a main chain of (1-5)-linked L-arbinose molecules and (1-4) linked D-glucose molecules. GIIIDO'2 IIa is contains the following repeating unit:



Two functionally different immune modulators, one methanol insoluble, high molecular weight (10kD) saccharide-containing fraction and the other, methanol-soluble, low molecular weight (10kD) fraction were isolated from aqueous bark extract.

Sulphurous Compounds: A number of cyclic tri- and tetra sulfides were identified from the steam distillate of the fresh matured leaves by GC-MS analysis. Several di- and trisulphides were also identified by capillary GC-MS analysis of the headspace volatiles from crushed seeds. Di-n-propyl disulphide was shown to be the major compound.

Table2.3: Substances isolated from various parts of the Neem tree (Philip S.J, 1995)

Name	**Structure**	**MP**	**Mol. formula**	**Mol wt.**	**Sources**	**Isol.**	**Ref.**
Azadirachtanin A	23	225	$C_{32}H_{40}O_{11}$	600	L	19	19
Azadirachtin	1	165	$C_{35}H_{44}O_{16}$	720	S	1	35,34
3-desa-acetyl-3-cinnamoyl	58	-	$C_{42}H_{48}O_{16}$	808	L	44	44
22-23-dihydro-23β-methoxy	59	-	$C_{36}H_{48}O_{47}$	752	S	43	43
1-tigloyl-3-acetyl-11-methoxy azadirachtin	57	-	$C_{34}H_{46}O_{16}$	734	B	44	44
Azadirachtol	61	-	$C_{32}H_{46}O_{6}$	526	F	62	62
3-tigloyl azadirachtol	60	204	$C_{33}H_{42}O_{14}$	662	S	44	44
Azadiradion	8	-	$C_{28}H_{34}O_{5}$	450	S	11b	11b
7-desaacetyl-7-benzoyl	9	-	$C_{33}H_{36}O_{5}$	512	S	15	15
7-desaacetyl-7-benzoylepoxy-	11	-	$C_{33}H_{36}O_{6}$	528	S	15	15
7-desaacetyl-7-hydroxy	-	160	$C_{26}H_{32}O_{4}$	408	F	65	65
1β, 2β-Diepoxy	15	110	$C_{28}H_{34}O_{7}$	482	S	15	15
4α,6α-Dihydroxy-A-homo-	41	177	$C_{26}H_{36}O_{6}$	468	L	32	32
17-Epi-	13	-	$C_{28}H_{34}O_{5}$	450	S	12	12
Epoxy	10	199	$C_{28}H_{34}O_{6}$	466	S	11b	11b
17β-hydroxy	12	-	$C_{28}H_{34}O_{5}$	466	S	12,57	12

Table (continued)

Name	Structure	MP	Mol. formula	Mol. wt.	Sources	Isol.	Ref.
1α-methoxy-1-2-dihydro-	16	235	$C_{29}H_{36}O_7$	498	S	15	15
Azadirone	7	-	$C_{26}H_{36}O_4$	436	S	11b	11b
Gedunin	42	218	$C_{26}H_{34}O_7$	482	S	11b	-
7-desacetyl	44	-	$C_{26}H_{32}O_6$	440	S	11a	11a
7-desacetyl-7-benzoyl	43	278	$C_{33}H_{36}O_7$	544	S	15	15
Iso azadirolide	53	-	$C_{32}H_{42}O_{10}$	586	L	37	37
Iso nimbocinolide	61	-	$C_{32}H_{42}O_9$	570	L	65	65
Isonimolicinolide	51	100	$C_{30}H_{36}O_6$	526	F	36	36
Margocinolide	64	130	$C_{27}H_{32}O_8$	484	T	68	68
Iso-	-	-	$C_{27}H_{32}O_8$	484	T	68	68
Meldenin	17	240	$C_{28}H_{38}O_5$	454	S	13	13
meliantriol	5	176	$C_{30}H_{50}O_5$	490	S,L	7	7
nimbandiol	38	121	$C_{26}H_{32}O_7$	456	S	31	31
6-acetyl-	39	178	$C_{26}H_{34}O_6$	498	S	66	66
Nimbidinin	34	282	$C_{28}H_{34}O_6$	442	S	58,59	58,59
Nimbin	24	201	$C_{30}H_{36}O_9$	540	SBW	60	60
Desacetyl-	22	208	$C_{28}H_{34}O_8$	498	SBF	60	60
4-Epi-	-	-	$C_{30}H_{36}O_9$	540	S	69	69
Photooxidized-	47	180	$C_{30}H_{36}O_{10}$	556	S	13	13
Nimbinene	36	134	$C_{28}H_{34}O_7$	482	SLB	31	31
6-Desacetyl	37	141	$C_{28}H_{32}O_6$	272	SLB	31	31
Nimbiol	26	250	$C_{18}H_{24}O_2$	470	B	3	3
Nimbocinone	6	76	$C_{30}H_{46}O_4$	466	L	8	8
Nimbolide	35	245	$C_{30}H_{46}O_{10}$	642	L	30	30
Nimbolin A	31	180	$C_{26}H_{32}O_2$	674	W	29	29
Nimbolin B	32	243	$C_{26}H_{34}O_6$	408	W	29	29
Nimocinol	-	-	$C_{28}H_{34}O_7$	442	F	70	70
Nimolicinolic acid	52	92	$C_{26}H_{34}O_6$	482	F	36	36

Table (continued)

Name	**structure**	**MP**	**Mol. formula**	**Mol. wt.**	**Sources**	**Isol.**	**Ref.**
Nimolicinol	45	270	$C_{28}H_{34}O_8$	452	S	33	33
Nimolinone	62	-	$C_{28}H_{34}O_7$	596	F	63	63
Salannin	4	167	$C_{34}H_{44}O_9$	554	S	28	28
3-Desacetyl-	29	214	$C_{32}H_{42}O_8$	612	S	16	16
Photooxidized	46	244	$C_{34}H_{44}O_{10}$	556	S	13	13
Salannol	40	208	$C_{32}H_{42}O_8$	192	S	16	16
Scopoletin	-	204	$C_{10}H_8O_4$	192	T	65	65
Sugiol	2a	-	$C_{20}H_{28}O_2$	300	B	62	62
7-acetylniotrichilonone	14	208	$C_{28}H_{38}O_{10}$	452	S	15	15

Where: S= seed, W=wood, L=Leave, B=Bark, F=Fruit

*The complex structure of each compounds (column 1, table2.3) appear in appendix-A.

3. MATERIALS AND METHODS

3.1. Raw material preparation

Neem seed was obtained from Gambella region. Impurities like stones, dusts and metals were removed by hand. The fruit soaked for two hours and then the flesh off the seed easily and leaving the rinsed shell to dry before storage. The fruit was decorticated using decorticating machine (disc-mill). Then the shafts and other impurities were separated.

3.2. Sample analysis

3.2.1. Determination of moisture content of the seeds

20g, 30g, and 40g of the cleaned sample was weighed and dried in an oven at 100°C and the weight was measured every 2hrs. The procedure was repeated until a constant weight was obtained. The percentage moisture in the kernel was calculated using the following:

$$\mathbf{Moisture}\% = \frac{(\mathbf{W1} - \mathbf{W2}) * \mathbf{100}}{\mathbf{W2} * \mathbf{100}} \qquad (\mathbf{3.1})$$

Where: W_1 = Original weight of the sample before drying; W_2 = Weight of the sample after drying.

3.2.2. Size reduction and sieve analysis of the seeds

The moisture was removed by placing the sample in an oven at 50°C for 24 hours. The dried Neem kernel was crushed in attrition mill (Retsch GmbH) with sieve size 2mm. The sample was sieved using vibrating shaker (Retsch) with set of sieves sizes arranged in descending order 1.8mm, 1.4mm, 1mm, 0.85mm, 0.71mm, 0.425 and 0.2mm to obtain particular sizes of 0.4-0.71mm, 0.71-0.85mm and 0.85-1.4mm. This is because to investigate the effect of particles size on yield and quantity of oil.

Methods

Extraction using solvent has several advantages. It gives higher yield and less turbid oil than mechanical extraction, and relative low operating cost compared with supercritical fluid extraction.

3.3. **Solvent extraction**

Experimental work was conducted using two different laboratory setups (soxhlet and agitated vessel extraction). In solvent extraction process, we used soxhlet extraction (used solvent n-hexane) and agitated-extraction vessel (by ethanol and n-hexane). The result from soxhlet extraction like extraction time was used as the starting parameter for the agitated-extraction method.

3.3.1. **Soxhlet extraction**

300ml of normal hexane was poured into round bottom flask. 40g of the sample was placed in the thimble and was inserted in the centre of the extractor. The Soxhlet was heated to 69.9^{o}C.. This was allowed to continue for two, four, six and eight hours. The experiment was repeated by placing the same amount of the sample into the thimble again by varying particle sizes (0.4-0.71mm, 0.71-0.85mm and 0.85-1.4mm). The weight of oil extracted was determined for each run hours. At the end of the extraction, the resulting mixture *(miscella*) containing the oil was heated to recover solvent from the oil.

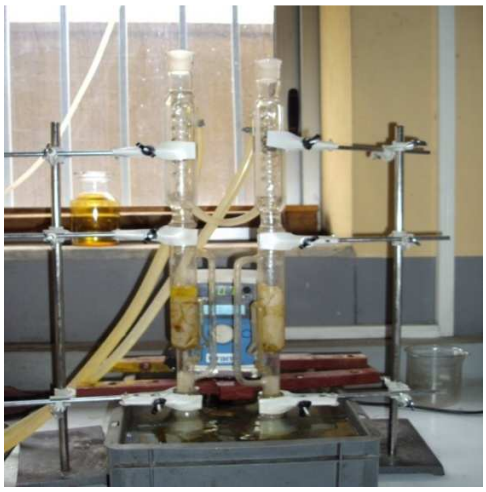


Figure3.1: Laboratory setup of soxhlet extraction unit

Determination of the yield of Neem oil extracted

30g (W_1) of the sample was placed in the thimble and about 300ml of normal hexane was poured into the round bottom flask. The apparatus was heated at 69.9°C and allowed for 2hrs, 4hrs, 6hrs and 8hrs continuous extraction using Soxhlet apparatus. The experiment was repeated for

different particle size with one replica. At the end, the cake was weighed and dried in the oven at 100^0C until the constant weight (W_2) is attained and the percentage of oil extracted was determined as:

$$\%\mathbf{yield} = \frac{(\mathbf{W1}-\mathbf{W2})*\mathbf{100}}{\mathbf{W1}*\mathbf{100}} \qquad (\mathbf{3.2})$$

Where: W_1=Sample weight initially placed in the thimble and W_2= sample weight after dried in the oven.

3.3.2. **Agitated Extraction Vessel**

Operation of Agitated Vessel Extractor

Round bottom flask fitted with a condenser and a stirrer was inserted in to a bath heated with thermostat as shown in fig. 3.2.

In this setup crushed Neem kernels powder (W_1) were put in to the vessel and n-hexane was added in to the vessel with kernel powder to solvent ratio of 1:5 (W/W) stirred for 3 hours. The same procedure was repeated for ethanol as a solvent at different particle sizes as mentioned above. Extractions were conducted at three different temperature levels (30^o, 40^o, and 50^oC) because to study the effect of temperature on quantity and quality of the oil. The samples were taken and centrifuged to separate the solid fraction from solution. The residue can be used as fertilizer. Filtrate (miscella) was heated and evaporated to obtain solvent-free oil in rotary-evaporator (RV OS basic) and the recovered solvent was recycled.

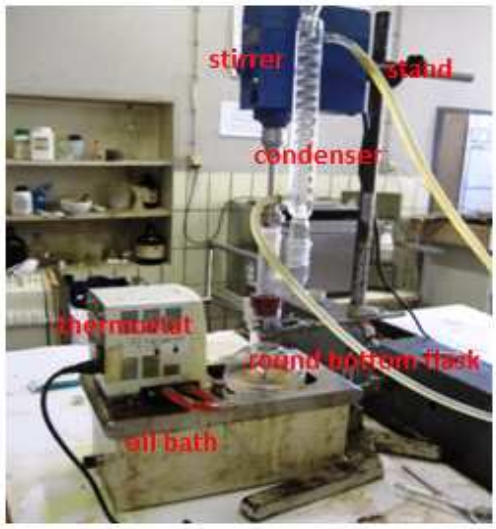


Figure3.2: Agitated extraction vessel laboratory set-up

Determination of yield

The cake was weighed (W_2) and dried in the oven at 100^0C until the constant weight (W_2) is attained and the percentage of oil extracted was determined as:

$$\%\mathbf{yield} = \frac{(\mathbf{W1} - \mathbf{W2}) * \mathbf{100}}{\mathbf{W1} * \mathbf{100}} \qquad (\mathbf{3.3})$$

Where: W_1=Sample weight initially placed in the vessel and W_2= sample weight after dried in the oven.

Determination of Specific Gravity

The density of the oil was determined by using density bottle. A clean and dry bottle of 25ml capacity was weighed (W_0) and then the bottle was filled with the oil, stopper inserted and reweighed to give (W_1). The oil was substituted with water after washing and drying the bottle and weighed to give (W_2). The expression for specific gravity (Sp.gr) is:

$$\mathbf{Sp.gr} = \frac{(\mathbf{W1} - \mathbf{W0})}{(\mathbf{W2} - \mathbf{W0})} = \frac{\mathbf{Mass\ of\ the\ substance}}{\mathbf{Mass\ of\ an\ equal\ volume\ of\ water}} \qquad (\mathbf{3.4})$$

Determination of viscosity of the oil

45m1 of oil was poured into a test tube and a viscometer was used to measure the viscosity at a temperature of 35^{o}C.

Determination of boiling temperature of the oil

25ml of Neem oil poured in to beaker and a thermometer was inserted and placed on a heating mantle, it was observed that the oil in the beaker started circulating leading to boiling of oil and read temperature on thermometer.

Determination of Refractive Index

Refractive Index was measured using refractometer. Few drops of the sample were transferred into the glass slide of the refractometer. Water at 30°C was circulated round the glass slide to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. This was repeated and the mean value noted and recorded as the refractive index.

Determination of pH Value

2g of the sample was poured into a clean dry 25ml beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. It was then cooled in a cold-water bath to 25°C. The pH electrode was standardized with buffer solution and the electrode immersed into the sample and the pH value was read and recorded.

3.3.2.1. Sensory quality evaluation of the product

The quality of neem oil can be expresses based on the average molecular weight of triglycerol present in the oil and the amount of free fatty acid present in the oil which is determined through determination of Saponification value, acid value, Iodine value and pH-value.

i) Determination of Saponification Value

Indicator method was use as specified by ISO 3657 (1988). 2g of the sample was weighed into a conical flask; 25ml of 0.5N Ethanolic potassium hydroxide was then added. The content which

was constantly stirred was allowed to boil gently for 30min. A reflux condenser was placed on the flask containing the mixture. Few drops of phenolphthalein indicator was added to the warm solution and then titrated with 0.5M HCl to the end point until the pink colour of the indicator just disappeared. The same procedure was used for other samples and blank. The expression for saponification value (S.V.) is given by:

$$\textbf{Saponification Value}(\textbf{S.V}) = \frac{\textbf{56.1} * \textbf{N} * (\textbf{V0} - \textbf{V1})}{\textbf{M}} \qquad (\textbf{3.5})$$

Where V_0 = the volume of the solution used for blank test; V_1 = the volume of the solution used for determination S.V; N = Actual normality of the HCl used; M = Mass of the sample.

ii) Determination of Acid Value

25ml of Toluene and 25ml of ethanol was mixed in a 250ml beaker. The resulting mixture was added to 2g of oil in a 250ml conical flask and few drops of phenolphthalein were added to the mixture. The mixture was titrated with 0.1M KOH to the end point with consistent shaking for which a dark pink colour was observed and the volume of 0.1M KOH (V_0) was noted. The Acid value was calculated as:

$$\textbf{Acid value}(\textbf{AV}) = \frac{\textbf{V} * \textbf{C} * \textbf{56.11}}{\textbf{M}} \qquad (\textbf{3.6})$$

Where V =Volume of potassium hydroxide (ml), C=Concentration of potassium hydroxide, 56.11 =Molecular weight of potassium hydroxide, M= sample weight

iii) Determination of Iodine value

The method specified by ISO 3961 (1989) was used. 0.4gm of the sample was weighed into a conical flask and 20ml of carbon tetra chloride was added to dissolve the oil. Then 25ml of Dam's reagent was added to the flask using a safety pipette in fume chamber. Stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2 hours and 30 minutes. At the end of this period, 20ml of 10% aqueous potassium iodide and 125ml of water were added using a measuring cylinder. The content was titrated with 0.1M sodium-thiosulphate solutions until the yellow colour almost disappeared. Few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop wise until

blue coloration disappeared after vigorous shaking. The same procedure was used for blank test and other samples. The iodine value (I.V) is given by the expression:

$$\textbf{Iodine value}(\textbf{I.V}) = \frac{\textbf{12.69} * \textbf{C} * (\textbf{V1} - \textbf{V2})}{\textbf{M}} \qquad (\textbf{3.7})$$

Where: C = Concentration of sodium thiosulphate used; V_1 = Volume of sodium thiosulphate used for blank; V_2 = Volume of sodium thiosulphate used for determination, M = Mass of the sample.

3.4. **Design of the Experiment**

Data analysis has performed by DESIGN EXPERT software using general factorial design method. For soxhlet extraction we had two factors, time with four levels and particle size with three levels while in agitated mixing extraction the factors to be studied are solvent types, particles size, and temperatures with levels two, three and three respectively.

This design of the experiment helps us to differentiate the significance of the main and the interaction factors. This program software also used to develop the mathematical model that will describe the effects of the main and interaction factors on the response.

4. RESULTS AND DISCUSSION

4.1. Determination of Moisture Contents

The fresh fruit was collected on October, 2011, after four months it was decorticated and by taking 20.0043, 30, and 40 grams of Neem kernel, the moisture content of the sample was obtained (table4.1) using equation 3.1.

Table4.1: Moisture content determination of Neem kernel

	Drying time(hour)							%moisture content
	0	2	4	6	8	10	12	
Sample weight in grams	20.0043	18.8537	18.744	18.713	18.682	18.624	18.624	6.900
	30	28.49	28.321	28.108	28.072	28.048	28.049	6.503
	40	38.152	38.102	38.048	37.825	37.321	37.320	6.700

The moisture content of the seed kernel of sample with 20.004, 30, and 40gms was 6.9, 6.503 and 6.7%, respectively. Thus, the average moisture content of the three samples will be 6.701%.

4.2. **Soxhlet extraction**

4.2.1. **Percent yield of soxhlet extractor**

The yield can be calculated using equation 3.2 and shown in table4.2.

Table4.2: Total %yield for soxhlet extractor for different particle size and time using n-hexane as a solvent.

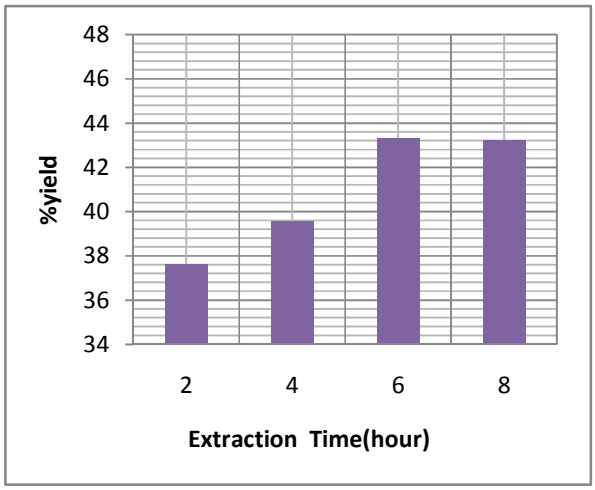
Particle size (mm)	Cake wt, replica. avg.,&%yield	Extraction time (hours)			
		2	4	6	8
0.85-1.4	cake wt(gm)	18.8	18.2	17.0	16.9
	replica(gm)	18.6	18.0	16.9	17.1
	Avg.	18.7	18.1	16.9	17.0
	%yield	37.6	39.5	43.3	43.2
0.85-0.71	cake wt(gm)	18.4	17.3	16.3	16.2
	replica(gm)	18.2	17.7	16.5	16.4
	Avg.	18.3	17.5	16.4	16.3
	%yield	38.9	41.7	45.2	45.5
0.425-0.75	cake wt(gm)	18.2	16.9	16.0	15.9
	replica(gm)	17.9	16.7	15.9	16.1
	Avg.	18.1	16.8	15.9	16.0
	%yield	39.7	43.9	46.8	46.5

The maximum extraction of Neem oil was 46.785% at particle size ranges from 0.425-0.75mm for the extraction time of 6hours and the minimum yield obtained was 37.625% at maximum particle size and minimum extraction time.

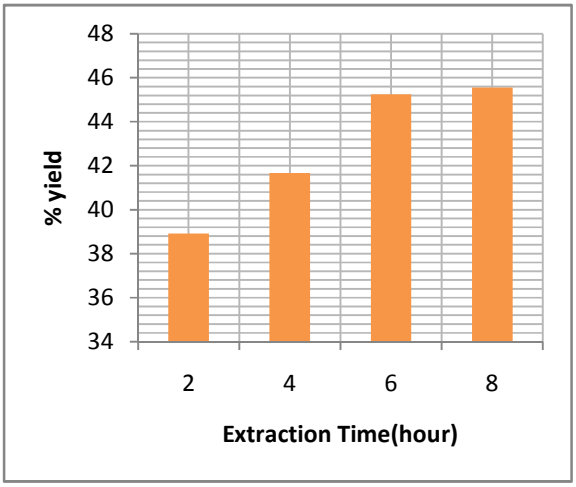
Effect of extraction time on percent yield of oil

Percent yield of Neem oil can be affected by extraction time, temperature, solvent type, particle size and other components in the seed. Extraction time plays a great role on the percentage yield of Neem oil using n-hexane as a solvent. Figures4.1 (a), (b), and (c) show that as the contact time increases the oil yield also increases this continues till transfer of oil from the kernel powder to the solvent attains zero. In other word, when the maximum amount of extractable oil is obtained, the oil yield level remains invariable even by extending the reaction time. So that in the soxhlet extraction the maximum oil yield could be finding at an extraction time of 6 hours and above. As shown graph of Figure 4.1, extracting the oil beyond six hours is wasting time because using n-hexane as a solvent can find maximum yield at this time.

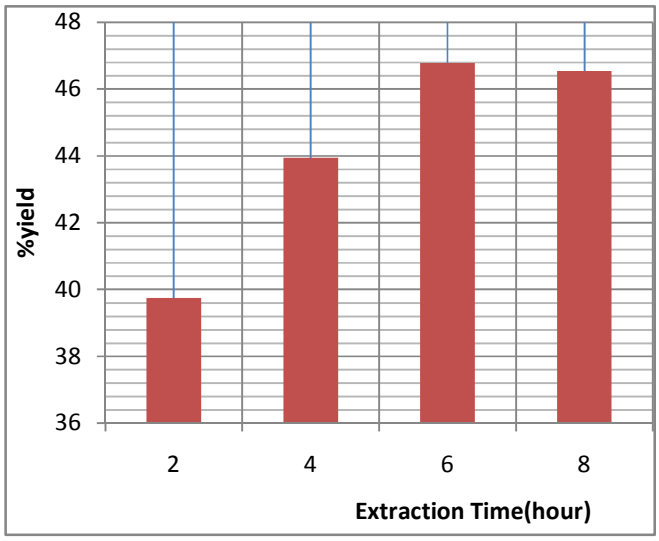
The extraction rate is fast at the beginning of the extraction but gets slow gradually. The reason is that when the kernel powder is exposed to the fresh solvent, the free oil on the surface of seeds is solubilized and oil gets extracted quickly inducing a fast increase in the extraction rate. Furthermore, since the oil concentration is low in the solvent at the beginning of the extraction process, the oil diffuses quickly from the kernel to the liquid phase due to the difference in concentration (driving force) of the oil. As the time passing by, the concentration of oil increases in the solvent resulting in a decrease in the diffusion rate.



(a)



(b)



(c)

Figure 4.1: The effect of time on Neem oil yield at particle size (a) 0.85-1.4mm, (b) 0.71-0.85mm and (c) 0.425-0.71mm

Effect of particle size on percent yield of oil

Particle size plays a great role on the yield of Neem oil. Smaller particle size gives high yield while samples with large particle size deliver low yield. That means less oil is extracted from the larger particles (>0.85 mm) compared to the small size of the particles. The reason is that larger particles with smaller contact surface area, have more resistant to solvent entrance and oil diffusion towards the solvent. Therefore, less amount of oil will be transferred from inside the larger particles to the surrounding solution in comparison with the smaller ones. Thus, an increase in particle size will decrease the oil yield.

Nevertheless, we know that when the particle is too small (very fine particle size) i.e., below <0.425 mm, the extracted oil become small in its amount, even though the contact surface area for small particle is supposed to be significantly higher than that for the larger particles. This may be due to the agglomeration of the fine particles which reduces the effective surface area available for the free flow of solvent towards inside the solid particles.

The following table (table 4.3) shows analysis of variance (ANOVA), this will tell us the significance of different factors.

Table4.3: Analysis of variance (ANOVA) results for an experiment in soxhlet extraction

Source	**Sum of squares**	**Degree of fredom**	**Mean square**	**F-value**	**p-value Prob>F**
Model	216.26	11	19.66	84.64	<0.0001
A-Time	168.89	3	56.30	242.36	<0.0001
B-particle size	44.37	2	22.19	95.52	<0.0001
AB	3	6	0.50	2.15	0.1217

The Model F-value of 84.64 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A-time, B-particle size are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The P-value of AB (interaction factors) is 0.1217 >P-value thus, the interactions of size and time are not significant in the model terms.

Figure4.2 below shows that there is no interactions among each factors. This shows us an increament in time will increase the quantity of Neem oil extracted. Extraction beyond six hours didn't give a significant change on oil yield.

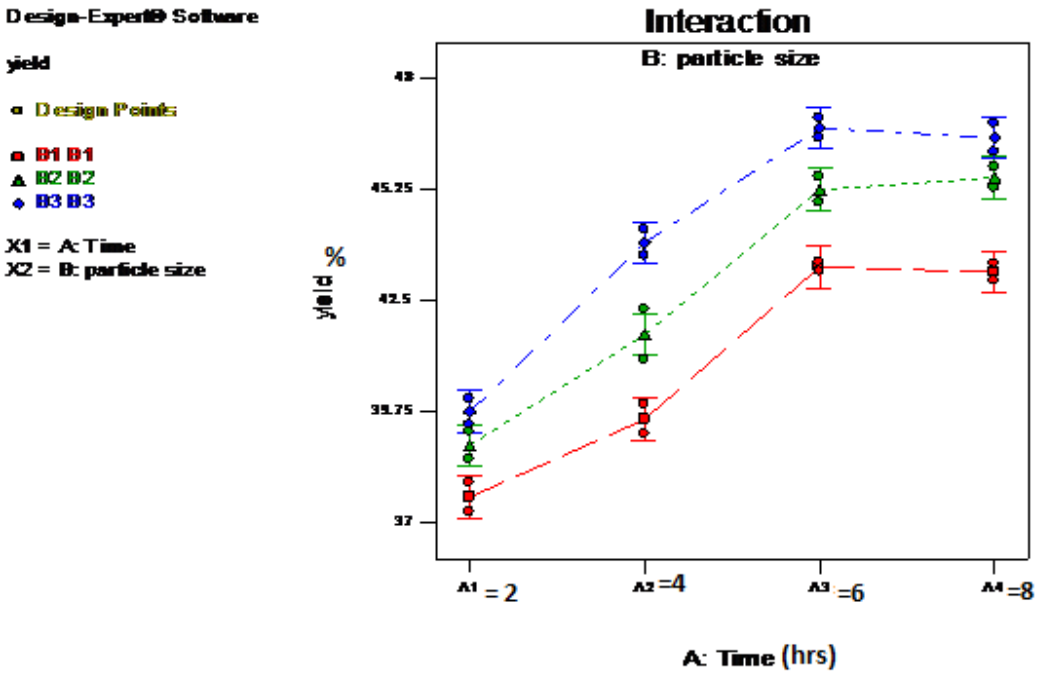


Figure4.2: Effects of time, particle size and their interactions on oil yield

Where: B1, B2 and B3 are codes for particle sizes ranges from 0.85-1.4mm, 0.75-0.85mm, and 0.425-0.71mm, respectively. A1, A2, A3, and A4 are codes for extraction time(hours) two, four, six and eight hours, respectively. Design points are points on the graph which helps to develop mathematical model of the predicted response based on these points.

Design -expert was applied to analyze results on the extraction process and a first order regression equation, with the interaction terms, of the form:

$$\%Yield = +42.68 - 3.92 * A[1] - 0.96 * A[2] + 2.45 * A[3] - 1.74 * B[1] + 0.17 * B[2] + 0.61 * A[1]B[1] - 0.42 * A[2]B[1] - 0.039 * A[3]B[1] - 0.017 * A[1]B[2] - 0.23 * A[2]B[2] + 0.045 * A[3]B[2] \quad (4.1)$$

was obtained growing the individual effect of time and particle sizes as parameter and their interactions in the entire extraction process was found to be ineffective. Therefore, the final

equation in terms of coded factor without the interaction effect is given by a first order regression equation:

$$\%Yield = +42.68 - 3.92 * A[1] - 0.96 * A[2] + 2.45 * A[3] - 1.74 * B[1] + 0.17 * B[2] \quad (4.2).$$

Where: A[1]= the difference of time level-1 from the over all average.

A[2]= the difference of time level-2 from the over all average.

A[3]= the difference of time level-3 from the over all average.

B[1]= the difference of particle size level-1 from the over all average. and

B[2]= the difference of particle size level-2 from the over all average

Diagonstics.

The following figure4.3 shows the relation between the actual value of the experiment and the value predicted by the model equation developed by the Design Expert software.

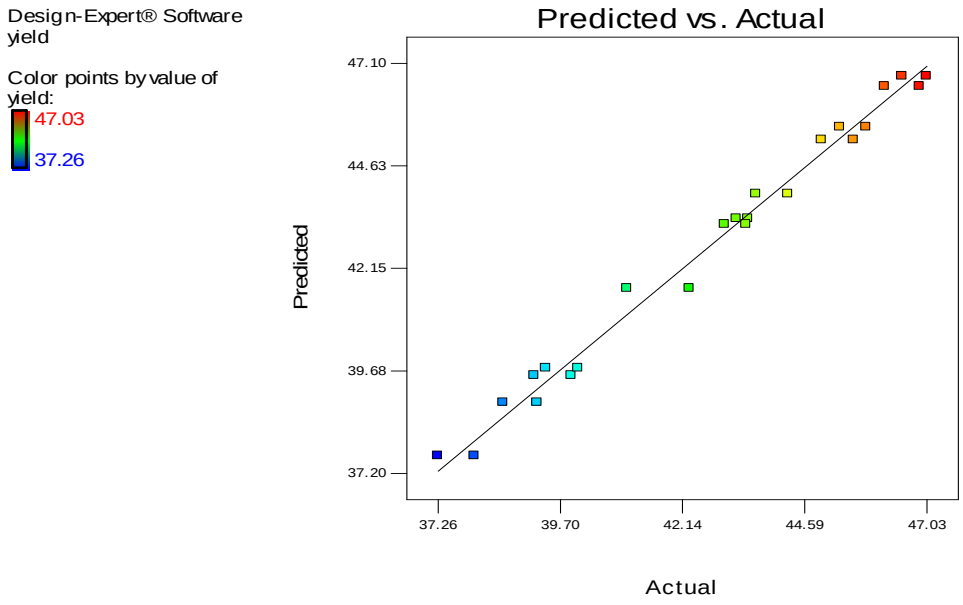


Figure4.3: Predicted vs. actual value of yield for soxhlet extraction.

The residue for equation (4.2) that will describe the difference between the actual values of the model and the predicted one was shown in the following table4.4

Table4.4: Difference between the actual (experimental) value and predicted value.

standard order	Actual value%	Predicted value%	Residual
1	37.99	37.02	0.97
2	37.26	37.02	0.24
3	39.93	39.98	-0.05
4	39.19	39.98	-0.79
5	43.46	43.38	0.08
6	43.23	43.38	-0.15
7	43.42	43.36	0.06
8	42.99	43.36	-0.37
9	38.57	38.93	-0.36
10	39.25	38.93	0.32
11	42.29	41.89	0.4
12	41.04	41.89	-0.85
13	45.57	45.3	0.27
14	44.93	45.3	-0.37
15	45.82	45.27	0.55
16	45.3	45.27	0.03
17	40.06	40.33	-0.27
18	39.42	40.33	-0.91
19	43.62	43.3	0.32
20	44.26	43.3	0.96
21	47.03	46.7	0.33
22	46.54	46.7	-0.16
23	46.19	46.68	-0.49
24	46.89	46.68	0.21

4.3. Agitated Mixing Extraction

The yield and the quality of Neem oil differ due to different reasons, possibly due to using solvent types, particle size, extraction time, temperature etc. This section mainly discussed on determination of physical properties of the oil, the effect of temperature and solvent type on the amount and quality of the oil. Three factors and four responses are added as an input to the DESIGN EXPERT software and analyzed the data.

4.3.1. Determination of Physical properties of the oil

Specific Gravity: Specific gravity of the oil is determined by specific gravity bottle.

- ✓ Weight of empty dry bottle, W_o = 34.562gm
- ✓ Weight of 25ml oil with the bottle, W1 = 57.212gm
- ✓ Weight of 25ml oil with the same bottle, W2 =59.562gm

Then, using equation 3.4, the specific gravity of the oil was 0.906.

pH value of Neem oil: the value of pH of the oil was measured using pH meter and result is shown in table4.5

The physical properties of Neem oil was summarized in table 4.5.

Table4.5: Physical Property Determination, Experimental Result of the Characterization of Extracted Oil

Physical property	**Value**	**Unit**
Refractive index at 28°C	1.462	-
Boiling point at room temp.	129	^oC
Odour	Garlic-peanut	-
Colour	yellow	-
Specific gravity	0.906	-
Viscosity	34.5	mPas
pH	5.7-6.5	-

4.3.2. **Determination of percentage yield**

The quantity of oil extracted for agitated vessel extractor was calculated by using equation 3.3 on Microsoft excel sheet and the results are shown below on table 4.6 and 4.7 used for n-hexane and ethanol, respectively.

Table4.6: Percentage yield of Neem oil at different particle size and temperature using n-hexane as a solvent

Temperature in ^{o}C	Particle size in millimeter					
	0.85-1.4		0.71-0.85		0.425-0.71	
	Input size=50gm		Input size=45gm		Input size =60gm	
	Wt. cake(gm)	%yield	Wt. cake(gm)	%yield	Wt. cake(gm)	%yield
30	31.9	36.1	28.3	37.1	36.5	39.2
40	30.6	38.7	26.4	41.2	33.1	44.8
50	29.9	40.3	25.0	44.4	31.6	47.3

Table4.7: Percent yield of neem oil extraction at different particle size and temperature using Ethanol as a solvent

Temperature in ^{o}C	Particle size in millimeter					
	0.85-1.4		0.71-0.85		0.425-0.71	
	Input size=50gm		Input size=45gm		Input size =60gm	
	wt cake(gm)	%yield	wt cake(gm)	%yield	wt cake(gm)	%yield
30	39.6	20.8	34.5	23.2	43.8	26.9
40	37.8	24.4	33.2	26.1	41.3	31.1
50	35.1	29.8	31.3	30.4	40.1	33.2

In this batch extraction method, the maximum oil was obtained using n-hexane as a solvent at the smaller particle size and higher extraction temperature. As experimental results show, see tables 4.6 and 4.7, the maximum oil found was 47.3% for n-hexane but 33.2% for ethanol.

Effect of temperature on oil yield

The quantity of oil can be affected by temperature. From the experimental result of this thesis, for instance, let's look at Figure4.4 line (a, light blue color) for particle size ranges from 0.85-1.4mm, %yields of Neem oil using ethanol at a temperature of 30, 40 and 50 ^{o}C are 20.8, 24.4, and 29.8%, respectively. This result will show us increasing temperature will raise yield. Based on the findings, for both solvents, the yield was found to enhance with increasing temperature. This was because of rising the temperature, both the diffusion coefficient and the solubility of the oil in to both solvents is enhanced, thus heat treatment improves the extraction Neem oil. The higher extraction temperatures the easier to break the molecule inside the seed; as a result, the yield also gets high.

Effects of solvent type on oil yield

Consider Figure 4.4 and let's take particle size ranges from 0.85-1.4mm, then the percentage yield of Neem oil was differ in the two solvents, at this particular case, the maximum oil yield using ethanol was found 29.8% at a temperature of 50^{o}C and a minimum yield of 20.8% at 30^{o}C while at the same operating condition n-hexane resulted a maximum yield of 40.3% and a minimum of 36.1%. This is due to solvent type used i.e. oil extraction by using n-hexane gives higher yield than ethanol. This is because n-hexane has high capacity to dissolve non-polar compounds in the oil than ethanol. Therefore, based on the findings, hexane is a better solvent for Neem oil extraction.

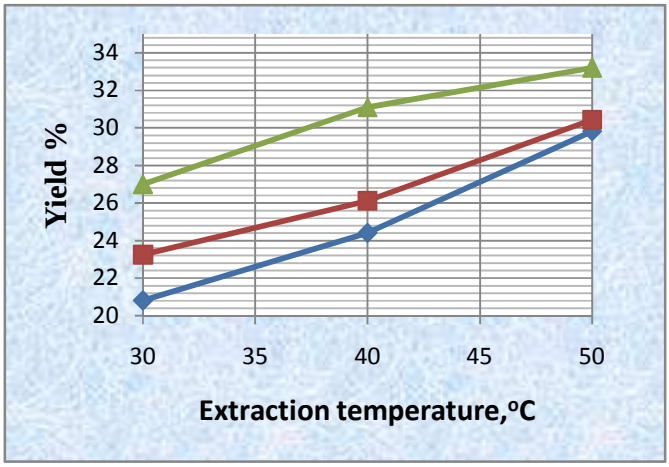


Figure4.4: The effect of temperature on % yield at particle size (a) 0.85-1.4mm, (b) 0.71-0.85mm and (c) 0.425-0.71mm using Ethanol as a solvent

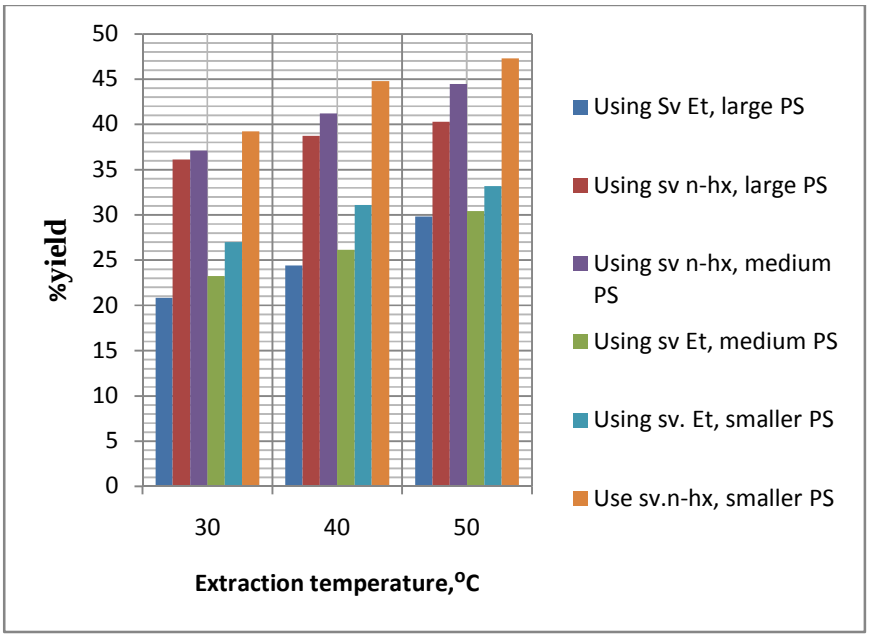
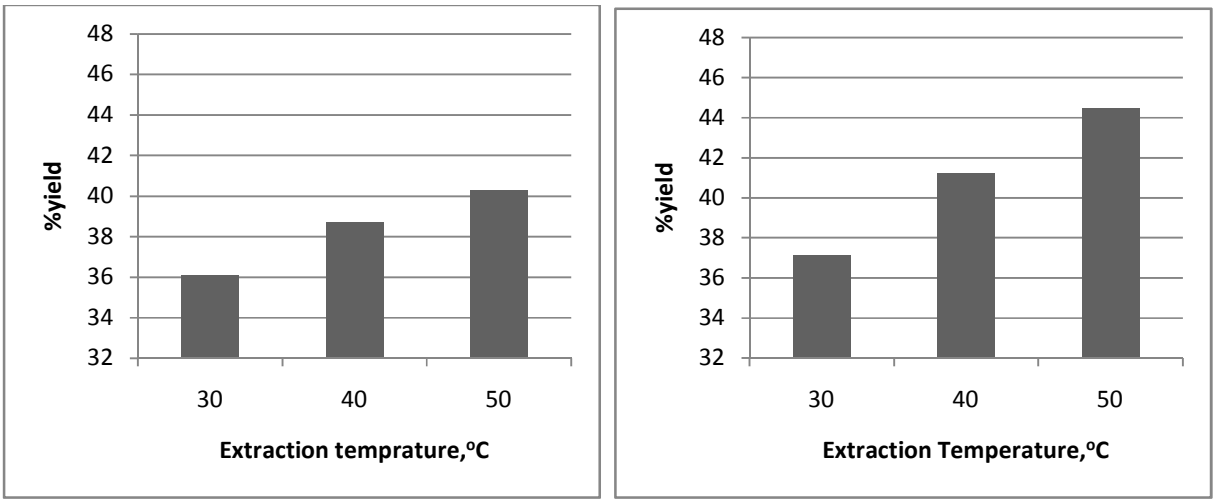


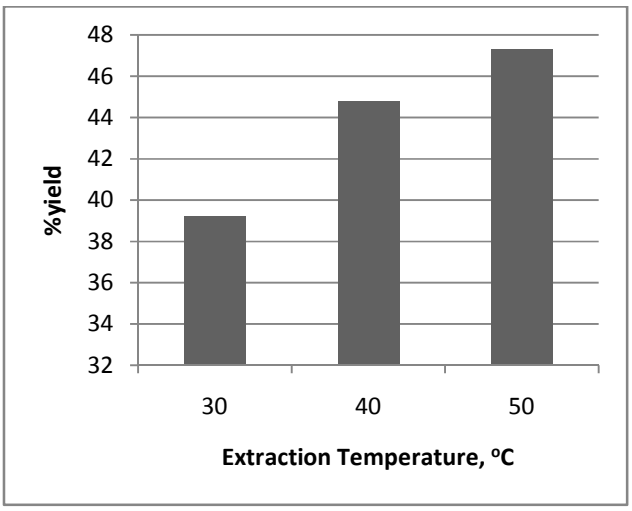
Figure 4.5: Comparison of %yield using solvents ethanol and n-hexane at different particle size, temperature and solvent type.

Effects of particle size on oil yield

The effect of increasing and decreasing particle size on oil yield has been shown on Figure 4.5 above. It is quite clear that there is an increase in the oil yield to a maximum value due to reduce in particle size and a further increase in the particle size results in a drop in oil yield. The oil yield increased from 36.1% at particle size between 0.85-1.4mm to 47.3%, was a maximum at particle size of 0.425-0.75mm and using n-hexane solvent, the results of n-hexane extracts are comparable from the previous literature (Maria Yuliana Liauw, F. A. Natan, P. Widiyanti, D. Ikasari, N. Indraswati and F. E. Soetaredjo). Increasing the particle size leads to increasing diffusional resistance and hence reduction in percent of decomposition of the seed kernel powder. Figures4.5 shows us as the variations in percentage yield results due to the type of solvent (n-hexane oil extract and ethanol extract) used, there is also a change in quantity of the oil varied due to a change in particle size. In all the three cases higher particle size results the production of the lesser quantity oil.



(a) (b)



(c)

Figure4.6: The effect of temperature on Neem oil yield at particle size (a) 0.85-1.4mm, (b) 0.71-0.85mm and (c) 0.425-0.71mm using n-hexane as a solvent

So far we discussed the effects of temperature and solvent type on quantity of oil. Then here, we will analyze the resulted data to determine the significant factors of the experimental work by using DESIGN-EXPERT software.

Table4.8: Analysis of variance (ANOVA) table for the response on percentage yield of Neem oil

source	Sum of squares	df	Mean square,	F-value	P-value Prob>F
Model	1083.27	13	83.33	53.41	0.0008
A-solvent type	841.87	1	841.87	539.644	<0.0001
B-particle size	89.32	2	44.66	28.63	0.0043
C-temprature	147.32	2	73.66	47.22	0.0017
AB	0.93	2	0.46	0.3	0.7578
AC	1.76	2	0.88	0.56	0.6089
BC	2.08	4	0.52	0.33	0.8437

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. From this we can conclude, there is no interaction effect, the only dominating factors are the main effects which are solvent type, particle size and temperature.

The final equation in terms of coded factor without the interaction effect is given by a first order regression equation:

$$\%Yield = +34.19 + 6.84 * A - 2.49 * B[1] - 0.42 * B[2] - 3.60 * C[1] + 0.21 * C[2] \quad (4.3)$$

Where: B[1]= the difference of particle size level-1 from the over all average

B[2]= the difference of particle size level-2 from the over all average

C[1]= the difference of temperature level-1 from the over all average

C[2]= the difference of temperature level-2 from the over all average

From the report of DESIGN EXPERT software the solutions for 18 combinations of categoric factor levels selects for maximum oil yields are n-hexane, 0.425-0.75 mm particle size, and at the extraction temperature of 50^{o}C.

Table4.9 shows us there is no interaction effects among the main factors. P-value greater than 0.1000 indicate the model terms are not significant. Therefore, the interaction effect p-value is greater than 0.1000. the effects are not significant. And there are only the main effects which dominate the extraction yields.

4.3.3. **Quality Evaluation of the Product**

The quality of the product could determine based on the values of Saponification, acid and iodine as a result of extraction temperature variation. The effect of temperature on oil's Saponification, acid and iodine values are discussed below. The effects of solvent type in general on oil qualities were presented at the end of this chapter.

4.3.3.1. **Saponification value of the oil**

The saponification value is the number that expresses in milligrams of the quantity of potassium hydroxide required to neutralize the free acids and to saponify the esters present in 1 g of the substance. The test method to determine saponification value of oil is standardized as in ASTM D1959-97 Standard Test Method for saponification value of oils and fatty Acids.

- ✓ Preparation of 0.5NKOH-Ethanol (Ethanolic potassium hydroxide): to prepare 0.5N of Ethanolic KOH solution, 14.027gm of KOH dissolved in 500ml of ethanol.
- ✓ Preparation of 0.5N of HCl: to prepare this solution, 43.7ml of HCl poured on 1000ml of distilled water.

Figure4.7: Gentle heating Neem oil with 25ml of Ethanolic-KOH solution

After gentle heated the oil with Ethanolic-KOH for 30min. Immediately five drops of phenolphthalein added and the amount of HCl require saponifying the oil and form a color change for ethanol extract are 20.8, 18.8 and 17.8ml, but for n-hexane extracts 21.4, 19.9 and 18.9ml for the following corresponding temperatures 30, 40 and 50^{o}C. Thus, determination of saponification value found using equation 3.5 and the results obtained was shown in table 4.9:

Table4.9: Saponification value of Neem oil at different extraction temperature

Temp. ^{o}C	The volume of the solution required for titration(ethanol)ml	Sap. Value using Ethanol extract (mgKOH/g)	The volume of the solution required for titration (n-hex)ml	Sap. Value using N-hexane extract (mgKOH/g)
30	20.8	175.712	21.4	184.028
40	18.8	194.3	19.9	209.296
50	17.8	205.835	18.9	221.93

Effects of temperature on Saponification value

Saponification value indicates the average molecular weight of triglycerides in the oil. Temperature can affect both the yield and the quality of Neem oil. The quality of oil can be determined by studying the effect of temperature on Saponification value. Figure4.8 shows that as temperature increase Saponification value also increase this is because temperature causes lipids to breakdown, therefore reduced the average molecular weight of the oil. The quality of oil decreases as extraction temperature increases since oxidation of oils and fats is facilitated by the presence of heat and light. Since Saponification value is the amount of potassium hydroxide required neutralizing the free fatty acid containing in the oil and the experimental result showed us SV increase with temperature, this told us as temperature increase free fatty acids too, in turn the quality of oil also decreases. Oxidation processes may also lead to the formation of insoluble compounds, which may cause an obstacle on the effects of active ingredient (azadirachtin) on its proper functioning.

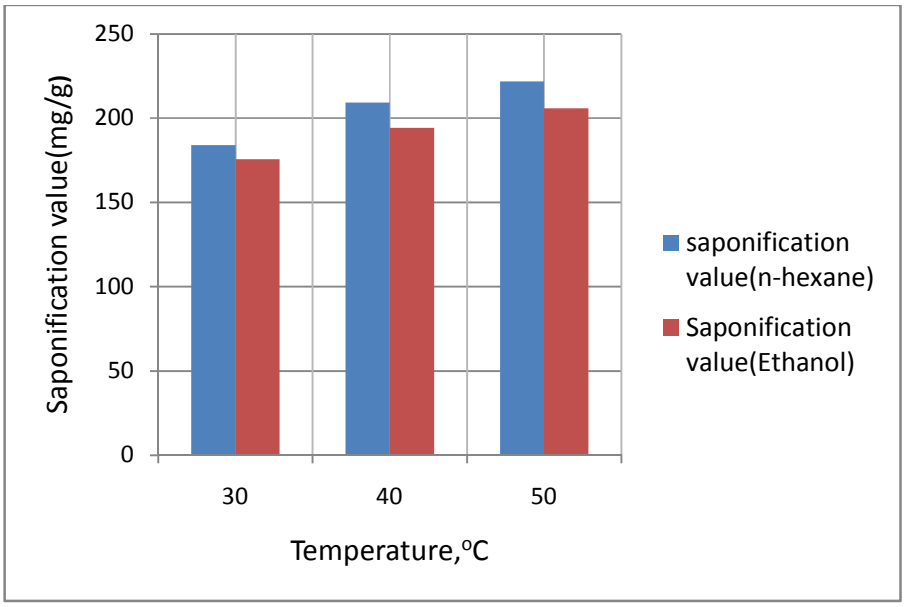


Figure4.8: Effect of temperature on Saponification value for extractions using ethanol and n-hexane.

To characterize the main effects and the interaction effects on the Saponification value, we used DESIGN EXPERT software.

Table4.10: ANOVA table on the response of Saponification value

source	Sum of squares	df	Mean square,	F-value	P-value Prob>F
Model	4435.95	5	887.19	34730.51	<0.0001
A-solvent type	758.53	1	758.53	29693.75	<0.0001
C-temprature	3617.42	2	1808.71	70805.07	<0.0001
AC	60.00	2	30.00	1174.34	<0.0001

The Model F-value of 34730.51 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, and AC are significant model terms. In terms of actual factors main effects are solvent type, temperature and the interaction effect is solvent type-temperature are significant.

Thus, the model equation interms of coded factor that predicts the saponification value(SV) with out the insignificant interactions is given by :

Saponification value(SV)= +198.44+6.49*A-18.88*C[1]+3.36*C[2]-2.5*AC[1]+1.01*C[2] (4.3)

Where: A =solvent type, C= temperature, AC= interaction effects of temperature and solvent type. C[2]= the difference of temperature level-2 from the over all average, and C[1]= the difference of temperature level-1 from the over all average

Diagonstics.

The following graph shows the relation between the actual value of the experiment and the value predicted by the model equation developed by the DESIGN EXPERT software.

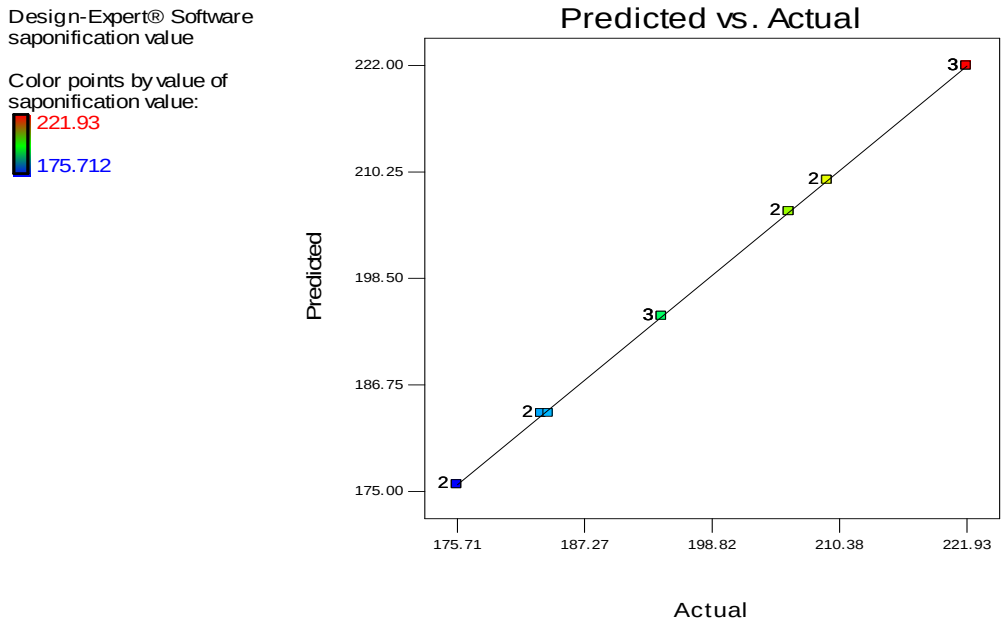


Figure4.9: Predicted vs actual experimental value for Saponification value.

4.3.3.2. **Acid value of the oil**

Acid value indicates the amount of free acid present in the oil. It can be calculated using equation 3.6 and the results are summarized in the following table 4.11:

Table4.11: Acid values of Neem oil at different extraction temperature

Temp. ^{o}C	The volume of the solution required for titration(n-hexane) ml	Acid Value using N-hexane extract (g/g)	The volume of the solution required for titration (ethanol) ml	Acid Value using Ethanol extract (g/g)
30	27.2	96.4	9.2	32.8
40	28.8	102	10.2	36
50	34.4	122	11.3	40

Effects of temperature on Acid value

Temperature can affect both the yield and the quality of Neem oil. The quality of oil can be determined studying the effect of temperature on Acid value. Figure4.10 shows that as temperature increase acid value increase this is because the extraction temperature influences the hydrolysis of Neem oil into free fatty acids and glycerol. The decomposition of the glycerides in the oil is also affected by the heat treatment. As transition theory states' increasing of the reaction temperature has affected the production of fatty acids which clearly showed an increase in conversion of fats in to free fatty acids. Vegetable oil contains lipase enzyme which has an optimum temperature about 35-45^{o}C. Lipase enzyme hydrolyzes oil become free fatty acid and glycerol. The decrease oil quality is due to increased activities of lipase enzyme at the lower temperature. Therefore, as temperature increase oil quality decrease since temperature affects the active ingredient.

Effects of solvent type on acid value

As Figure4.10 shows the type of solvent affects the oil quality. N-hexane as non-polar solvent is suitable for free fatty acid extraction compared to ethanol, which is polar solvent, is able to extract bioactive compounds.

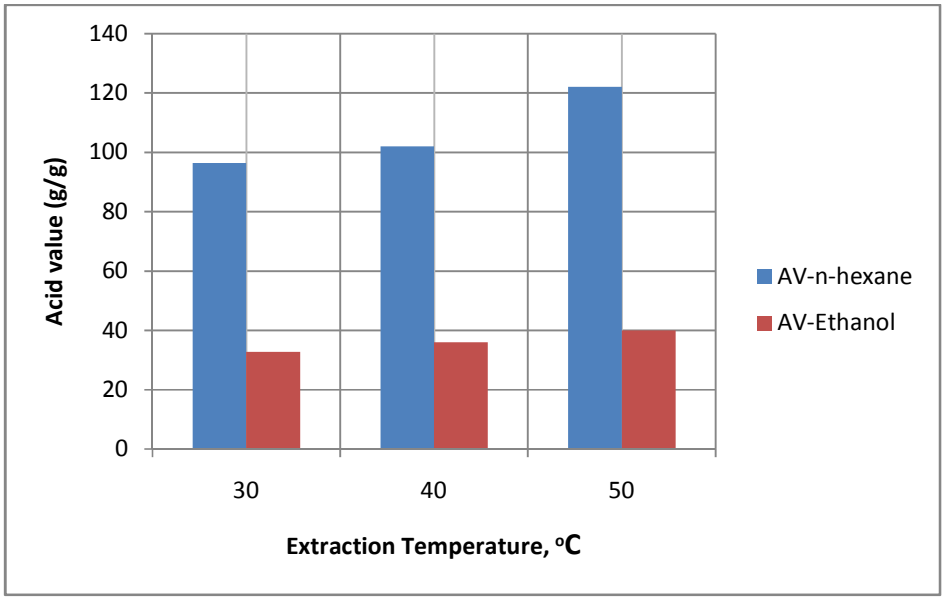


Figure4.10 Effect of temperature and solvent type on Acid value

Table4.12: ANOVA on the response of Acid value

source	Sum of squares	df	Mean square,	F-value	P-value Prob>F
Model	23552.08	5	4710.42	6.366E+007	<0.0001
A-solvent type	22387.28	1	22387.28	6.366E+007	<0.0001
C-temprature	864.64	2	432.32	6.366E+007	<0.0001
AC	300.16	2	150.08	6.366E+007	<0.0001

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, AC are significant model terms. Other interactions are not significant that is why they are removed from the model equation.

Final Equation in Terms of Coded Factors:

$$Acid\ value(AV) = +71.53 + 35.27A - 6.93C[1] - 2.53C[2] - 3.47AC[1] - 2.2AC[2] (4.3)$$

Where the experimental model variables in equation 4.3 are described above in saponification model equation 4.2.

ANOVA table 4.12 shows significant factors are A-solvent type and C-temperature but particle sizes do not have effect on acid value. There is an interaction between the main factors AC.

4.3.3.3. **Iodine value of the oil**

Iodine value of Neem oil extracted using the two solvents, ethanol and n-hexane has been conducted in EHNRI-Ethiopia and the result is shown in the table4.13 below:

Table4.13: Iodine values of Neem oil at different extraction temperature and solvent type.

Temp. ^{o}C	Iodine value using Ethanol solvent (g/g)	Iodine value using N-hexane solvent (g/g)
30	66.58	72.62
40	65.79	68.41
50	61.32	62.83

Effects of temperature on Iodine value

It is a measure of unsaturation in lipid, which again determines the degree of flow. From the Figure4.11 shown below we observed that the iodine value of the oil decreases as it was heated from a temperature of 30 to 50^{o}C. Unsaturated compounds contain molecules with double or triple bonds, which are very reactive toward iodine. This suggests higher temperature results the loss of unsaturation (double or triple bonds) in the fatty acids of the triacylglycerols as a result, decreased in iodine value due to loss of double and triple bonds in the oil. The more iodine is attached, the higher is the iodine value, and the more reactive, less stable, softer, and more susceptible to oxidation and rancidification. Decrease in iodine value indicates lipid oxidation and this might be due to metallic ions present among other factors, which enhances or promotes oxidation.

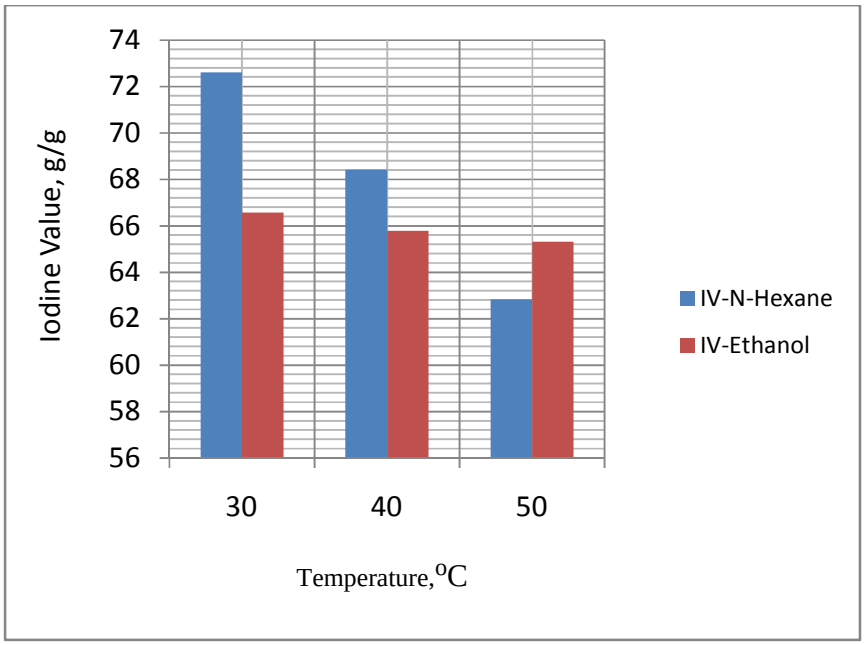


Figure4.11: Effects of temperature and solvent type on Iodine value.

The following table shows us the analysis of variance which was performed using DESIGN EXPERT software. This table directs to study the main effects temperature and solvent type on Iodine value of Neem oil and the significance of their interactions.

Table4.14: Analysis of Variance (ANOVA) table responses on the main effects and their interactions.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	166.28	5	33.26	2.993E+006	< 0.0001
A-solvent type	*19.03*	*1*	*19.03*	*1.713E+006*	*< 0.0001*
C-temprature	*91.91*	*2*	*45.95*	*4.136E+006*	*< 0.0001*
AC	*55.34*	*2*	*27.67*	*2.490E+006*	*< 0.0001*

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

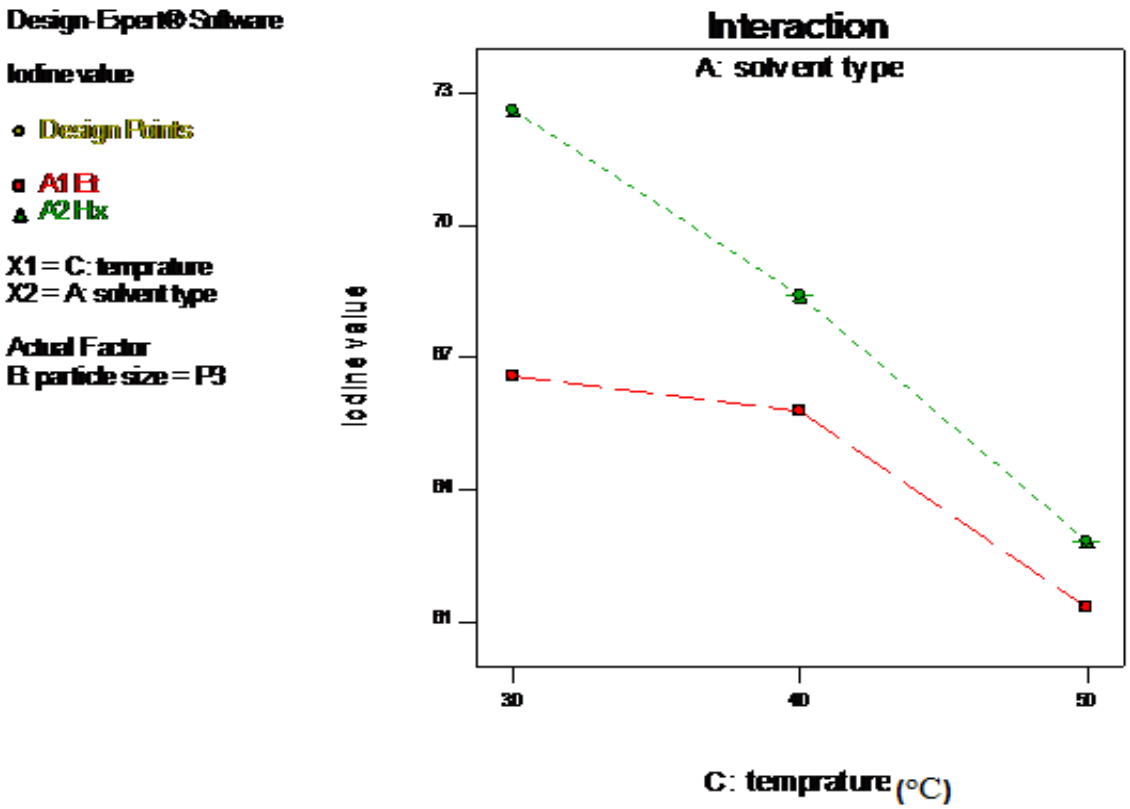


Figure4.12: An interaction between effects of temperature and solvent type ^{o}C

As the ANOVA table4.15 indicates the lines in the above graph shows there is an interaction between temperature and solvent type because the lines are not parallel. Figure 4.12 shows the iodine value or number attached in the oil decrease due to increments in temperature for both normal hexane (green color) and ethanol (red color) extracts.

Final Equation in Terms of Coded Factors:

Iodinevalue(IV)=+66.92+1.03*A+2.68*C[1]+0.17*C[2]+1.99*AC[1]+0.28*AC[2] (4.4)

ANOVA table 4.14 shows significant factors are A-solvent type and C-temperature and interactions of temperature and solvent type AC, but particle sizes do not have effect on Iodine value.

Effects of solvent types on quality of Neem oil

The study concludes that ethanol can be considered as an alternative solvent to n-hexane for oil extraction where the efficiency of extraction is comparable to that of n-hexane. The oil extracted by n-hexane and ethanol was tested for some parameters and it is observed that there is no much variation in the quality of the oil extracted by n-hexane and ethanol except that the color of oil extracted by ethanol is darker than the oil extracted by n-hexane; this indicates that ethanol is a good solvent for oil. Acid value which is the indicator of free fatty acid present in the oil is slightly high in the oil extracted by ethanol. Major variations were not observed in other parameters of oil like Iodine value, Saponification value etc.

5. MATERIAL BALANCE AND COST ESTIMATION

5.1. **Material balance**

Assumption: Seed collected on the harvesting period

Note: All material balances had performed based on the experimental work in the laboratory.

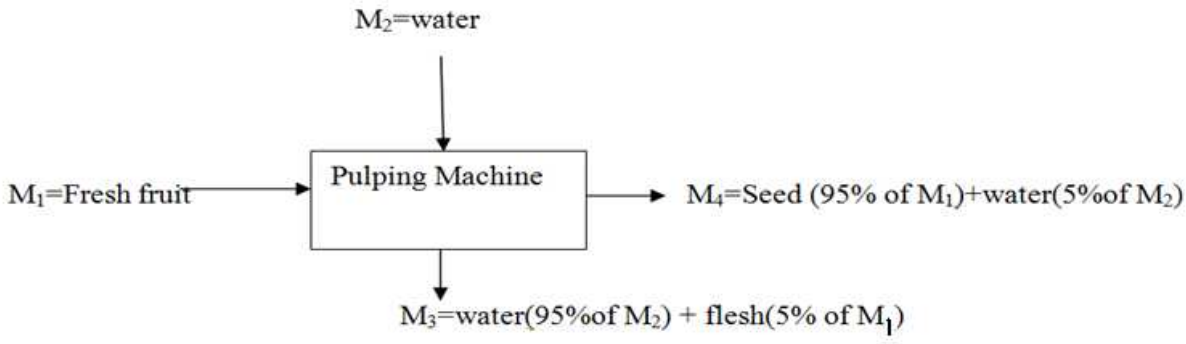
- **Balance on pulping machine**
  - Weight of the flesh from the fresh fruit=5% of fresh fruit
  - Amount of water for pulping=50% of Kg of fresh fruit

Basis: 1000kg/hr of fresh fruit

Total Material Balance

Accumulation=Output + Consumption – Input – Generation (5.1)

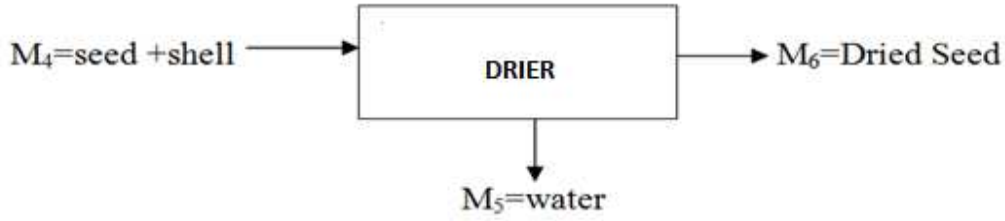
Since there is no reaction, the generation and consumption terms are zero, no accumulation



Input = Output

But the amount of water flow rate required for pulping 1000kg/hr fresh fruit will be 500kg/hr=M_2.

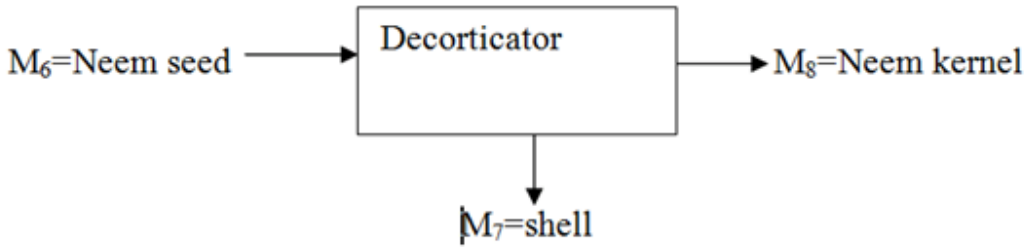
- ✓ Water in stream three = 95% of M_2= 475kg/hr of water
- ✓ Amount of water leaving with seed (stream 4) =5% of M_2 = 25kg/hr of water, assume that all water contents are removed from the seed by drying using sun light for 24hours.



- ✓ Weight of the flesh= 5% of wt. fresh fruit=50kg/hr of flesh
- ✓ Neem fruit with the shell=95% of M_1=950kg/hr of Neem seed

➢ **Balance on decorticating machine**

From laboratory work, wt. Neem kernel= 60-55% of Neem seed, let's take the average value 57.5%



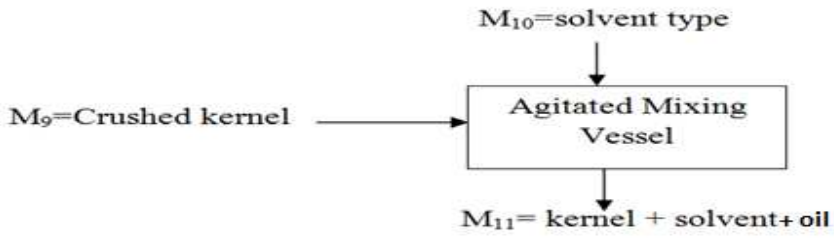
- ✓ Wt. of shell=42.5% of Neem seed=403.75 kg/hr of shell have been removed, and can be used as an adsorbent.
- ✓ Wt. of Neem kernel=57.5% of Neem seed= 546.25kg/hr Neem kernel send to the storage tank

➢ **Balance on size reduction Machine**

Here by assuming that, there is 1% of Neem kernel loss, then = 5.4625kg/hr of kernel lost

Then, the amount of raw material left to the extraction vessel with particle size ranges from 0.425-0.75mm will be 540.79kg/hr=M_9

- **Balance on Agitated Vessel Extraction**



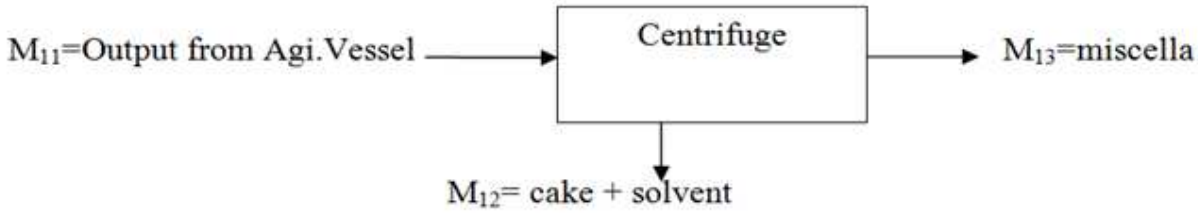
In the laboratory, for 30-40 g of Neem kernel, the amount of solvent required should be five times. Thus, 250ml of solvent was used, therefore, for 540.79kg/hr of Neem kernel 3379.87lt of solvent will be required which is 2213.8kg solvent required.

- ✓ Therefore M_{11}= wt. of solvent, oil and cake= 2754.6kg/hr

- **Balance on Centrifuge**

  From the experimental work, cake from the centrifuge contains 6.1% solvent.

  For processing 40gm Neem kernel, there is 2.44gm of the solvent loss. Thus, to process 540.78kg Neem kernel there is a loss of 32.447kg of solvent.



- ✓ The amount of solvent in the oil will be 2181.35kg/hr and separated by distillation and the solvent will recycle.

- **Balance on Distillation Unit**

  Oil balance around the column

$$0.114*2431.19 = 0.98*B + 0.002* D \quad *$$

  Solvent balance

$$0.884*2431.19 = 0.01*B + 0.99* D \quad **$$

From these two equations the bottom product and the distillate flow rate contains 282.8 Kg/hr and 2168.02Kg/hr, respectively.

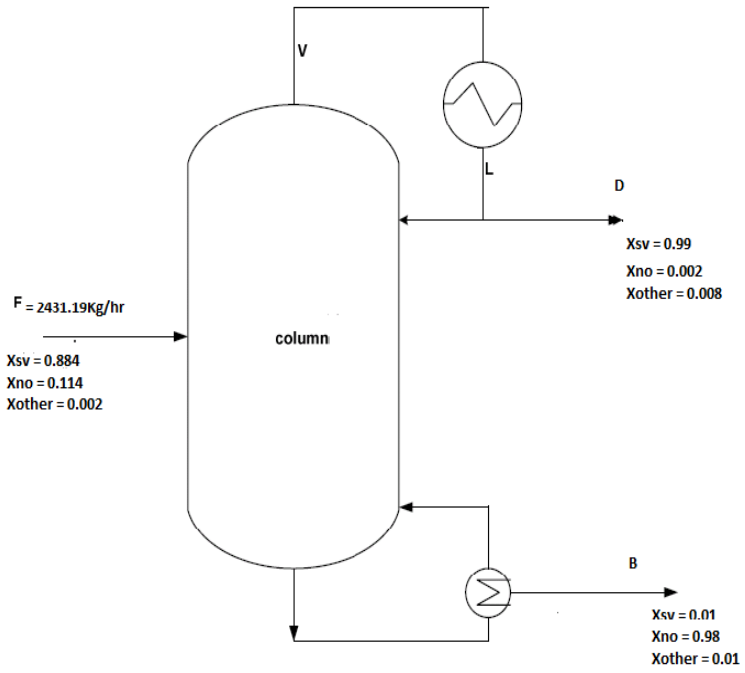


Table5.1: Summary of Material balance

Equipment	**Input type**	**Flow rate(kg/hr)**	**Output type**	**Flow rate(kg/hr)**	**Capacity (kg/hr)**
Pulping machine	• Fresh fruit • water	1000 500	• water +flesh • water+ seed	525 975	1500
Evaporation (sun light)	Seed and water	975	• water • seed	25 950	975
Decorticator	Seed	950	• kernel • shell	546.25 403.75	950
Pulverizer	Kernel	546.25	• loss • crushed kernel	5.4625 540.79	546.25
Agitated Mixing machine	• kernel • solvent	540.78 2213.8	Oil + cake+ solvent	2754.59	2754.59
Centrifuge	Oil+cake+solvent	2754.59	• miscella • cake	2431.19 324.95	2754.59
Distillation	miscella	2431.19	• oil • solvent	249.95 2129.05	2431.19

*Energy balance is not included in this document. But the rough estimates of the cost will be calculating using the given percent values of the variables for economic evaluation of the project found in the following text book (plant design and economics for chemical engineers, Max S. Peters, Klaus D. Timmerhaus).

5.2. Cost Estimation

5.2.1. Equipment cost

Using rapid capital cost estimation method

- ✓ *cost of pulping machine*

  Machine having capacity 95kg/hr costs 1528dollars*16.94= 25884.82br.

  Cost relative to capacity is given by using six-tenth rule

Cost of equipment A= cost of equipment B (Capacity A/Capacity B) $^{0.6}$, where Equipments A and B are of the same type.

Therefore Cost of pulping machine= 1528(1500/95)$^{0.6}$ =8000.99dollar*16.94=135.696.86br.

- ✓ *Cost of Decorticator*

Machine with capacity 500kg/hr costs 1667dollar, then using six tenth rule, cost of the same machine with capacity 950kg/hr will be=41554.07br.

- ✓ *Cost of pulverizing machine*

Machine with capacity of 300kg/hr costs 236dollars, using six-tenth rule, the cost of the same type of machine with capacity 546.25kg/hr will be=5734.56br.

- ❖ Using equation with machine size,from Max S. Peters, Klaus D. Timmerhaus.

$$\mathrm{Ce} = C * S^n$$

Where: Ce = purchased equipment cost, S= characteristics size, C= cost constant from 6.2 of Max S. Peters, Klaus D. Timmerhaus., n= index for that type of equipment

- ✓ *Cost of Agitated vessel*

Mass flow rate=2754.59kg/hr, density= 1.2gm/ml=1200kg/m^3, then volumetric flow rate=2.3m^3/hr, the extraction takes place for three hours, then volume of the vessel will be 6.9m^3. From Max S. Peters, Klaus D. Timmerhaus, C=31000dollars, n=0.45

Using the above equation, the purchased cost of agitated vessel will be 1,252,440.75br.

- ✓ *Cost of centrifuge*

Volume= 6.9m^3, let's take the diameter =1.1m, using the above equation, cost of centrifuge be 391,314br.

- ✓ *Cost of distillation unit*

Cost of recovery unit at the height of the column 10 m from (Coulson and rechardson(V-6 page 255)) = 284,539birr.

- ✓ *Costs of storage tanks* for the Neem oil and the seed
    - For the oil 250kg/hr, assume the plant operate 8hrs per day, thus, volume of the tank required will be 2.207m^3, from Max S. Peters, Klaus D. Timmerhaus, C=2400, n=0.6

Using the above equation, cost of the oil storage tank is 65451.3br.

- For the seed: 950kg/hr*300day*8hrs= 2280000kg/hr, assume the density of the seed be 1200kg/m^3, thus the volume of the storage tank 1900m^3, from Max S. Peters, Klaus D. Timmerhaus, C=2400, n=0.6, using equation above, the cost of the storage tank for the seed is 3,774,759.6br.

- ✓ *Costs of three pumps* with flow rate, 2.179ft^3/min, 2.182ft^3/min, and 0.162ft^3/min will be calculated using above equation, results 313,933.87, 314,279.52 and 66,007.4br, respectively. And total=694,220.3br.

Table5.2: Summary of costs of the equipments

No.	Equip. type	Required Capacity or Size		Cost	
				Dollar	**Birr**
1	Pulping machine	1500kg/hr	-	8000	135696.86
2	Decorticator	950kg/hr	-	2450.12	41554.07
3	Pulverizer	546.25kg/hr	-	338.12	5734.56
4	Agitated vessel	-	$6.9m^3$	73933.93	1,252,440.75
5	Centrifuge	-	1.1m (dia.)	23100	391,314
6	Distillation	-	$3.8841m^2$	16796.87	284,539
7	Storage tank-1	-	$1900m^3$	222568.34	3774759
8	Storage tank-2	-	$2.207m^3$	3859.16	65451.3
9	Pumps(3)	Flow rates $2.179ft^3$/min, $2.182ft^3$/min, and $0.162ft^3$/min		40932.79	694220.3
Total purchased equipments cost					6,645,709.84

5.2.2. **Estimation of capital investment cost**

3.2.2.1. **Fixed capital investment (FCI) estimation**

Direct cost

A. Costs of equip. + installation + instrumentation +piping+ electricity+ painting(50-60%of FCI)
   I. Purchased equipment cost (PEC) =6,645,709.84Br.
   II. Installation including painting (30% of PEC) =1,993,712.95Br.
   III. Instrumentation and control (6% of PEC) =398,742.59Br.
   IV. Piping (5% of PEC) =332,285.49Br.
   V. Electricity (40% of PEC) =2,658,283.9Br.
B. Building, process and auxiliary (10% of PEC) = 664,570Br.
C. Service facilities (40% of PEC) =2,658,283.9Br.
D. Land (4% of PEC) = 265,828.4Br.

DIRECT COST (DC) = A + B + C + D = 15,617,417.03Br.

Indirect cost

A. Eng'g and supervision (20% of DC) = 3,123,483.41Br.
B. Construction expense and contractor fee (18% of DC) = 2,811,135.07Br.

INDIRECT COST (IC) = A + B = 5,934,618.48Br.

Fixed Capital Investment (FCI) = DC + IC = 21,552,035.51Br.

Working Capital investment (WCI) = 15% of TCE *

TCI = FCI + WCI **

From the above two equations and the value of FCI, the WCI = 3,803,300.38Br.

3.2.2.2 Total production cost (TPC) estimation

- Total fresh fruit required : 1000kg/hr = 2,400,000 kg/yr
- Price of fruit = 1.156 Br/kg
- The total costs of fresh fruit = 2,774, 400 Br/yr
- Total fresh n- hexane solvent required

  Total crushed kernel = 540.78kg/hr * 8 * 300 = 1,297,672 kg/yr From the experimental work, for 0.045kg of crushed kerne, I used 0.25lt solvent. Then, for 1,297,672 kg of kernel we will use 7,210, 400lt solvent. Assuming that the recovered solvent can be used again at least one more times. Thus, 3,605,200lt solvent will require with make-up of 356,544lt. Therefore, total solvent required 3,961,744lt/yr. and this costs 182,240,224 Br.

  A. Fixed Charges (FC)
     1. Depreciation = 10% of equipment cost + 2.5% of building = 681,185.234Br.
     2. Local taxes = 2.5% of FCI = 538,800.9Br.
     3. Insurance = 0.7% of FCI = 150,864.24Br.

Total Fixed Charge = 1,370,850.38Br.

  B. Production cost (PC)

     FC = 15% of TPC, TPC = 9,139,002.5Br.

1. Raw material and inputs = 185,014,624Br.
2. Operating labor (15% of TPC) = 1,370,850.38Br.
3. Direct supervisor and clerical labor(20% of operating labor) = 274,170.08Br
4. Utilities (15% of TPC) = 1,370,850.37Br
5. Maintenance and repair (6% of FCI) = 1,293,122.13Br
6. Laboratory Charge (15% of operating labor) = 205,627.56Br.

Total product cost = 189,529,244.4Br.

C. Plant overhead cost (POC) (10% of TPC) = 913,900.25Br

MANUFACTURING COST = FC + PC + POC = 191,813,995.1Br.

General expenses

A. Administration cost (4% of TPC) = 365,560.1Br.
B. Distribution and selling cost (11% of TPC) = 1,005,290.27Br.
C. Research and development cost (5% of TPC) = 456,950.125Br.

GENERAL EXPENSE = 1,827,800.49Br.

TOTAL PRODUCTION COST = Manufacturing cost + General expense = 193,641,795.6Br.

Total product cost /kg of Neem oil = 193,641,795.6Br /600,000kg = 322.73Br/kg of Neem oil.

Whole selling price of 1lt (0.906kg) Neem oil = 20dollar/L = 339.20Br/L.

Total income = 224,635,759.7Br/yr

Gross income = Total income – total production Cost = 30,993,965.99Br.

Let take the tax rate in Ethiopia 35% of gross income, tax = 10,847,888.1Br.

Net profit = Gross income – tax = 20,146,077.89Br.

Rate of return

$$\text{Rate of return on investmnet} = \frac{\text{Net profit} * 100}{\text{Total capital investment}} = 79.45\%$$

$$\text{payback period} = \frac{\text{FCI}}{\text{Net profit+Depreciation}} = 1.03\text{yrs, around one year}$$

Neem oil extraction using n-hexane is profitable as it is clearly observed from the above cost estimation. The rate of return on investment 79.45% implies the plant returns 79.45% of its total capital investment in one year. The payback period tells us the plant return its total investment cost in around one year and then it will become profitable. The income statement and the other indicators of profitability show that the project is viable. The project can be implemented after detailed feasibility study has been done.

6. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

This work was intended to study the influence of different factors (Particle sizes, solvent type, temperature, and time) on the quality and quantity of Neem oil. Variability of these operating conditions are the pre-dominant factors for the quality and quantity of the oil.

There are different methods of essential oil extraction from Neem seed kernel. In this thesis, soxhlet extraction and agitated vessel extraction were used. In soxhlet extraction the solvent used for the extraction was n-hexane and the maximum oil yield obtained was 46.785% at the particle size of 0.425-0.71mm and the extraction time of 6hours, the observed quantitative difference in the quantity of the oil was due to particle size and extraction time variability. Thus, determination of the appropriate size of the particles and optimal time for the recommended particle size needs to have a consideration to get the maximum amount of the required product.

For agitated mixing extraction, both the yield and the quality of the oil have studied for solvents, n-hexane and ethanol. Based on the analysis of the experimental result obtained, the quantity of oil extracted from Neem seed kernel was found to be 47.32% for n-hexane and 33.198% for ethanol at 50^{o}C and 0.425-0.71mm particle size. The result obtained was decreased as we compare it from the literature using ethanol as a solvent. The quality of the oil could be affected due to several reasons like impurities with the seed and the solvent and the operating conditions. From the investigation, temperature was the dominant factor for the change in quality of the oil. Unlike on iodine value, temperature has the direct relationship with Saponification and acid value and the laboratory result is comparable with the (Maria Yuliana Liauw, F. A. Natan, P. Widiyanti, D. Ikasari, N. Indraswati and F. E. Soetaredjo). Thus, investigation of optimal operating temperature has to be taken in to consideration.

Based on the rough economic analysis of solvent extraction method, the project is profitable since the rate of return on investment was 79.45%, this show us the project returns its 79.45% of the initial investment in one year and the payback period is around one year.

6.2. **Recommendation**

- ✓ Here in the experimental work, the solvent to solid ratio has not been studied. This is due to the current market price of the solvent rise.
- ✓ To install small scale Neem oil extraction industry, solvent extraction method requires high investment cost; this is due to the requirement of equipment with well designed control system and instrumentation to recover the solvent and the need of high electrical power to separate the solvent from the oil. Finally, I recommend the project to be implemented after detail feasibility studies have been done.
- ✓ From the point of application of Neem oil:
    - Since rural areas of the Ethiopian people use Neem tree for malaria treatment. The people need to be fully aware of which parts of the Neem tree use for their specific purpose, Infants and very old people are not recommended to use Neem oil for internal use.
    - It is advisable to use extracts of Neem seed using n-hexane as a solvent for insect sides because of the toxicity of n- hexane.

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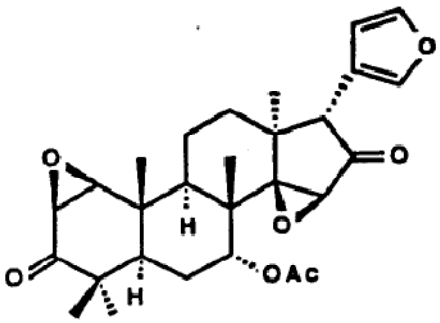
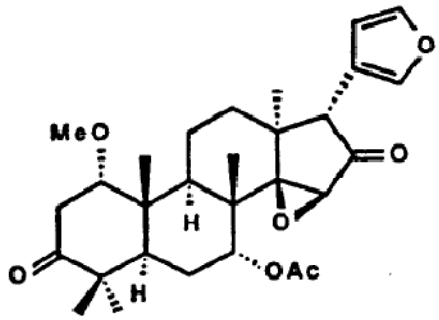
APPENDICES

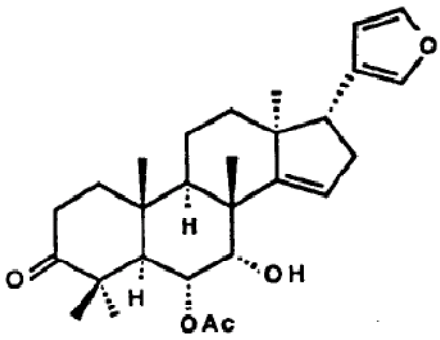
Appendix A. Chemical compounds found in Neem oil



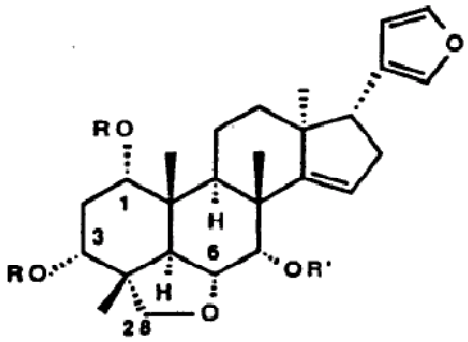
(15) diepoxyazadiradione



(16) 1- α -methoxy-1,2-dihydroazadiradione



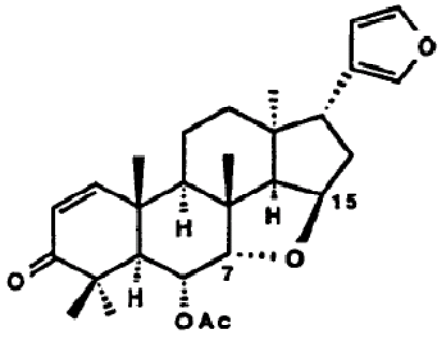
(17) meldenin



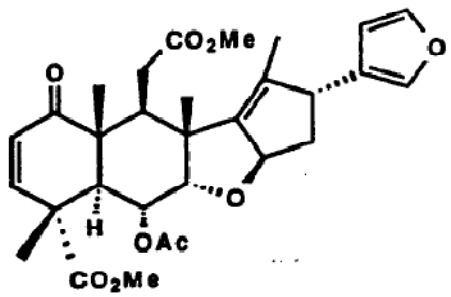
(18) R=R'=H vilasinin

(19) R=R'=Ac vilasinin triacetate

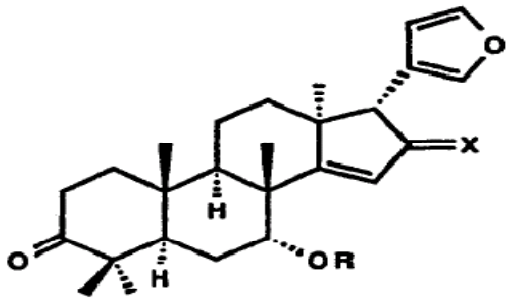
(20) R=Ac, R'=H vilasinin-1,3-diacetate



(21) vepinin



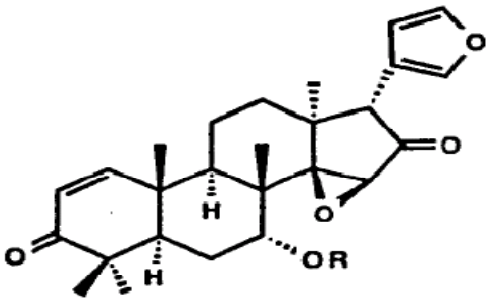
(22) nimbin



(7) X=H,H R=OAc azadirone

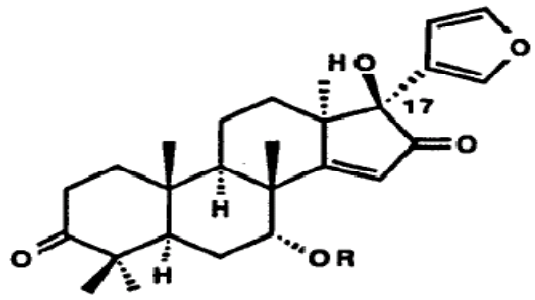
(8) X=O R=Ac azadiradione

(9) X=O R=COPh azadiradione benzoate

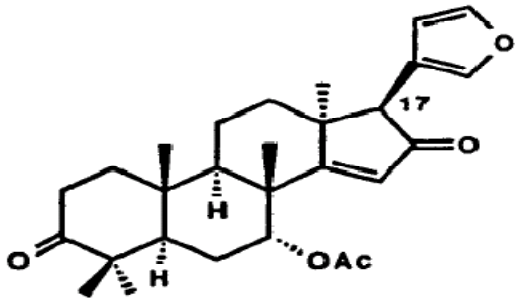


(10) R=Ac epoxyazadiradione

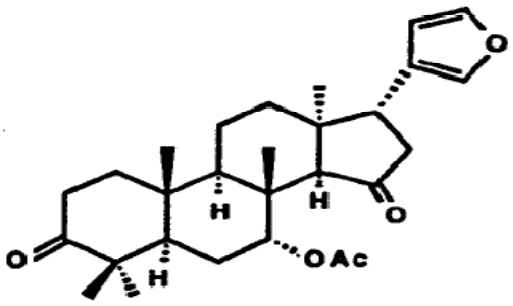
(11) R=COPh epoxyazadiradione benzoate



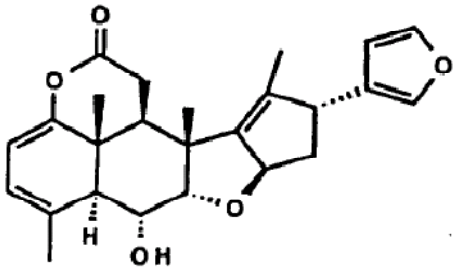
(12) 17-β - hydroxyazadiradione



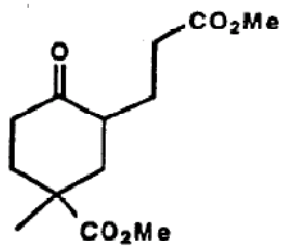
(13) 17-epiazadiradione



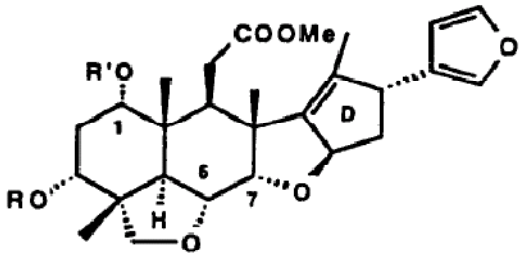
(14) 7-acetylneotrichilenone



(27) pyronimbic acid



(28) nimbin part structure

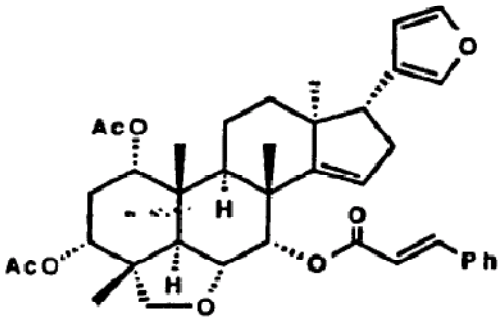


(4) R=OAc, R'=tigloyl salannin

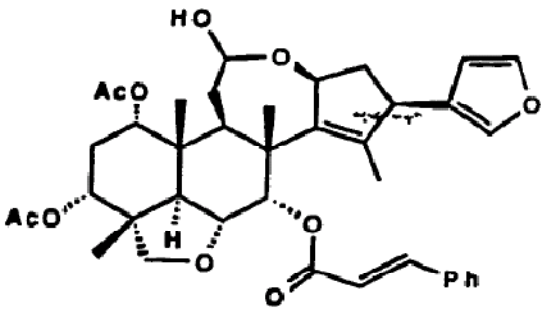
(29) R=H, R'=tigloyl deacetylsalannin

(30) R=R'=H deacetylsalannin

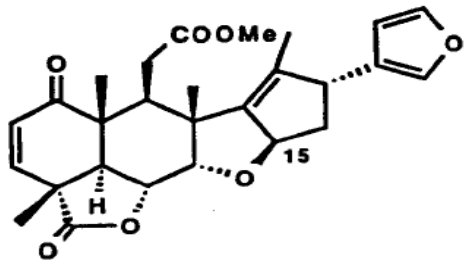
(40) R=H, R'=3-methylbutanoate salannol



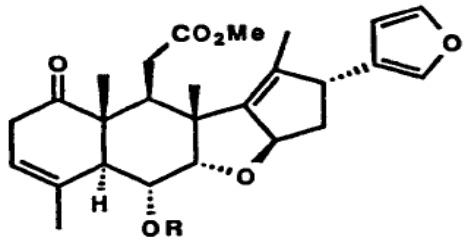
(31) nimbolin A



(32) nimbolin B

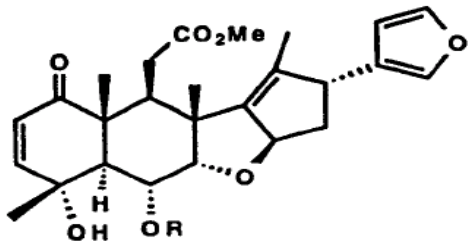


(35) nimbolide



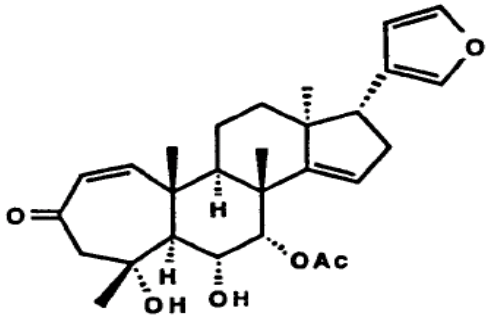
(36) R=Ac nimbinene

(37) R=H deacetylnimbinene

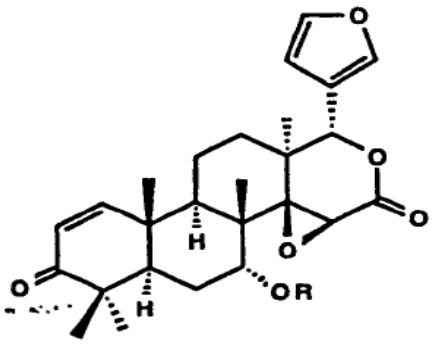


(38) R=H nimbandiol

(39) R=Ac 6-acetylnimbandiol



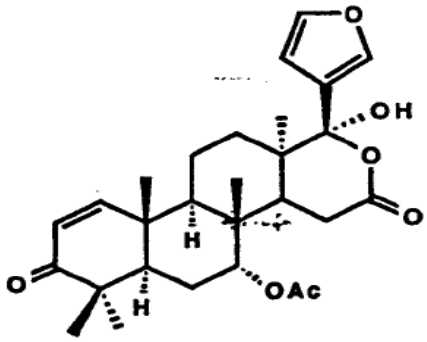
(41) 4α,6α -dihydroxy-A-homoazadirone



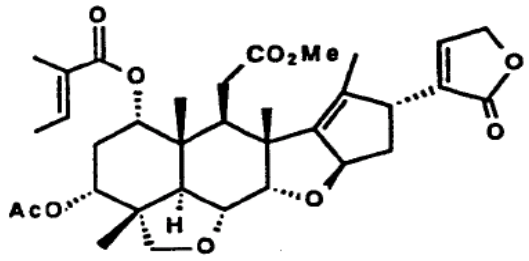
(42) R=Ac gedunin

(43) R=COPh 7-deacetyl-7-benzoylgedunin

(44) R=H 7-deacetylgedunin



(45) nimolicinol



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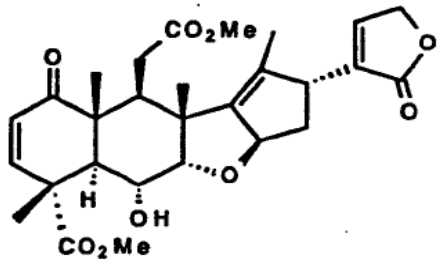

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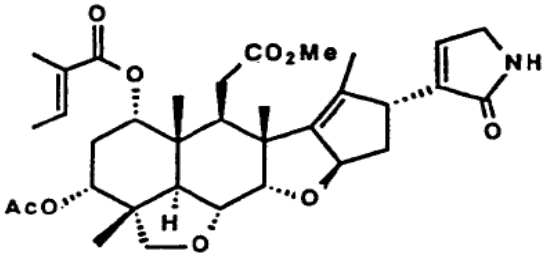
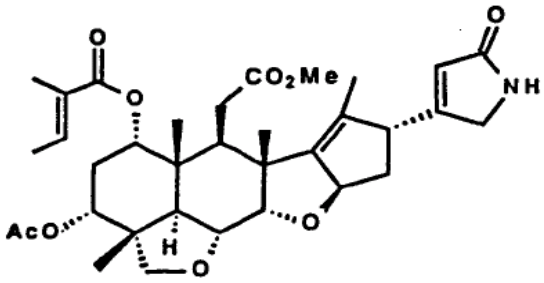


(48) salannolactam I



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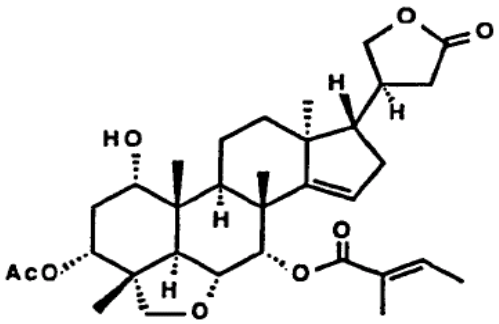
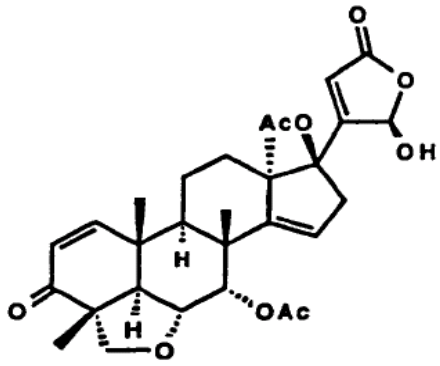
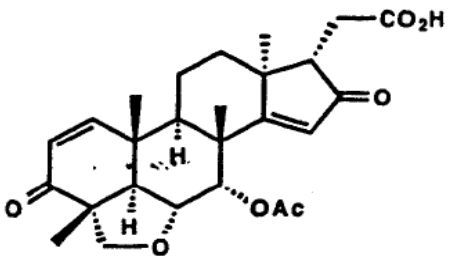


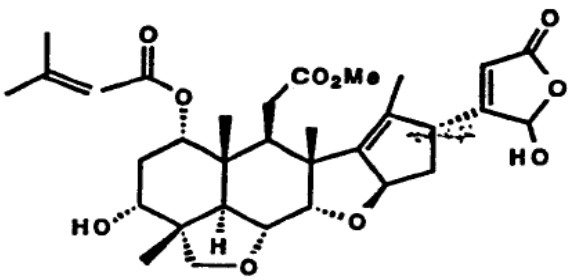
(50) 3-acetyl-7-tigloylvilasinnin lactone



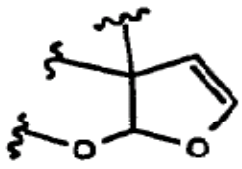
(51) isonimolicinolide



(52) nimolicinoic acid



(53) isoazadirolide



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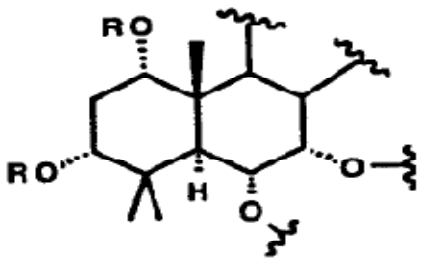

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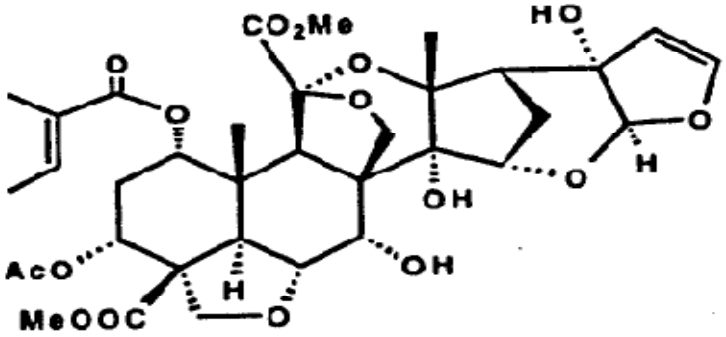
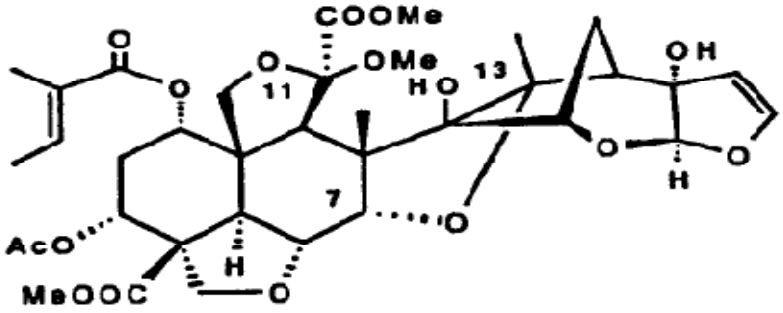
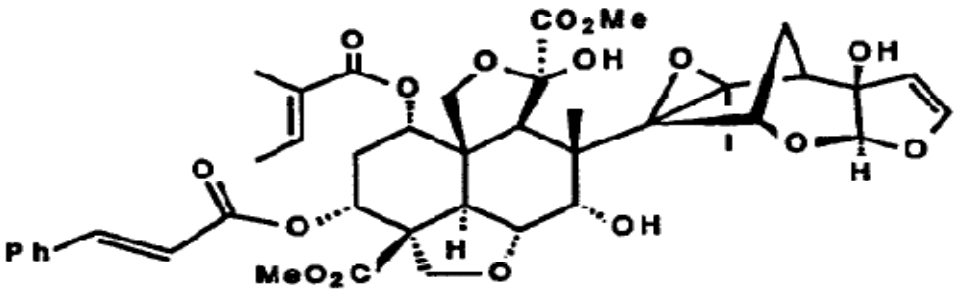


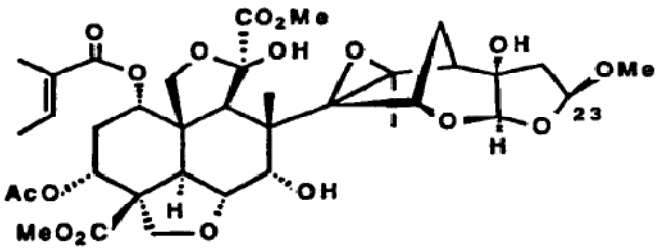
(56) Nakanishi's azadirachtin



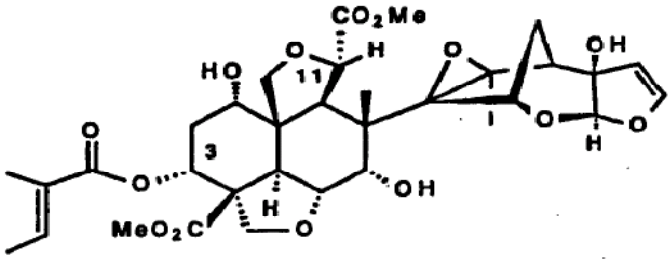
(57) 1-tigloyl-3-acetyl-11-methoxyazadirachtinin



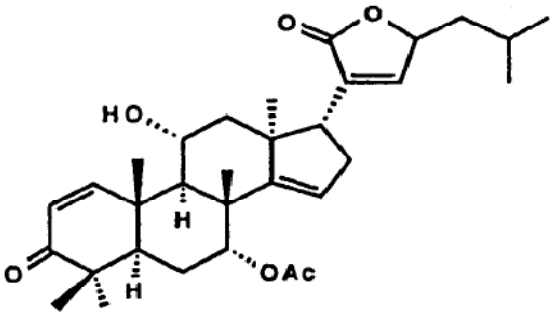
(58) 3-deacetyl-3-cinnamoylazadirachtin



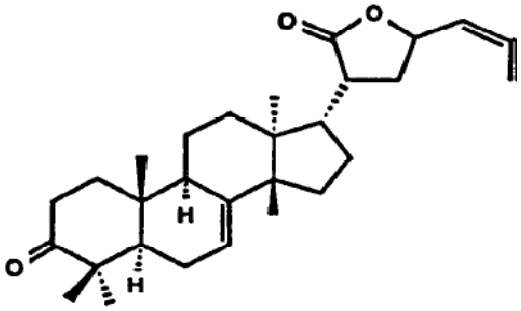
(59) 22,23-dihydro- β -methoxyazadirachtin



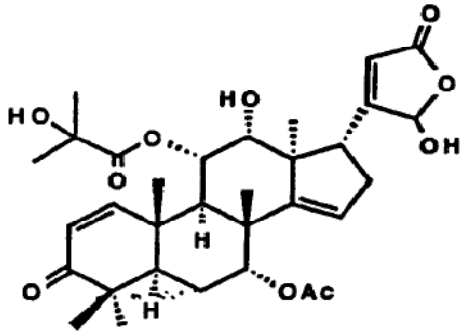
(60) 3-tigloylazadirachtol



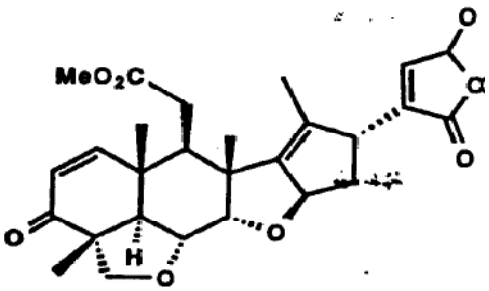
(61) azadirachtol



(62) nimolinone



(63) isonimbocinolide



(64) margosinolide

Appendix B

B1. Soxhlet Extractor Data Analysis

Table B1. Analysis of experimental (actual) data of the soxhlet extraction.

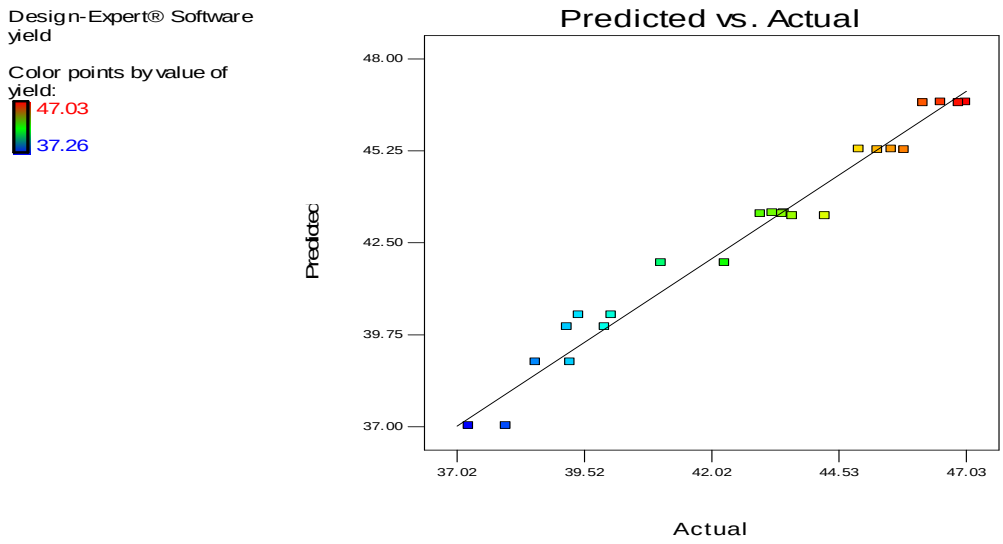
Std.	run	Factor 1 time(hr)	Factor 2: particle size(mm)	Response: %yield
2	1	A1	B1	37.26
18	2	A1	B3	39.42
11	3	A2	B2	42.29
19	4	A2	B3	43.63
20	5	A2	B3	44.26
24	6	A4	B3	46.89
6	7	A3	B1	43.23
4	8	A2	B1	39.19
13	9	A3	B2	45.57
22	10	A3	B3	46.56
1	11	A1	B1	37.99
13	12	A4	B2	45.82
23	13	A4	B3	46.193
7	14	A4	B1	43.42
17	15	A1	B3	40.06
8	16	A4	B1	42.993
14	17	A3	B2	44.93
21	18	A3	B3	47.03
9	19	A1	B2	38.507
12	20	A2	B2	41.04
10	21	A1	B2	39.25
16	22	A4	B2	45.295
5	23	A3	B1	43.46
3	24	A2	B1	39.93

The design data helps us to characterize results from the experiment to know the effects of each factors and their interactions and to develop the model which helps us to predict what will be the effects of the experiment if we change the values of the main factors and their interactions using DESIGN EXPERT software.

Table B2. Values for reasonable agreements

Std. Dev.	0.57	R-Squared	0.9736
Mean	42.68	Adj R-Squared	0.9662
C.V. %	1.33	Pred R-Squared	0.9530
PRESS	10.29	Adeq Precision	34.163

The "Pred R-Squared" of 0.9530 is in reasonable agreement with the "Adj R-Squared" of 0.9662."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 34.163 indicates an adequate signal. This model can be used to navigate the design space.



FigB1. Comparisons of the predicted model vs. Actual values of the experiment.

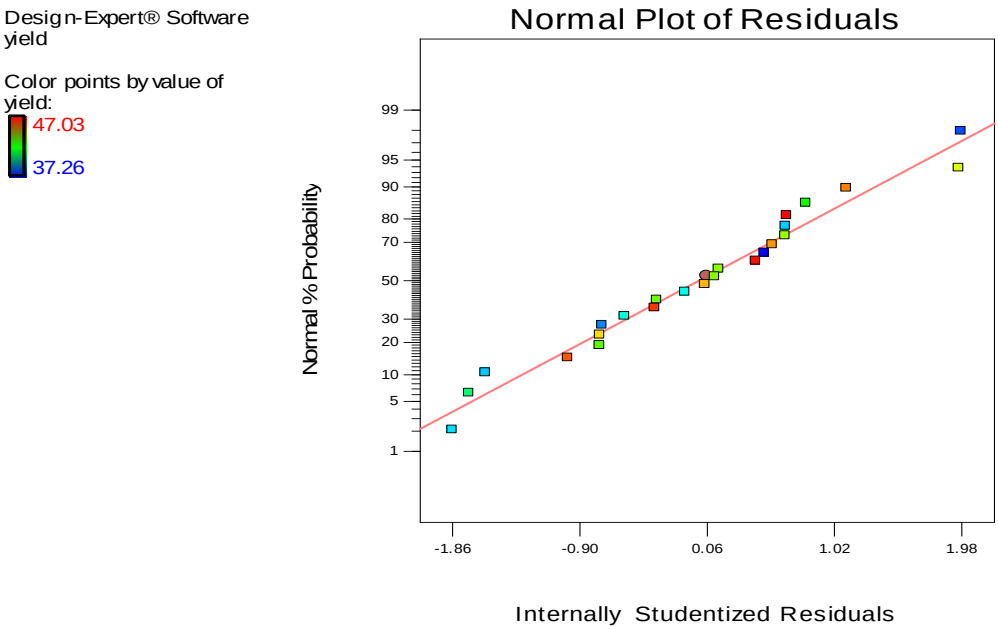


Fig.B2 Normal plots of the residual.

B2.Agitated Mixing Extraction data analysis

Table B3. Analysis of experimental data of the Agitated Mixing extraction.

Std	Run	Fact.1A:solvent type	Fact.2 B:particle size,mm	Fact. 3C: temp. ^{o}C	%yield Response1	SV(mg/g) Response 2	AV(g/g) response 3	IV(g/g) Resonse4
8	1	Et	P3	30	26.99	175.712	32.8	66.58
4	2	Hx	P2	30	37.1	183.35	96.4	72.62
18	3	Hx	P3	50	47.32	221.93	122	62.83
11	4	Et	P3	40	31.09	194.3	36	65.78
10	5	Hx	P2	40	41.22	209.256	102	68.41
3	6	Et	P2	30	23.25	175.72	32.8	66.58
1	7	Et	P1	30	20.82	175.72	32.8	68.41
12	8	Hx	P3	40	44.8	209.256	102	65.79
7	9	Et	P1	40	24.41	194.36	36	62.83
16	10	Hx	P2	50	44.44	221.93	122	72.62
2	11	Hx	P1	30	36.12	183.35	96.4	65.32
13	12	Et	P1	50	29.82	205.83	40	61.32
9	13	Et	P2	40	26.12	194.3	36	65.79
14	14	Hx	P1	50	40.28	221.93	122	62.82
15	15	Et	P2	50	30.44	205.83	40	61.32
6	16	Hx	P3	30	39.22	184.028	96.4	72.62
17	17	Et	P3	50	33.2	205.83	40	61.32
8	18	Hx	P1	40	38.72	209.256	103	68.41

The design data helps us to characterize results from the experiment to know the effects of each factors and their interactions and to develop the model which helps us to predict what will be the effects of the experiment on yield and quality if we change the values of the main factors like temperature, particle size, solvent type and their interactions using DESIGN EXPERT software.

Table B4. Values for reasonable agreements

Std. Dev.	0.96	R-Squared	0.9899
Mean	34.19	Adj R-Squared	0.9857
C.V. %	2.80	Pred R-Squared	0.9773
PRESS	24.76	Adeq Precision	47.172

The "Pred R-Squared" of 0.9773 is in reasonable agreement with the "Adj R-Squared" of 0.9857. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 47.172 indicates an adequate signal. This model can be used to navigate the design space.

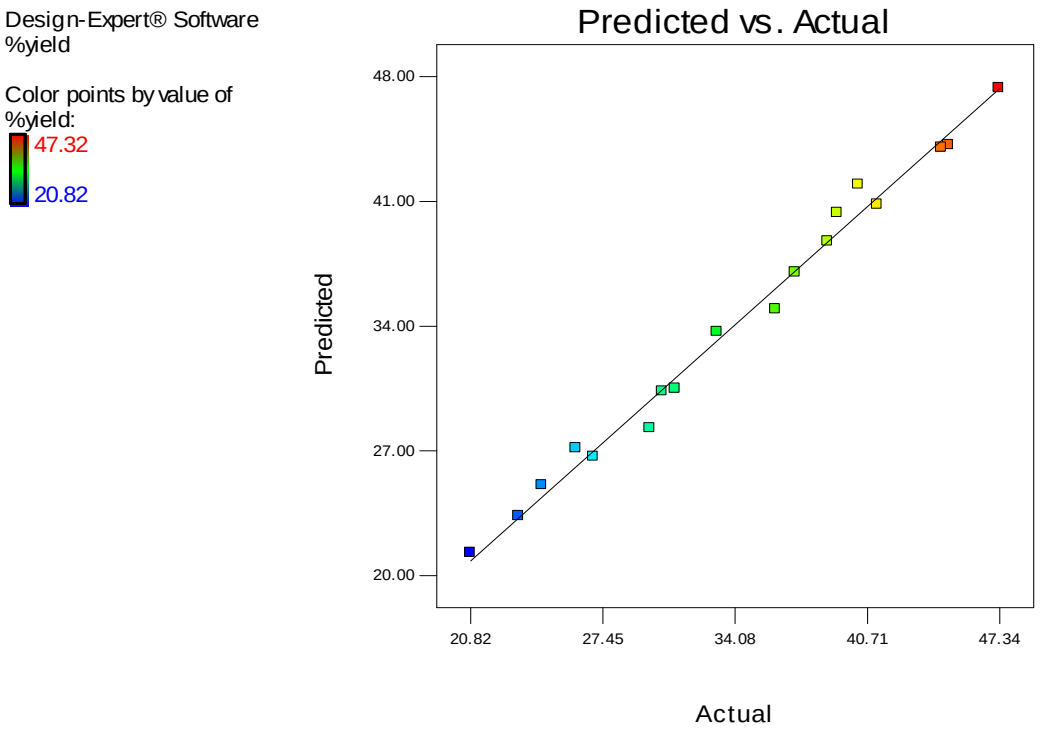


Figure B3. Show that predicted vs actual values of the experiment

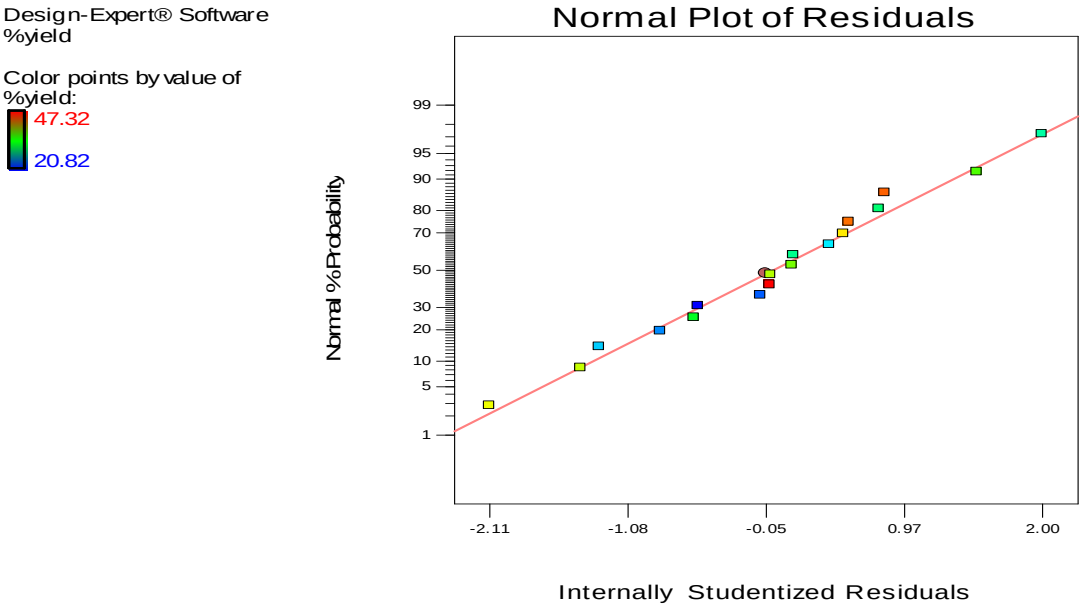


Fig.B4. Graph of Normal plots of residual

➢ **Responses on Saponification number**

Table B6. Values for reasonable agreements

Std. Dev.	0.16	R-Squared	0.9999
Mean	198.44	Adj R-Squared	0.9999
C.V. %	0.081	Pred R-Squared	0.9998
PRESS	0.69	Adeq Precision	500.806

The "Pred R-Squared" of 0.9998 is in reasonable agreement with the "Adj R-Squared" of 0.9999. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 500.806 indicates an adequate signal. This model can be used to navigate the design space.

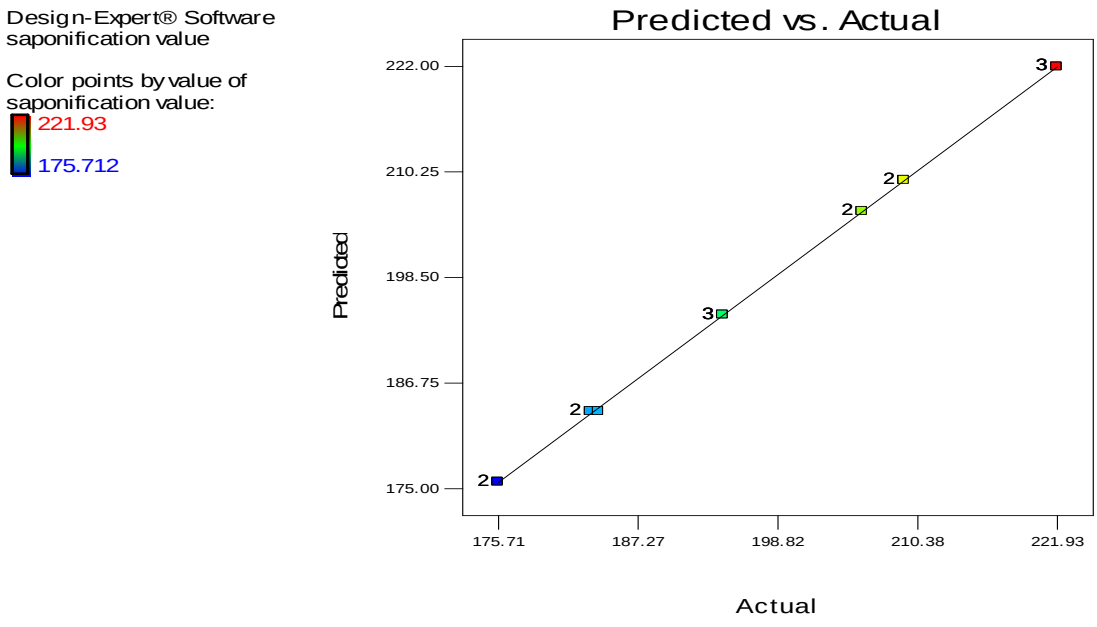


Fig.B7 Predicted vs Actual values of Saponification response

- **Response on Acid value**

Table B7. Values for reasonable agreements

Std. Dev.	0.000	R-Squared	1.0000
Mean	71.53	Adj R-Squared	1.0000
C.V. %	0.000	Pred R-Squared	1.0000
PRESS	0.000	Adeq Precision	500.806

The "Pred R-Squared" of 1.0000 is in reasonable agreement with the "Adj R-Squared" of 1.0000.

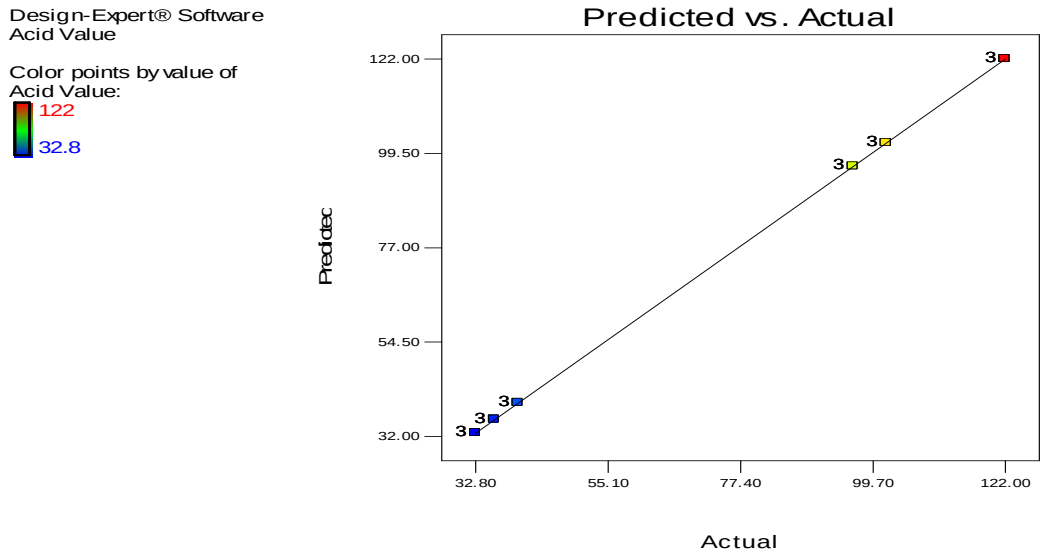


Fig.B8. Actual vs predicted value

Appendix C

Distillation unit design

Using the short-cut method of Fenske –Underwood-Gilland (FUG method)

From Material Balance

Components	Feed fraction	Bottom fraction	Top fraction
Solvent(n-hexane) LK	0.884	0.01	0.99
Neem oil(no) HK	0.114	0.98	0.002
other	0.002	0.01	0.008

- ✓ Heavy Key Component = Neem oil
- ✓ Light Key Component = N-hexane

Designing Steps of Distillation Column

- ✓ Calculation of Minimum number of stages, N_{min}
- ✓ Calculation of Minimum Reflux Ratio R_m.
- ✓ Calculation of Actual Reflux Ratio.
- ✓ Calculation of theoretical number of stages.
- ✓ Calculation of actual number of stages.
- ✓ Calculation of diameter of the column.
- ✓ Calculation of the height of the column.

Calculation of Minimum no. of Plates:

The minimum no. of stages N_{min} is obtained from Fenske equation which is:

$$\text{Nmin} = \frac{\ln[(xLK/xHK)D(xHK/xLK)B]}{\ln(\alpha LK/HK)\ \text{average}}$$

Average geometric relative volatility = 1.53 So, N_{min} = 10

Calculation of Minimum Reflux Ratio R_{min}

Using Underwood equations

$$\frac{\alpha_A x_{DA}}{\alpha_A - \theta} + \frac{\alpha_B x_{DB}}{\alpha_B - \theta} = R_m + 1$$

As feed is entering as saturated vapors so, q = 0, by trial, $\theta = 1.003$

Using equation of minimum reflux ratio

$$\frac{\alpha_A x_{fA}}{\alpha_A - \theta} + \frac{\alpha_B x_{fB}}{\alpha_B - \theta} = 1 - q$$

Putting all values we get, $R_m = 2.058$

Actual Reflux Ratio:

The rule of thumb is: $R = (1.2 \text{ ------- } 1.5) R_{min}$

$R = 1.5 R_{min}$

$R = 3.087$

Theoretical no. of Plates:

Gilliland related the number of equilibrium stages and the minimum reflux ratio and the no. of equilibrium stages with a plot that was transformed by Eduljee into the relation;

$$\frac{N - N_{min}}{N+1} = 0.75\left[1 - \left(\frac{R - R_{min}}{R+1}\right)^{0.566}\right]$$

From which the theoretical no. of stages to be N= 16stages

Location of feed Plate:

The Kirk bride method is used to determine the ratio of trays above and below the feed point.

$$\log\left(\frac{N_D}{N_B}\right) = .206\log\left[\left(\frac{B}{D}\right)\left(\frac{x_{HK}}{x_{LK}}\right)\left(\frac{(x_{LK})_B}{(x_{HK})_D}\right)^2\right]$$

From which,

Number of Plates above the feed tray = ND = 11

Number of Plates below the feed tray = NB = 5

Height of Distillation Column

To convert equilibrium stages to actual trays, one must take into account that equilibrium is not reached with actual trays. Equilibrium stages are converted to actual trays using overall tray efficiency.

$$E_o = \frac{N}{N_{act}}, overall\ tray\ effeciency$$

$$E_o = 0.492(\mu_L \alpha)^{-0.245}$$

μ_{avg}= Feed viscosity at average temperature (68.9^{o}C) = 0.34mNs/m^2

E_o = 0.492(0.34*1.508)$^{-0.245}$=0.579, therefore N_{act} = 28stages

Height of column H_c= (N_{act}-1) H_s+ ΔH+ plates thickness

No. of plates = 28, Tray spacing H_s = 0.30 m

ΔH= 0.5 meter each for liquid hold up and vapor disengagement

ΔH=1 m

Total thickness of trays = 0.005*28 = 0.14 m so,

Height of column = (28-1)*0.30+ 1+0.14 = 9.24 meters

Area of the column

Assumed tray spacing = 0.3 m

From Fig (15-5) Plant Design and Economics for Chemical Engineering, sieve tray flooding capacity, C_{sb} = 0.0760 m/Sec

Surface tension of Mixture = σ = 18.35 dynes/Cm

$V_{nf} = C_{sb}\left(\frac{\sigma}{20}\right)^{0.2}\left(\frac{\rho l - \rho v}{\rho v}\right)^{0.5}$, thus, V_{nf}=1.56 m/sec

Assume 90% of flooding then, V_n=0.9V_{nf}

So, actual vapor velocity, V_n=1.404 m/sec

Net column area used in separation is: $A_n = m_v/V_n$

Where: Volumetric flow rate of vapors = m_v

m_v = (mass vapor flow rate /(3600) vapor density)

$m_v = 2.02 m^3/sec$

Now, net area $A_n = m_v/V_n = 1.438 m^2$

Assume that down commer occupies 15% of cross sectional Area (A_c) of column thus:

$A_c = A_n + A_d$: Where, A_d = down commer area.

$A_c = A_n + 0.15(A_c)$ but, $A_c = A_n / 0.85$, $A_c = 1.69\ m^2$

So Diameter of Column is: $A_c = (\pi/4)D^2$, $D = (4A_c/\pi)$

D = 1.47 meter

Appendix D:

Laboratory equipments and samples photo.

D1. Rotary evaporator

D2. Centrifuge

D3. Output from Centrifuge

D4. Sample products

D5. Weighing balance