Extraction and Physiochemical Properties of Neem (*Azadirachta Indica* (L.) juss) Seed Oil

Assma Ebrahim Omer Adam

B.Sc. (Hons.) in Scientific Laboratories (Chemistry), Sudan University of Science and Technology (2010)

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Chemistry

Department of Applied Chemistry and Chemical Technology
Faculty of Engineering and Technology

June 2014
Extraction and Physiochemical Properties of Neem (*Azadirchta Indica* (L.) juss) seed oil

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<tr>
<td>Dr. Mohamed Elmubarak Osman</td>
<td>Main Supervisor</td>
<td>...........</td>
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<tr>
<td>Dr. Abobakr Khalid Ziyada</td>
<td>Co. supervisor</td>
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Examination Committee:

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<tr>
<td>Dr. Alnaiem Abdalla Ali</td>
<td>External Examiner</td>
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<tr>
<td>Dr. Mohammed Osman Babiker</td>
<td>Internal Examiner</td>
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Date of Examination: 5/7/2014
بسم الله الرحمن الرحيم

قال تعالى:

«والأرض مددناها وألقينا فيها رواسي وأنبتنا فيها من كل شيء موزون»

صدق الله العظيم

"سورة الحجر: الآية 19"
Dictation

I dedicate this work to:

My parents

Brothers and sisters

Friends…
Acknowledgment

Firstly, I thank Allah, almighty, who gave me health, strength and patience to complete this work. I would like to express my deep gratitude to my supervisor prof. Mohammed Elmubarak Osman for his guidance, support, valuable comments and advice. I would like to express my sincere thanks to the staff of national laboratory (Stack). Finally, I would like to thank my friends for their moral and continuous support.
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University of Gezira

Abstract

The versatility of the Neem tree (Azadirachta indica) is reviewed. This species, native to India, highly implanted in Sudan has tremendous potential for human use. Since Neem is a natural renewable resource producing extensive useful volatile oil, this research is concerned by extraction of volatile oil from Neem plant (Azadirachta Indica), and studies the chemical properties of extracted volatile oil and prepares cream with different concentrations of Neem oil, and tested as a mosquito repellent. Analysis of the cream as moisturizing cream satisfies the requirement of Sudanese Standards and Metrology Organization (SSMO).

The volatile oil was extracted from Neem fruit by Soxhlet extraction using Petroleum ether as the extracting solvent which is removed by rotary evaporator. The percentage yield of oil extracted was (37.08%). The result of study of chemical properties showed that: the oil has an acid value of (2.127), peroxide value of (0.00), iodine value of (47.35) and Saponification value of (138.45). The cream was prepared using a combination of solid base of Paraffin oil, Vaseline, wax and talk powder. Gum Arabic solution was used as stabilizing agent. Cresol as colouring agent. Neem oil 2% and 4% concentration of the oil in volume/weight of cream was added to obtain the final cream product. The effectiveness of cream as a mosquito repellent, has been tested. The result indicate. That the cream efficiency is 64.5%and 36.4% for the 10% volume/weight and 5% volume/weight respectively.is moderately active as mosquito repellent at 10% volume/weight concentration of the oil. Its economic exploitation will be beneficial, particularly to the Third World. In recent years, some useful commercial products have been developed from Azadirchta indica, and more considerable, scope for future product development. With a different concentration a potentially commercial product is suggested.
استخلاص زيت النيم وتحديد خواصه الفيزيوكيميائية

عصماء إبراهيم عمر ادم

ماجستير العلوم في الكيمياء (يوليو 2014م)
قسم الكيمياء التطبيقية وتقنية الكيمياء
جامعة الجزيرة

ملخص البحث

يختص هذا البحث باستخلاص الزيت الطيار من ثمار نبات النيم، وتحديد بعض الخواص الكيميائية للزيت المستخلص، كما يختص بتضبير كريم مرطب واستشار فعاليه الكريم في طرد الباعوض بعد اضافه زيت النيم له، وتحليله كريم مرطب وفقا للمواصفه السودانيه (م ق س د 4155/2008).

نتائج استخلاص الزيت الطيار من ثمار نبات النيم من خلال جهاز التقطير البخاري اوضحت ان نسبه الاستخلاص للزيت الطيار المستخلص (37.08%). نتائج تحديد بعض الخواص الكيميائية اوحتت الاتي: رقم الحموضه (2.127)، رقم البيروكسيد (0.00)، رقم التصبين (138.45)، رقم اليود (47.35). تم تحليل الكريم المحضر من الصمغ العربي وزيت النيم المستخلص وفق المواصفه السودانيه (م ق س د 4155/2008)، ووضحت النتائج انه مطابق للمواصفه السودانيه (م ق س د 4155/2008). كريم مرطب حيث انه ابيض اللون وراحته مقبوله وشكله مستحبل غير متجلب بريقه الزيت الهيدروجيني (4.82) والمحترق المائي له (53.9%) ومحترق الماء الدسم (57.05%). تمت دراسه اثر فعاليه الكريم المحضر بعد اضافه تركيز مختلفه من زيت النيم (2%, 4%) على عينات مختلفه من انثى الافوكيس، حيث كانت نسبه طرد الباعوض لكلا من التركيز (2%, 4%) هي (36.48%, 64.51%) على التوالي.
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CHAPTER ONE

Introduction
INTRODUCTION

1.1. Neem tree (Azadirchta Indica):

Neem is one of the Mahogany families Meliaceae, and it is known by the botanic name *Azadirachta Indica*, it is one of the most versatile medicinal plants having a wide spectrum of a biological activity, every part of the tree has been used as traditional medicine.

The Neem tree (*Azadirachta indica*) of family Meliaceae is native to the Indian subcontinent, and its seed and leaves have been in use since ancient times to treat a number of human ailments and also as household pesticide.

The tree itself is known as an air purifier and variety of medicinal and germicidal properties have been attributed to leaves, bark, seeds and other parts of the plant. Leaves of the tree are used as anti-inflammatory, anxiolytic, anti-androgenic, anti-stress, humoral and cell-mediated immunostimulant, anti-hyperglycemic, liver-stimulant, anti-viral and anti-malarial activities.

1.2. Botanical classification of Neem Tree:

*Family: Meliaceae*

*Sub family: Melioidear*

*Tribe: Melieae*

*Order: Rutales*

*Sub order: Retainer*

*Genus: Azadirachta*

*Species: Azadirchta indica*

*Common name: Neem, Nim and more than 100 names*.

1.3. Description of Neem Tree:
Neem is fast-growing tree, generally 15–20 m tall (sometimes up to 40 m tall), with a crown diameter up to 20 m. Neem is evergreen but can shed most of its leaves under dry conditions. The compound (pinnate) leaves are alternate, 20–40 cm long, with 20–30 dark green, serrated leaflets, each about 3–8 cm long. The terminal leaflet is often absent. Young leaves are reddish to purplish in colour.

Petioles are 70–90 mm long. The bark is deeply fissured. Flowers are cream coloured, perfumed and arranged in axillary clusters (each cluster is called an inflorescence). Each inflorescence is 15–25 cm long and comprises 150–250 individual flowers. Each flower is about 1 cm in diameter with five petals,

Neem has a strong root system with a deep tap root and extensive lateral roots. Suckers can be produced following damage to the roots.  

1.4. Distribution of Neem Tree:

Neem is a native of the Siwalik Deccan parts of South India. It grows wild in the dry forests of Andhra Pradesh, Tamil Nadu and Karnataka. It has spread to Pakistan, Bangladesh, Sri Lanka, Malaysia, Indonesia, Thailand, Middle East, America, Puerto Rico and Haiti. The largest known plantation of nearly 50,000 trees is at Arafat plains en route to Mecca in Saudi Arabia for providing shade to Hajj pilgrims.

1.5. Chemistry of Neem:

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 folklore medicinal values, the chemist took up the isolation of active principle from seed and other parts. Siddiqui was the first to report the isolation of three products vis. Nimbin, nimbidin and nimbinin from its oil.

The Neem constituent belonging to chemically diverse classes have been divided into two major sections:
I) Isoprenoids

II) Non-Isoprenoids.

The latter category comprises glycerides, polysaccharides, sulphur ones compounds, flavonoids and their glycosides, amino acid, aliphatic compounds etc.

I) **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, sugiol and nimbiol were reported first time. Structures of few compounds are given below:

\[
\begin{array}{cccc}
  & R_1 & R_2 & X \\
\text{Sugiol} & \text{Me} & \text{OH} & \text{H.H} \ (1) \\
\text{Nimbiol} & \text{ipr} & \text{OH} & \text{H.H} \ (2) \\
& \text{H} & \text{O} & \text{ipr} \\
\end{array}
\]

MARGOSONE (4)
ii) Triterpenoids:

The bitterness of Neem is due to 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 in 1960.

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, salsnin and azadirachtin.

iii) Protomeliacins:

The triterpenes containing C₅ side chains C-17 are supposed to be bio-genetic precursors of limonoids and hence known as protolimonoids or protomeliacins.

Meliantriol was first triterpenyl alcohol, isolated from both Neem oil and fresh fruits of Melia azedarachand shown to exhibit marked feeding inhibition against Desert locusts. The structure was well established and confirmed by its synthesis from melianone.

\[ \text{MELIANONE} \]
Siddiqui has added other protomeliacins Nimbocinone(6), Nimolinone(7) and Kulactone (8). Nimbocinone has been isolated from Neem leaves while most of the other constituents from fruit coats and whole fruits.

IV) Limonids with intact four rings and \( \gamma \)-hydroxy butenolide side chain:

The presence of a \( \gamma \)-hydroxybutenolide side chain in place of the furan ring is the characteristic of this group of compound. Two isomeric constituents,
nimocinolides (9&10), isonimocinolide (11&12) have been isolated from Neem leaves whereas isonimocinolide (13) 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 (14) and its deacetyl derivatives (15) have been found in Neem
bark also in addition to their co-occurrence in seed oil. Gedunin was shown to possess 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 characteristics features of this group are presence of oxygen function at C3 and C7.

Azadirachtin A
Butterworth and Morgan (1968) isolated Azadirachtin. Every part of Neem is a source of Azadirachtin A but seed kernel is good source and in Neem oil 0.03% is present. In seed kernel maximum concentration of Azadirachtin A is 50000 ppm.

vii) C-Secomeliacins

This is a large and important group containing the most complex compound and it is specifically found in Neem. There are 3 important sub-groups:

(i) Nimbin
(ii) Salanin
(iii) Azadirachtin

There were 22 members of nimbin and Salanin group have been isolated from Neem. Salanin has anti-feed ant properties.

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 was a cumbersome procedure.

\[ \text{Salanin} \quad R1=\text{Tiglica C=4}, R2=\text{Ac, R3=Me} \]
II) Non-Isoprenoids constituents:

i) (Poly) phenolic

a) 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.

b) Flavonolglycosides: The occurrence of glycoside of kaempferol and quercetin in flowers and leaves and that of myricetin in leaves is reported. They are quercetin-3-galactoside, kaempferol-3-glucoside and myricetin 3-L-arabinose.

c) Dihydrochalcone: From the Aqueous fraction of the fruits, nimbochalcin and a dihydrochalcone derivative was isolated.

d) Tannis: Condensed tannis to the extent of 15% were reported to occur in bark.

ii) Carbohydrates and Proteins

The gum exudates from the stem were found to be a very complex condens ate 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 investigated and it has been found the most abundant was aspartic acid. Other amino acids like serine and threonine were also found.

1.6 Phytopharmacology of Neem:

The Neem tree gives a mixture of approx 135 biologically active compounds. The kernels are having 45 percent oil. The general class of active compounds is the triterpenes and within this category the most effective are the limonoids, which are abundant in Neem oil. Neem also contains more than 20 sulphurous compounds responsible for the characteristic smell of crushed seeds and Neem oil. Neem is also effective against the organisms that cause athletes foot, skin and nail infections. The
chemicals found abundant in Neem tree and recognized for various pharmacological effects are listed in Table 1.6-14.

Table 1: Chemicals found in Neem and their pharmacological actions

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<th>Pharmacological</th>
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<tr>
<td>1</td>
<td>Gedunin</td>
<td>Vasodilator, antifungal</td>
</tr>
<tr>
<td>2</td>
<td>Nimbidin</td>
<td>Antibacterial and analgesic</td>
</tr>
<tr>
<td>3</td>
<td>Quercetin</td>
<td>Anti-protozoal, antioxidant</td>
</tr>
<tr>
<td>4</td>
<td>Salanin</td>
<td>Repellant, anti-feedant</td>
</tr>
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1.7. Medicinal uses:

The indigenous people of Nigeria consume the dried and powered tubulers of the terrestrial orchids as an energizing tonic. Neem also holds medicinal value. Each part of Neem is used in the medicines. It has been used in Ayurvedic medicines for more than 4000 years. Neem oil extracted from its seeds is used in medicines, pest control and cosmetics etc. Its leaves are used to treatment of Chickenpox. According to the Hindus, it is believed that the Goddess of the chickenpox, Sithala lives in the Neem tree. Neem tea is usually taken to reduce headache and fever. Its flowers are used to cure intestinal problems. Neem bark acts as an analgesic and can cure high fever as of malaria. Even the skin diseases can be cured from uses Neem leaves. Indians even believe that the Neem can even purify diseases.15-24.

1.7.1. Skin treatment:

Neem is effective in treating skin problems, infections and inflammations such as acne, boils, eczema, psoriasis, yeast infections and warts. To treat your skin problem, what you can do is apply Neem oil, cream, lotion or poultice on the affected skin. Neem is one of the medicinal herbs that are effective in treating scabies. The compounds in Neem help kill the mites and the eggs. There are several ways to do it but one effective method is to mix turmeric powder with Neem oil and apply the paste on the skin and leave it there to penetrate the burrows dug by the mites. It would be, really, of benefit to take Neem supplements in the form of capsule or add a few drops of Neem liquid extract into your beverage.
1.7.2. Treating head lice:

A university in Egypt did a treatment using Neem seed extract shampoo on 60 heavily lice infected children between the age of 4 to 15 years old. The shampoo was left on the children's scalp from between 5 minutes to 30 minutes. The study found that. Neem seed extract was effective against all stages of head lice and did not produce any side effect to the scalp and skin.

1.7.3. Other Neem Oil Uses:

Neem helps fight against malaria and is safer than taking malaria prevention prescription medication. Neem twigs can be used as a toothbrush. Since Neem extract has astringent and anti bacterial property, it is effective in teeth and gum health. Neem toothpaste and mouth wash are available to help fight tooth decay. It is also able to treat dandruff problems, dry and itchy scalps and also restore dry and damaged hair.

Because of its ability to boost the immune system, taking Neem supplement or drinking Neem tea may help hinder from the common cold.

Neem has shown to be effective in treating chicken pox by applying the paste on the skin or bathing in water that has been boiled with Neem leaves. Some alternative healing practitioners use Neem herb to treat swelling joints, gout, back pain and even gonorrhea and syphilis. Today the scientific research validates the traditional uses of Neem in the maintenance of general health. In addition, it has been used historically to treat bacterial, fungal, viral infections and to boost the immune system of human body. Another big plus for Neem products is their environmental friendliness. The tree grows on marginal soils, and every part of Neem tree can be used.

1.7.4. Other uses:

People in India use its twigs to brush their teeth. Neem is considered as the useful tree in rehabilitating the waste land areas. Neem seed pulp is useful for methane gas production. It is also useful as carbohydrate which is rich base for other industrial fermentations. Neem bark contains tannins which are used in tanning and dyeing. In south India its wood is used to make the furniture. The bark of the tree yields the fiber that is woven into ropes. Neem cake is widely used in India as fertilizer for sugarcane, vegetable and other cash crops. Many countries have been consistently growing the
Neem tree against the global warming. The worldwide Neem Foundation has helped in making the people aware about the importance of Neem and its uses globally\textsuperscript{25}.

\textbf{1.8Objective:}

- To extract volatile oil from Neem fruits (\textit{Azadirchta Indica}).
- To study the chemical properties of extracted volatile oil.
- To preparing a skin cream from Neem oil at different concentrations.
- To test the effectiveness of cream as a mosquito repellent.
CHAPTER TWO

Experimental
Material and method

2.1. Material:
2.1.1. Samples:
- Azadirachta indica seeds were donated by National Research Center - Khartoum.
- Gum Arabic is donated by the Natural Gums Research Center, Sudan University of Science and Technology.

2.1.2. Chemical
- Distilled water
- Ethanol (96%)
- Petroleum ether
- potassium iodide solution (0.1M)
- potassium hydroxide
- sodium thiosulphate (0.01M)
- phenolphthalein solution indicator
- hydrochloric acid (0.5)
- glacial acetic acid
- chloroform
- starch
- iodine mono chloride solution
- Wax
- Gum Arabic
- Color cresol
- Talc powder
- Vaseline
- Paraffin oil

2.1.3. Instruments:
- Soxhlet apparatus.
- Water bath
2.2 Methods

2.2.1 Extraction of oil:
83.5g of Azadirachta indica seeds kernel were removed coarsely powdered using mortar and pestle and extracted with petroleum ether using Soxhlet extraction for eight hours. The extract was then filtered through filter paper and the solvent was evaporated under reduced pressure using a rotary evaporator 26.

2.2.2 Physiochemical properties:
Physiochemical properties were done according the British Pharmacopoeia (2007):

2.2.2.1 Acid Value
2.637 g of Oil were dissolved in 50 ml of a mixture of equal volumes of ethanol (96 per cent) and light petroleum which were previously neutralized with 0.1 M potassium hydroxide, 0.5 ml of phenolphthalein solution used as indicator ,then it was titrated with 0.1 M sodium hydroxide until the pink colour persists (1 ml of titrant).

2.2.2.2 Saponification value
35g of potassium hydroxide were dissolved in 20 ml of water sufficient ethanol (96%) and added to produce 1000 ml.

2.026 g of Oil were weighted into a 200-ml flask, then 25.0 ml of the ethanolic solution of potassium hydroxide were added and boiled under a reflux condenser for 1 hour, the contents were frequently rotated. While the solution is still hot, the excess of alkali was titrated with 0.5M hydrochloric acid using 1 ml of phenolphthalein solution R1 as indicator .the operation was repeated without the Oil.

2.2.2.3 Peroxide value:
5.022 g of Oil were placed in a 250 ml conical flask fitted with a ground-glass stopper. 30 ml of a mixture of 2 volumes of chloroform and 3 volumes of glacial acetic acid were added and Shaken, then 0.5 ml of saturated potassium iodide solution were added and Shaken for exactly 1 min, 30 ml of water were added and Titrated with 0.01 M sodium thiosulphate, the titrate were added slowly with continuous vigorous shaking, until the yellow colour is almost discharged. Then 5 ml of starch
solution were added and the titration continued, shaken vigorously, until the colour is discharged. A blank test was carried out under the same conditions. The volume of 0.01 M sodium thiosulphate used in the blank titration must not exceed 0.1 ml.

2.2.2.4 Iodine Number:

0.268 g of Oil were dissolved in 10 ml of dichloromethane in a dry iodine flask. 20 ml of iodine monochloride solution were added; the stopper was inserted, previously moistened with dilute potassium iodide solution, and left in the dark for 30 minutes. 15 ml of dilute potassium iodide solution were added in the top cup, the stopper was carefully removed, the stopper were rinsed and the sides of the flask with 100 ml of water, shaken and titrated with 0.1M sodium thiosulphate using starch mucilage, which was added towards the end of the titration, as indicator. At the same time the operation was carried out in exactly the same manner, but without the Oil.

2.2.3 Cream preparation:
10g of Paraffin oil, 30g of Vaseline and 50g of wax were accurately, weighted and heated in water bath at 75°C. To 70g from the above mixture (placed in the water bath at 75°C) 2g of talc powder and 1g of cresol (dissolved in 10 ml of distilled water) were added to form 83g of the base cream. The emulsion (oil in water) is prepared by adding 5ml and 10ml of oil extract to 95 and 90ml of water (containing 3g of Gum Arabic) respectively. The base cream is divided into three lots of 27.66g one lot is used as control. To the second and third lots 20ml of 5 and 10% (oil in water) emulsion were added, making a total weight roughly 50g of cream gives an equivalent concentration of 2 and 4% of oil in cream respectively.

2.2.4 Analysis of cream
The cream was analyzed by SSMO, and some tests has been carried out; (colour, smell, shape, pH, water content, adipose content).

2.2.5 Effectiveness test for Neem Oil
Sample of skin cream was prepared as carrier for Neem oil with concentration of (2%, 4%), three cages with equal dimensions was prepared and has been placed at the same conditions; temperature, light percentage, and then a test has been carried out to Anopheles arabiensis which aged (2-5) days, 15 of which has been locked on the cages, and volunteers hands has been painted with the cream.
and then penetrated into the cages for 5 minutes each hour and then continued for 6 hours, same practice has been repeated three times for every concentration.

2.2.6 Calculation

The percentage of landing =

\[
\text{The percentage of landing} = \frac{\text{Total number of landing for control} - \text{Total number of landing for treatment}}{\text{Total number of landing for control}} \times 100
\]

The percentage of biting =

\[
\text{The percentage of biting} = \frac{\text{Total number of biting for control} - \text{Total number of biting for treatment}}{\text{Total number of biting for control}} \times 100
\]
CHAPTER THREE

Result and Discussion
RESULTS AND DISCUSSIONS

3.1 Result

Table 3.1: Extraction Oil yield

<table>
<thead>
<tr>
<th>Weight of sample</th>
<th>Weight of oil</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>83.5g</td>
<td>30.969g</td>
<td>37.08%</td>
</tr>
</tbody>
</table>

Table 3.2: Physiochemical properties of extracted oil

<table>
<thead>
<tr>
<th>Physiochemical properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid value</td>
<td>2.127</td>
</tr>
<tr>
<td>Saponification value</td>
<td>138.45</td>
</tr>
<tr>
<td>Peroxide value</td>
<td>0.00</td>
</tr>
<tr>
<td>Iodine number</td>
<td>47.35</td>
</tr>
</tbody>
</table>

Table 3.3: Result of cream analysis:

<table>
<thead>
<tr>
<th>Properties</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>White</td>
</tr>
<tr>
<td>Shape</td>
<td>Emulsion homogeneous textures</td>
</tr>
<tr>
<td>Smell</td>
<td>Acceptable</td>
</tr>
<tr>
<td>pH</td>
<td>9-5</td>
</tr>
<tr>
<td>Water content</td>
<td>53.9%</td>
</tr>
<tr>
<td>Adipose content</td>
<td>57.05%</td>
</tr>
</tbody>
</table>
### Table 3.4: variation of concentration with number of landing and biting mosquitoes

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment (2%)</th>
<th></th>
<th>Treatment (4%)</th>
<th></th>
<th>Control</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Landing</td>
<td>Biting</td>
<td>Landing</td>
<td>Biting</td>
<td>Landing</td>
<td>Biting</td>
</tr>
<tr>
<td>0 hr.</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1 hr</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>2 hr</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>3 hr</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>4 hr</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>8</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>5 hr</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>6 hr</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>22</td>
<td>22</td>
<td>45</td>
<td>62</td>
<td>49</td>
</tr>
</tbody>
</table>

### Table 3.5: percentage of concentration of effect as mosquitoes repellent

<table>
<thead>
<tr>
<th>Concentration</th>
<th></th>
<th>Percentage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Landing</td>
<td>Biting</td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>36.48%</td>
<td>55.10%</td>
<td></td>
</tr>
<tr>
<td>4%</td>
<td>64.51%</td>
<td>8.16%</td>
<td></td>
</tr>
</tbody>
</table>
3.2 Discussions:

From the practical results obtained, we figure out the below:

The obtained percentage of the extracted volatile oil from Neem tree (*Azadirachta indica*) is (37.08%) which existed within the range defined on scientific scholars. The acid value which practically obtained (2.127) may indicate for non partial decomposition for oil component, and it’s in range of acid value for some volatile oils for example (Mentha Piperita) with acidity that falls in range of (1.9-2.9). Neem oil does not contain peroxide, the Iodine Number of Neem oil is (47.35) which indicates high numbers of unsaturated double bonds and Saponification value of Neem oil (138.45) which indicates a high content of fatty acid the comparison to some other volatile oils. pH value for prepared cream is (4.82) falls within the Sudanese Standards and Metrology Organization (SSMO) standard of (9-5), the Water content of the prepared cream is (53.9%) which falls within SSMO standard with maximum value of (85%), Adipose content for prepared cream is (57.05%) which falls within SSMO standard with minimum value of (15%). The Colour of the prepared cream is white and the shape of Emulsion homogeneous textures, acceptable smell and these physical properties in compliance with SSMO standard. The cream which was prepared with concentration of (2%) from extracted Neem oil produced effectiveness percentage of repelling (35.48%) and the cream which was prepared with concentration of (4%) from extracted Neem oil produced effectiveness percentage of repelling (64.51%).
3.3 Conclusion:

- Neem seed oil was extracted and used as an emulsion (oil/water) in gum Arabic.
- The emulsion is added to the base cream prepared to obtain a mosquito repellent at 2 and 4% concentration of oil in base cream. The cream 4% oil concentration is significantly effective in repelling *Anopheles arabiensis* mosquitoes.
3.4 Recommendations:

- Active ingredient of Neem oil has to be isolated.
- It suggested that each ingredient can be tested on its own as a mosquito repellent is to establish which component as the most effective repellent.
- Safety and stability studies recommended to determine shelf life and safety of use.
CHAPTER FOUR

References
References
