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# Comparative Analysis of Methanol, Acetone, Ethanol and N-hexane Used in the Extraction of Oil from Neem Seeds (Response Surface Methodology)

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***Abstract-*** In the course of this project, a comparative analysis is carried out between four solvents used in the extraction of oil from neem seeds. The solvents are; ethanol, acetone, methanol and n-hexane. The main apparatus used is the soxhlet extractor, a laboratory apparatus specifically designed to extract lipids (oil) from a solid material. The neem seed was grounded in order to increase the surface area for oil collection. The neem seed powder was carefully waded in a thimble and put in the extraction chamber. The set up was allowed for a period of time using the first solvent and then when the extraction process was complete, the waded thimble was weighed to determine the amount of oil extracted and the other solvents were also used until the last one. At the end of the analysis, it was found that the solvent acetone yielded the highest oil as it extracted 5.44g (21.76%) of oil in 40mins. N-Hexane gave the next highest yield as it extracted 4.18g (16.72%) of oil and the extraction time was the shortest at 26mins. Using methanol as solvent gave an oil yield of 3.93g (15.72%) and the time of extraction was the longest as it peaked 58mins. Ethanol gave the lowest oil yield of 3.34g (13.36%) and the extraction time was 50mins. This comparative analysis was carried out on the four solvents to ascertain the solvent that can give optimum yield of neem oil from neem seed, started in the month of April and ended in May, 2021, In the Laboratory of chemical engineering department, school of engineering technology, federal university of technology Owerri imo state, Nigeria. From the results, acetone is best suited for the extraction of oil from neem seeds as more oil yield was recorded.

***Keywords-*** *Acetone Solvent, Neem Seed Oil Yield, Soxhlet Extraction of Neem Oil, N-Hexane Solvent Extraction*

## I. INTRODUCTION

From time immemorial, man depended on plants as medicine. From a historical perspective, it is evident that the fascination for plants is as old as mankind itself. The plant kingdom represents a rich store house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years. Plants are the richest source of drugs for traditional medicine, modern medicines, nutraceuticals food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999). The use of plant product as medicines could be traced as far back as the beginning of human civilization. The earliest mentioned medicinal used plant in Hindu culture is found in “Rigveda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository human knowledge. The active principle isolated, have provided leads in the development of several lifesaving drugs, which are in use today (Rastogi and Mehrotra, 2002).

The isolated active compounds of the plants are secondary metabolites chemical compound that occur naturally in plant with no nutritional value to human life. These active compounds are generally called phytochemicals. These phytochemicals play protective roles in plants, each chemical labelled phytochemical works in different ways, not all are the same for human, and not all come from the same plants. Some have shown more promise than others in fighting disease and illness in humans. There are some basic types of these active compounds that are found in different fruits and vegetables. We have some of them like antioxidants, they are present in onions and some other fruits and tea, they act as preventive measure for premature cell death and some forms of cancer and aging. Isoflavones or plant oestrogen; they are found in soy and soy products; they are helpful in the year just before and after menopause. Capsaicin is found in hot pepper and it has been shown to significantly reduce prostate tumours in size, at least in mice. Taking capsaicin on a regular basis by eating spicy foods with hot peppers may prove an excellent preventative agent to prostate cancer and benign growth of prostate. This experiment was carried out on AzadirachtaIndica leaves and seeds.

The Neem plant(AZADIRACHTA INDICA)popularly known as "Dogoyaro" in most parts of Nigeria, is a versatile tree in the mahogany family(Meliaceae)which is mainly found in India. The Neem plant is well known as one of the most versatile medicinal plants having a wide spectrum of biological activity. AzadirachtaIndica is a very useful traditional medicinal plant in the sub-continent and each part of the tree has some medicinal properties. The plant is native to Asia, but has now naturalized in West Africa and is widely cultivated in

Nigeria as an ornamental as well as medicinal plant. The neem tree has a straight stem and the tree can reach a height of about 30 metres and more, though 15 to 20 metres is the average. The neem tree is noted for its drought resistance. It is evergreen, but in severe drought it may shed most or nearly all of its leaves. It has white and fragrant flowers. The fruit is smooth and varies in shape from elongate oval to nearly roundish. The fruit skin is thin and the bitter-sweet pulp (mesocarp) is yellowish-white and very fibrous. The white, hard inner shell (endocarp) of the fruit encloses one, and sometimes two or three, elongated seeds (kernels) having a brown seed coat. Neem can grow in many different types of soil, but it thrives best on well drained deep and sandy soils and grows rapidly in the tropic and semi-tropic climate countries including Nigeria. It is also observed that this tree could survive in very dry and arid condition.

Neem oil is a vegetable oil pressed from the fruits and seeds of the neem. It is the most important of the commercially available products of neem for organic farming and medicines. Neem oil is generally red as blood, and has a rather strong odour that is said to combine the odours of peanut and garlic. It is composed mainly of triglycerides and contains many triterpenoid compounds, which are responsible for the bitter taste. It is hydrophobic in nature and in order to emulsify it in water for application purposes, it must be formulated with appropriate surfactants. The neem oil is used in India to manufacture soaps. Neem cake is widely used as animal feed and as fertilizer for cash crops, particularly sugarcane and vegetables.

Clinical studies with the dried neem leaf extract indicated its effectiveness to cure ringworm, eczema and scabies. Lotion derived from neem leaf, when locally applied, can cure these dermatological diseases within 3–4 days in acute stage or a fortnight in chronic case. Neem leaves are dried in India and placed in cupboards to prevent insects from eating the clothes. The leaves are boiled and the decoction taken frequently to treat malaria and high fever.

The vast uses of neem can be attributed to the fact that they contain biologically active substances including Flavonoids, Alkaloids, Tannis, Saponins and Steroids, which are collectively known as Phytochemicals. These organic chemicals are stored in matured cells of the various organs such as roots, stems, leaves, fruits and seeds. This invariably means that every organ of the Neem tree possesses a certain level of healing power, due to the phytochemicals distributed amongst them in different proportions. To the plants, phytochemicals act as natural defence system and are partly responsible for aroma, colour and flavour. These compounds are also responsible for the diverse activities of neem ranging from its insect repellent properties to its inhibitory effect on the growth of various pathogens such including bacteria, fungi and viruses.

Phytochemicals are non-nutritive plant chemicals which occur naturally in plants that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect them but recent research demonstrates that they can also protect humans against diseases. There are more than thousand known phytochemicals. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavonesin soy and flavonoids in fruits. Alkaloids (examples are Caffeine, Theobromine, Theophylline). Organ sulfides (examples are Allicin, Glutathione, Indole-3-Carbinol, Isothiocyanates). Tannins, steroids, Glycosides etc...

The activity of phytochemicals includes:

(i) Antioxidant - Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals with antioxidant activity includes: allylsulfides (onions, leeks, and garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes).

(ii) Hormonal action - Isoflavones, found in soy, imitate human oestrogens and help to reduce menopausal symptoms and osteoporosis. Stimulation of enzymes - Indoles, which are found in cabbages, stimulates enzymes that make the oestrogen less effective and thus could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries).

(iii) Interference with DNA replication - Saponins found in beans interfere with the replication of DNA cell, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens.

(iv) Physical action - Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanins are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health.

Phytochemicals are naturally present in many foods but it is expected that through bioengineering new plants will be developed, which will contain higher levels. This would make it easier to incorporate enough phytochemicals with our food.

Extraction with a good non-polar solvent extracts a good portion of the composition of the natural products. Separation of extracts and solvent will result in a partial loss of very volatile compounds (Anyikwa et al, 2019)

Neem contains several active ingredients and they act in different ways under different circumstance. These compounds bear no resemblances to the chemicals in today's synthetic insecticides, chemically, they are distant relative of steer birth control pill and many valuable pharmaceuticals containing only compound of carbon, hydrogen and oxygen, they have no atoms of phosphorous, sulphur or nitrogen (such as one commonly found in synthetic pesticides) their mode of action is thus, quite different (Ruskin, 1992).

Neem products are unique in that (at least for most insects) they are not upright killers instead, they alter an insect behavior or life processes in ways that can be extremely sublet. However, the insect can no longer feed or breed or metamorphosis, and can cause no further damage (Schmutterer, 1995).

The growing accumulation of experience demonstrates that neem products work by intervening at several stages of an insect's life. The ingredients from this tree appropriate the shape and structure of hormones vital to the lives of insects (not to mention some other invertebrates and even some microbes). The bodies of these insects absorb the neem compounds as blocks their endocrine systems. The resulting deep seated behavioral and physiological aberration has the insects so confused on brain and body that they cannot reproduce and their population plummet (Schmutterer, 1995).

The precise effects of the various neem tree extracts on a given insect's species are often difficult to pin point. Neems complexity of ingredients and its mined modes of action vastly complicate classification. Moreover, the studies to date are hard to compare because they have used different test insects' formulation (NRC, 1992: "Neem: A tree for solving global problems")

In addition, the material used in various test have often been studied and stored differently taken from differing parts of the tree or produced under different environmental conditions.

But, for all the uncertainly over details, various neem extracts are known to act on various insects in the following ways:

(i) Disrupting or inhibiting the development of eggs, larvae or pupa.

(ii) Disrupting muting and sexual communication

## II. Chemical composition of Neem 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 (Kumar *et al* 2010). The neem constituent belonging to chemically diverse classes have been divided into two major sections:

a) Isoprenoids

b) Non-Isoprenoids.

The latter category comprises glycerides, polysaccharides, sulphurones compounds, flavonoids and their glycosides, amino acids, aliphatic compounds etc.

### *A. Isoprenoids*

#### *1) 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.

#### *2) Triterpenoids*

The bitterness of neem is due to the occurrence of limonoids which are the tetranortripenoids based on apo-euphalskeleton. The term limonoid is derived from limonin, the first tetranortripenoids 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 (Meliaazedarach) 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, salaninandazadirachtin.

#### *3) Protomeliacins*

The triterpenes containing C8 side chains C-17 are supposed to be biogenetic 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 protomeliacinsnimbocinone, nimolinone, and kulactone, etc.

Nimbocinone has been isolated from Neem leaves while most of the other constituents from fruit coats and whole fruits.

#### *4) Limonoids with intact four rings and Û-hydroxybutenolide side chain*

The presence of a Û-hydroxybutenolide side chain in place of the furan ring is the characteristics of this group of compounds. Two isomeric constituents, nimocinolides, isonimocinolide have been isolated from Neem leaves whereas isonimolicinolide from fresh fruits. Nimocinolides showed mild insect growth regulating properties.

#### *5) 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 possess both anti-fungal and anti-malarial property.

#### *6) Azadironeand itsnaturalanalogues*

This group consists of limonoidsin which all rings of the triterpenoid skeleton remain intact. Characteristics features of this group are presence of oxygen function at $C_3$ and $C_7$. Figure 1 below shows the structure of azadirahtin.

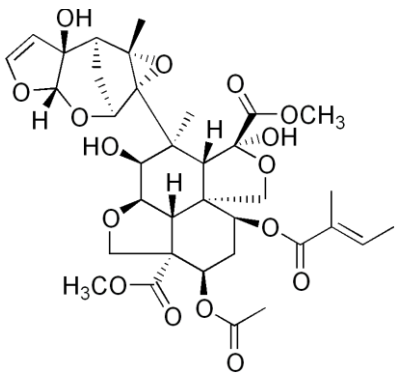


Figure 1. AzadirachtinA

TABLE I. PHYSICAL PROPERTIES OF AZADIRACHTIN

| | |
|---|---|
| Chemical name | AzadirachtinA |
| Chemical class/Use | Tetranortriterpenoid/Insect growth inhibitor) |
| Specific gravity | 1.066 |
| Solubility in water | 0.00005 |
| Boiling point | 78-212degreesF |
| Flashpoint | >145 F (Azatin);55.6degreesF (Margosan) |
| Vapor pressure | >2mmHgat25°C(Azatin);44mmat20°C (Margosan) |

*7) C-Secomeliacins*

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

## III. MATERIALS AND METHODS

### A. Materials

The equipment used in this research work includes:

*a) Weighing Balance:*

This is a device that is used to mass or weight of an object.

*b) Beakers*

A beaker is a common container in most laboratories that is used for stirring, mixing and heating chemicals. Most beakers have spouts on their rims to aid in pouring. They also commonly have lips around their rims and markings to measure the volume they contain, although they are not a precise way to measure liquids.

*c) Conical Flask*

A conical flask is a glass laboratory flask of a conical profile with a narrow tubular neck and a flat bottom used in chemistry laboratories for experiments that involve collecting and measuring liquids, mixing solutions and cultivating media. The design and construction of the flat-bottomed flask allows it to stand alone on the lab bench.

*d) Measuring Cylinder*

This is a piece of laboratory glassware used to measure the volume of liquids. It is used to accurately measure the volume of chemicals for use in reactions.

*e) Cork/Stopper*

This is a piece of buoyant material used in laboratories because of its impermeability and general absence of toxic elements. It is partially inserted inside a container to form a seal.

*f) Soxhlet Extractor*

A soxhlet extractor is a piece of laboratory equipment that is designed for the extraction of a lipid (oil) from a solid material. Soxhlet extraction is used when the desired compound has a limited solubility in a solvent and the impurity is insoluble in that solvent. It allows for a smooth and effective operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material.

*g) Volumetric Flask*

A volumetric flask (measuring flask or graduated flask) is a piece of laboratory apparatus, a type of laboratory flask, calibrated to contain a precise volume at a certain temperature. Volumetric flasks are used for precise dilutions and preparation of standard solutions. These flasks are usually pear-shaped, with a flat bottom, and made of glass or plastic.

*h) Retort Stand*

A retort stand, also called a clamp stand, a ring stand, or a support stand, is a piece of scientific equipment intended to support other pieces of equipment and glassware — for instance, burettes, test tubes and flasks.

*i) Test Tube*

A test tube, also known as a culture tube or sample tube, is a common piece of laboratory glassware consisting of a finger-like length of glass or clear plastic tubing, open at the top and closed at the bottom. Test tubes are widely used by chemists to handle chemicals, especially for qualitative experiments and assays.

*j) Grinder*

A grinder performs the function of grinding or homogenizing rigid, soft, wet, dry, flexible, fragile and fibrous materials.

*k) Heating Mantle*

The heating mantle is an auxiliary device which is used in laboratories. The heating mantle helps to heat or control the temperature of different samples or chemicals in the laboratory. The device has a crocheted net inside which, in which you can hang the round bottom flask inside to heat it.

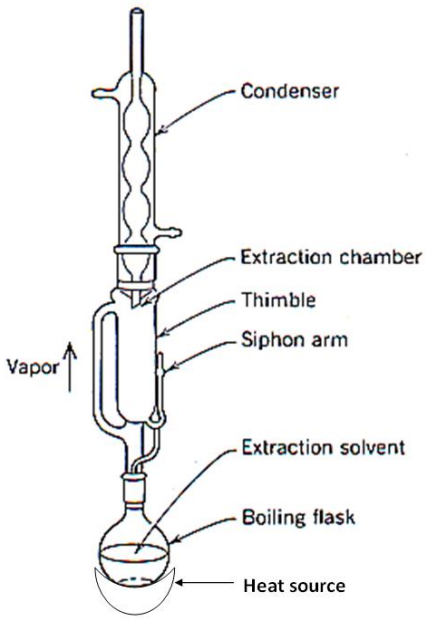


Figure 2. Soxhlet Extractor

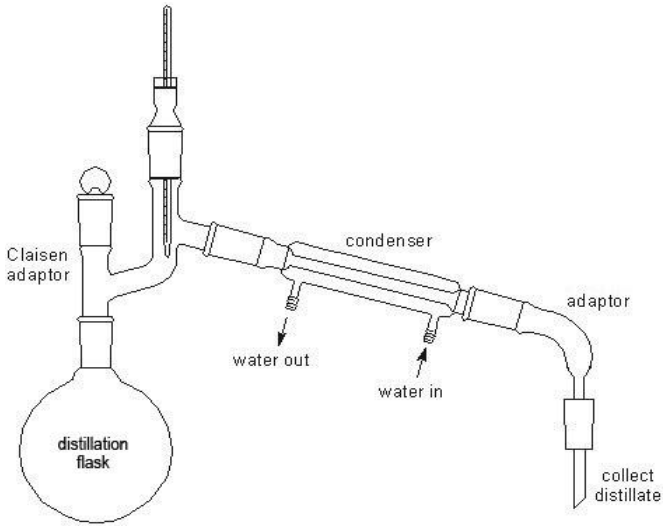


Figure 3. Distillation Apparatus Setup

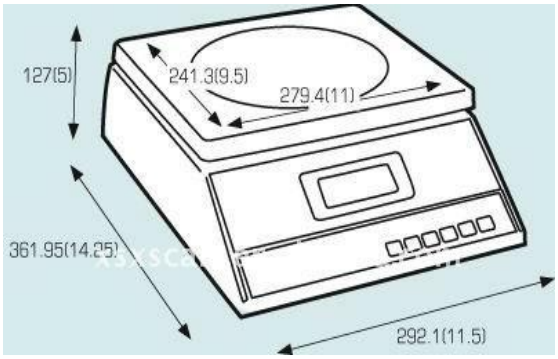


Figure 4. Weighing Balance

*1) Reagents*

The reagents used are shown in Table 2.

TABLE II. LIST OF MATERIALS USED AND QUANTITY

| Materials | Quantity |
|---|---|
| Neem Seed | 25g |
| Ethanol | 250ml |
| n-Hexane | 250ml |
| Methanol | 250ml |
| Acetone | 250ml |

## B. Method

*1) Experimental procedure*

The process involved in the Extraction of Oil is presented in Figure 5.

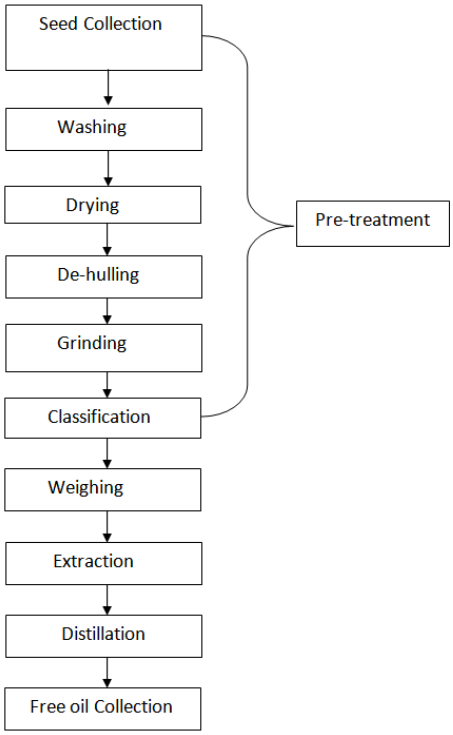


Figure 5. Flowchart of Neem Oil Extraction

*2) Pretreatment process*

Before the real extraction, some preparations have to be made on the neem to be used. The preparations will make the neem seed suitable for the extraction.

- Seed collection
- Drying
- De-hulling
- Size reduction
- Classification

*a) Seed collection*

The fruits containing the seeds were picked from the ground and plugged from the tree and washed clean.

*b) Drying*

The washed seeds are then dried for some days to reduce the moisture content from the seed.

*c) De-hulling*

The shell of the dried seeds where then removed to release the embedded inside.

*d) Size reduction*

After the neem seed is property dried and de-hulled it is then grinded into powder using glass mortar and pestle.

*e) Classification*

The granted seeds were separated using tray to blow of the chaff.

*3) Experimental Procedure Using Soxhlet Extractor*

The dried neem seed was crushed in a grinder to reduce the size. This is because the sample with the smallest particle size range yields high quality of oil.

The weight of the flask and the thimble were noted. 25g of the sample was added into the thimble and weighed and recorded as $W_1$.The thimble was folded carefully and then inserted into the extractor.

250ml of the first solvent (methanol) was then poured into the extractor flask which gave 4/5 of the volume of the flask.

The soxhlet apparatus was then heated up using a heating mantle.

The solvent evaporates and then condenses into the extractor which is poured back in the flask, this process continued for a period of time.

After heating and cooling for the required time, the waded thimble containing the sample was brought out and the oil distilled off the solvent or evaporation of the solvent was allowed to occur while the oil was collected as extract and the solvent as raffinate.

The weight of thimble after extraction was measured and recorded as $W_2$. The different between the weight of the thimble before and after extractor gives the appropriate weight of the oil extracted.

Repeat the above steps using solvents: n-hexane, ethanol, and acetone.

The percentage of Oil Extracted is shown in equation 1:

$$\frac{\text{Weight of Oil Extracted}}{\text{Total Weight of Sample}} \times 100 = \%\ \text{Oil Extracted} \quad (1)$$

## IV. Presentation of Results and Discussion

### A. Results

The results obtained from the extraction of oil using methanol as solvent are shown in Table 3:

TABLE III. TABLE OF RESULTS FOR METHANOL SOLVENT

| Sample | Analysed mass (g) | Mass of neem seed powder + thimble (g) | | Mass of oil extracted (g) | Extraction Time (mins) |
|---|---|---|---|---|---|
| | | Before Extraction ($W_1$) | After Extraction ($W_2$) | | |
| Neem seed powder | 25 | 26.18 | 22.25 | 3.93 | 58 |

The results obtained from the extraction of oil using ethanol as solvent are shown in Table 4:

TABLE IV. TABLE OF RESULTS FOR ETHANOL SOLVENT

| Sample | Analysed mass (g) | Mass of neem seed powder + thimble (g) | | Mass of oil extracted (g) | Extraction Time (mins) |
|---|---|---|---|---|---|
| | | Before Extraction ($W_1$) | After Extraction ($W_2$) | | |
| Neem seed powder | 25 | 26.79 | 23.45 | 3.34 | 50 |

The results obtained from the extraction of oil using acetone as solvent are shown in Table 5:

TABLE V. TABLE OF RESULTS FOR ACETONE SOLVENT

| Sample | Analysed mass (g) | Mass of neem seed powder + thimble(g) | | Mass of oil extracted (g) | Extraction Time (mins) |
|---|---|---|---|---|---|
| | | Before Extraction (W1) | After Extraction (W2) | | |
| Neem seed powder | 25 | 25.94 | 20.50 | 5.44 | 40 |

The results obtained from the extraction of oil using n-hexane as solvent are shown in Table 6:

TABLE VI. TABLE OF RESULTS FOR N-HEXANE SOLVENT

| Sample | Analysed mass (g) | Mass of neem seed powder + thimble(g) | | Mass of oil extracted (g) | Extraction Time (mins) |
|---|---|---|---|---|---|
| | | Before Extraction (W1) | After Extraction (W2) | | |
| Neem seed powder | 25 | 27.13 | 22.95 | 4.18 | 26 |

Fig 6 and 7 show the graphical representation of the oil extracted and the time of extraction respectively.

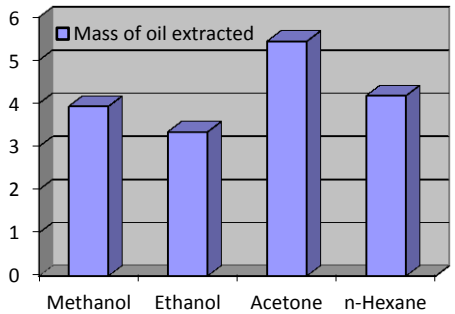


Figure 6. Chart showing the mass of oil extracted for each solvent

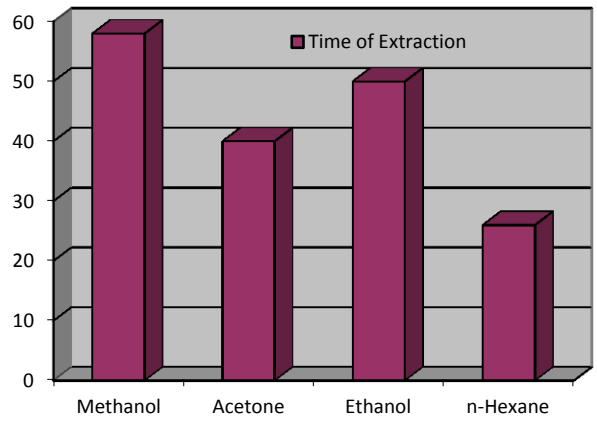


Figure 7. Chart showing the time of extraction for each solvent

### *B. Discussion*

From the results obtained in Tables 3-6, it can be ascertained that the solvent used for the soxhlet extraction process which gave the highest yield of oil was found to be acetone, which extracted 5.44g of neem oil. The percentage of oil extracted was calculated to be 21.76%. The solvent which gave the second highest yield of oil was found to be n-hexane, which extracted 4.18g of oil from the neem seed powder. Its percentage yield oil was found to be 16.72%. Methanol was found to give the penultimate yield of oil as it extracted 3.93g of oil with its corresponding percentage oil yield as 15 72%. And lastly, ethanol was the solvent that extracted the least oil from the neem seed powder, being 3.34g and the percentage oil yield is 13.36%.

A variation in of contact time or time of extraction was also observed between the four solvents during extraction. In descending order, methanol took the longest time, 58 mins, for the extraction process to come to completion, followed by ethanol which took a total of 50 mins. The process of extraction of oil from neem seed powder using acetone as solvent took a total of 40mins. N-Hexane was found to extract oil the fastest with a contact time of 26mins.

## V. CONCLUSION

In conclusion, the solvent extraction method was adopted in this project. A comparative analysis was carried out between 4 solvents in the extraction process to determine which solvent would give the highest yield of oil.

Based on the comparative analysis carried out and the experimental results obtained, unfortunately, a direct relationship cannot be established between contact time/time of extraction and the quantity of oil extracted from the neem seed powder. Hence, the quantity of oil extracted is solemnly based on the nature of the solvent used for the extraction process.

The mass of oil extracted and percentage oil yield for each solvent used are as follows; 5.44g and 21.76% for acetone, 4.18g and 16.72% for n-hexane, 3.93g and 15.72% for methanol and 3.34g and 13.36% for ethanol. The time of extraction in the same order is 40mins, 26mins, 58mins and 50mins respectively.

It can therefore be inferred that the best solvent to be used in extracting oil from neem seed based on this comparative analysis is acetone and the solvent which extracts oil the fastest is n-hexane.

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