PHYSICO-CHEMICAL PROPERTIES OF NEEM SEED KERNEL EXTRACT

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ABSTRACT

The extract from the seeds of Neem tree (Azadirachta indica) is known for its use by traditional medicine practitioners and its local use as insect pest repellent but little is known of its physical and chemical properties. This research studies the physical and chemical properties of Neem Seed Kernel Extract so as to verify the properties that enhance its suitability as traditional medicine and insect pest repellent. The NSKE was found to be better than synthetic insect pest repellent for its low toxicity, biodegradability, availability, stability, un-expiring characteristics, low production cost and ease of application. Chromatographic and spectrophotometric analysis revealed that NSKE contains four methyl esters with 11-14-Eicosadienoic acid being the most active in the vapour phase. These were found to produce phago-repellent effect. The oil content of 43.91 % shows that Neem Seed Kernel contains an appreciable quantity of oil and hence can be utilized for large – scale production. A conductivity value of 8.1×10^{-6} mscm $^{-1}$ shows that the oil is stable. A flash point of 260 ^{o}C show the extract is non flammable and hence can be burnt in lanterns. An iodine value of 65.80mg/100g shows that the oil is non-drying and capable of withstanding oxidative rancidity. Being non-drying, the oil is good for soap, lubricants and grease but not good for paints and varnishes. A peroxide value of 8.7 meq/kg Shows that the extract can withstand deterioration. A free fatty acid level of 5.39 % indicates that the extract is not prone to lipase action rancidity and hydrolytic action rancidity. A low acid value of 3.56 mg/g shows that the extract is not acidic and hence could not cause harm to the skin. This shows that NSKE can be used as cream. A Saponification Value of 198.26 mg/g shows that the extract can be used for production of soap. It is recommended that research be encouraged on the use of Neem seed Kernel Extract for the production of these products.

Keywords: *Physical property; Chemical Property; Spectrophotometric; Insect Pest Repellent; Neem Seed Kernel Extract; Physico-Chemical properties.*

1.0. Introduction

Sampson (1999) state that the extract from the seeds of neem tree (Azadirachta Indica) is known for its use by traditional medicine practitioners and its local use as mosquito repellent but little is known about its chemical properties. The aim of this research is to study the physical and chemical properties of the Neem Seed Kernel Extract (NSKE) whose sample is sometimes referred to as sample oil and identify the properties that make it suitable as mosquito repellent. Erakhrumen (2011) state that though Neem Seeds are readily available in Nigeria, most of the seeds produced by the Neem tree are currently underutilized. This research encourages large scale production of Neem Seed Kernel Extract. Stability studies by Aremu & Femi-Oyewo (2009) reveal that Neem oil cream formulations maintain physical and chemical integrity. Sampson (1999) state that besides stability, Neem Seed Kernel Extract has a high flash point (260 ^{o}C) and hence can be burnt in lanterns. Moreover, it does not easily get deteriorated as it possesses low conductivity, low Iodine number (non-drying oil), low Peroxide Value and low Percentage of Free Fatty Acid.

A low acid value show the extract can be rubbed as cream. A high Saponification Value show the oil can be utilized for production of Soap. In this Study, experiments are conducted to find the values of these parameters as well as the components in the Neem Seed Kernel Extract.

2.0. Materials and Methods

2.1. Materials and Equipment used

Equipment and materials used for this study were: Soxhlet extractor for extracting oil from grounded neem seed kernels; WPA CM 35 conductivity meter; Model F Lovibond tintometer; No. 18 capillary tube viscometer; Jenway 3015 pH meter; Schimadzu GC-MS equipment; Ati Matson infra red spectrometer; Abbe refractometer; Schimadzu 160 A U.V spectrophotometer; Abbels closed cup apparatus; NMR spectrophotometer; Reagents; hexane, to mention just a few of them.

2.2. Measurement of Physical Properties

2.2.1. Colour

The extract was put into a one-inch glass cell to almost full the cell and placed in the liquid compartment of a Lovibond tintometer. The colour filters were adjusted till the colour of the two halves from the viewing glass matched with each other. The colour reading was taken from the calibration of the colour filters on a decimal scale for three primary colours: Red, Yellow and Blue.

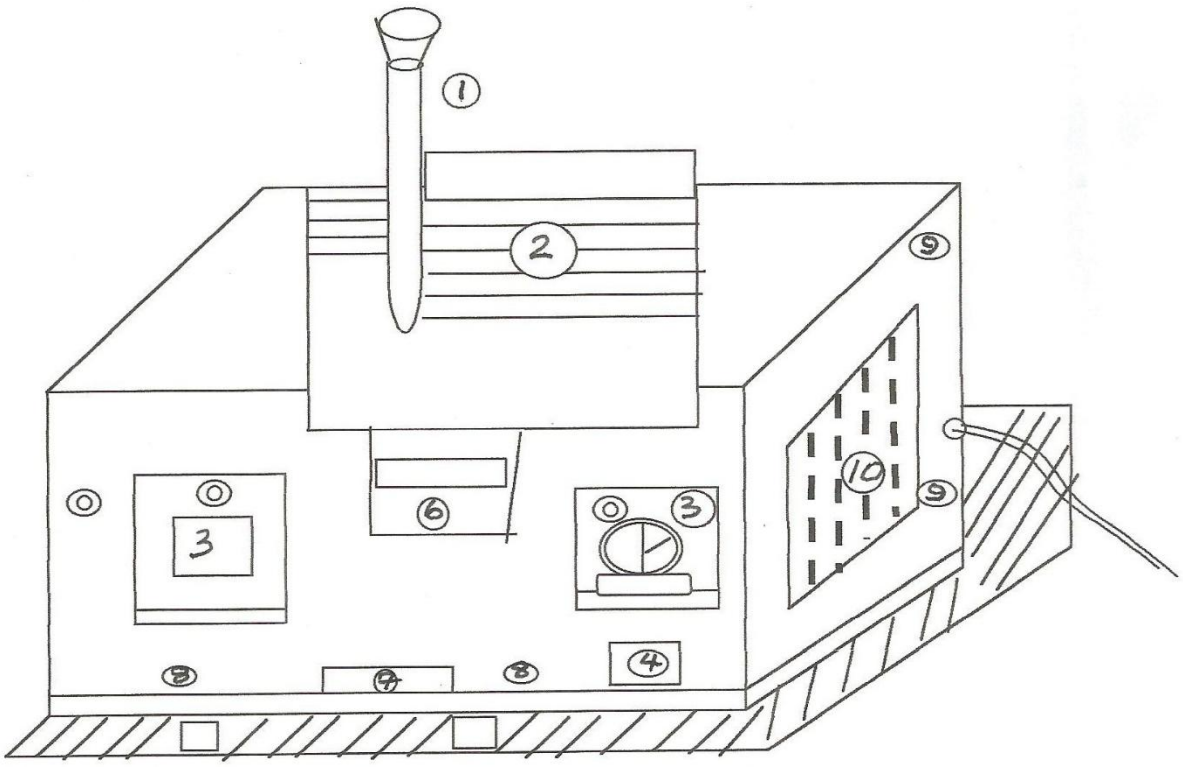


Fig.1: Lovibond Tintometer (model F)

1. Viewing tube
2. Glass colour filters
3. Lamp housing and vents
4. House lapse meter
5. Diffusing Screen (Hidden optical system)
6. Liquid Sample Compartment
7. Solid sample position
8. Detachable leg retaining screws
9. Brightness control unit

10. Brightness scale (visual density)

The tintometer work by the principle of subtractive colour mixing. The colour of the oil is due to pigments in the oil and not reflection of colours of light. Mixing of colours of light is termed additive colour mixing. Light reflect one colour and absorb all other colours but pigments reflect some colours and absorb some colours. In both cases, the colour reflected is what is seen but for oils it is only a combination of colours that can be seen, that is why the three components Red Yellow and Blue are read together.

2.2.2. Odour

The extract was smelled with the nostrils and the odour assessed by sensory evaluation.

2.2.3. Taste

A little of the extract was rubbed on the tongue and the taste assessed by sensory evaluation.

2.2.4. Conductivity and Resistivity

The sensor of a conductivity meter (WPA CM 35) was cleaned using distilled water and dried. The sensor was then dipped into a sample of the extract (sample oil) and the conductivity in Siemen per centimetre read by the help of a pointer.

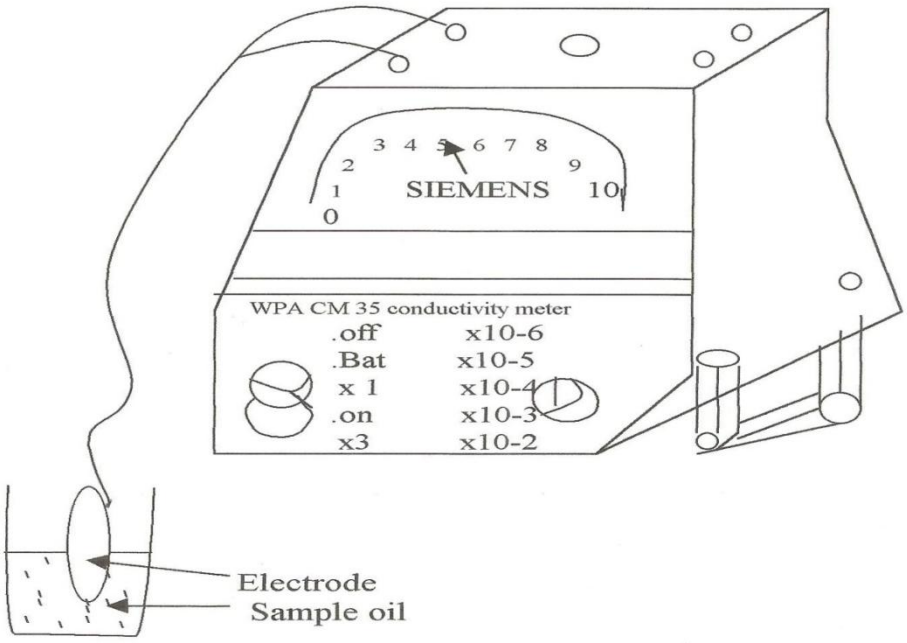


Fig.2: WPA CM 35 Conductivity Meter

The unit Siemen per cm was converted to micro ohm per centimetre.

The reciprocal of the conductivity gave the resistivity of the extract.

A liquid or material is said to be conductive if it can conduct heat and electricity. It is desired that the extract should be of low conductivity to avoid alteration in handling and processing parameters such as temperature, flow rate, and viscosity, which could affect process and quality control. The presence of impurities increases the conductivity of the extract, so a measure of the conductivity predicts the purity of the extracted oil.

2.2.5. Viscosity

A capillary tube viscometer No. 18 was filled with a liquid of known viscosity e.g. water, and sucked from the reservoir bulb to the measuring bulb. A stop watch was used to measure the time (Q) between the fiducial marks, the liquid took to drop through the constriction of the capillary tube. The density of water ρ was measured by taking the mass and dividing it by the volume of the same mass of water. The viscosity of water (μ) was obtained from Douglas (1990). Using the formula $\mu=K\rho Q$, the constant for the viscometer $K=\mu/\rho Q$ was calculated. The density (ρ) and efflux time (Q) for the extract were then measured after drying the viscometer. Since K, had already been calculated, the viscosity of the extract was obtained. From the formula :

$$\mu = K\rho Q \qquad (1)$$

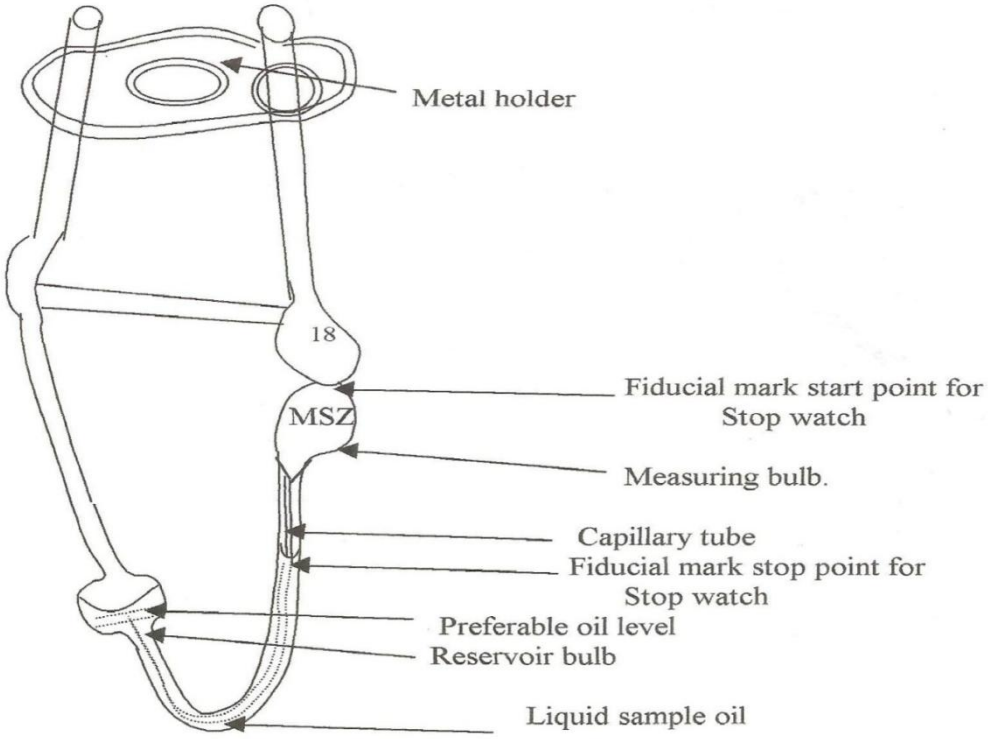


Fig.3: No. 18 Capillary tube viscometer

The operation of a viscometer depends on the principle that the time required for a definite volume of liquid to be discharged when made to flow through a capillary tube under a given pressure depends on the viscosity of the liquid. A

liquid is said to behave Newtonian if it maintains a constant viscosity at a constant temperature and non-Newtonian if vice versa. The ratio of viscosity to density is called kinematic viscosity. To achieve correct results:

i. The liquid must be pure and free from contamination, which could block the tube.

ii. Bubbles must not be present in the liquid as this can affect the efflux time.

iii. Flow through the capillary tube must be laminar.

2.2.6. Refractive Index

0.5 g of the sample oil was poured into a beaker and covered. The beaker containing the sample was put into a refrigerator to reduce the temperature to 20 ^{0}C. Two drops of the sample was taken on a glass rod and placed on the main prism of an Abbe refractometer. The supplementary prism was gently closed to come in contact with the main prism and the visual field viewed through the eyepiece near the door of the laboratory which was opened so that light can come in. The refractive index was read from a scale in the visual field of the refractometer.

The visual field is made up of scale ranges:

"1' 1.333 to 1.404 on left side

"2" 1.404 to 1.464 on centre

"3" 1.468 to 1.520 on right side

On a scale division = 0.001

A scale range selector was adjusted to locate the setting position, which gives bright and dark sides. A boundary line, which separates brighter and darker sides at the upper and lower portions, appeared in the field of vision.

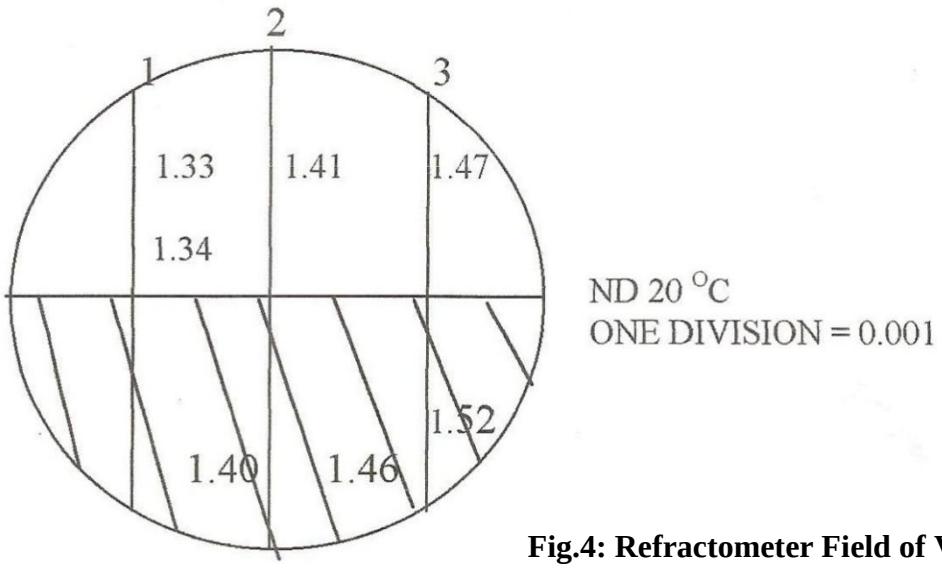


Fig.4: Refractometer Field of Vision

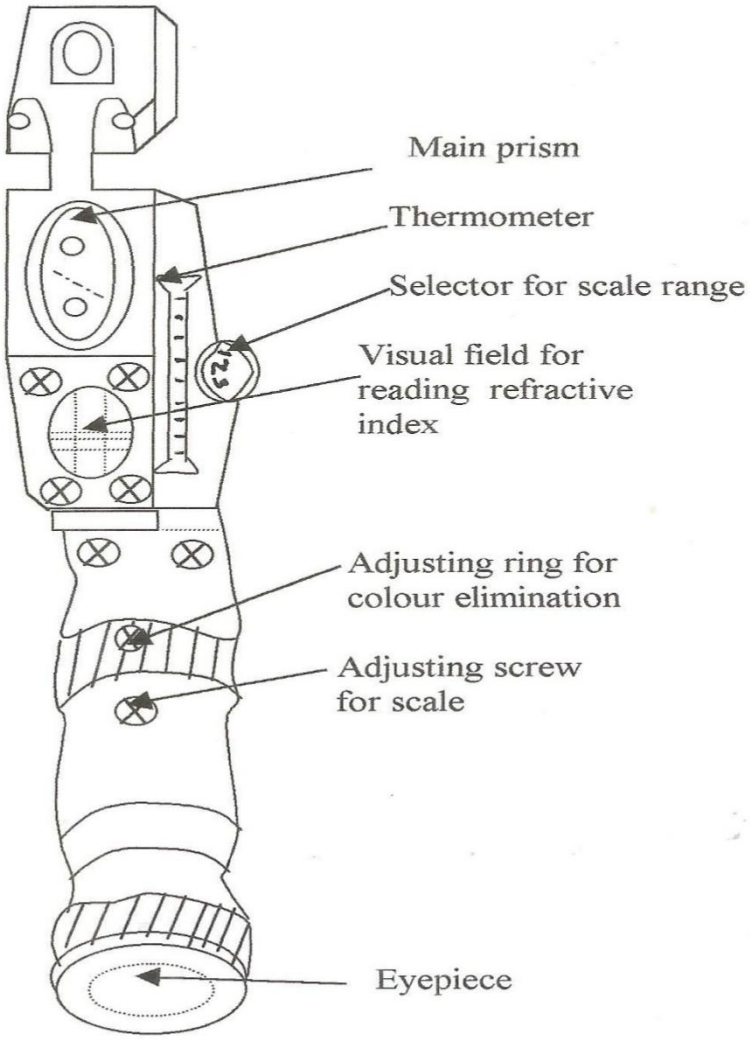


Fig.5: Abbe Refractometer.

The calibration, which is shown by the boundary line, indicates the refractive index. Refractive index, the ratio of sine of angle of incidence to sine of angle of refraction is constant for each type of oil. It can therefore be used for identification or investigation of the purity of the oil. To achieve correct results:

i. The refractometer end should be pointed in the direction of bright light.

ii. Eyepiece should be rotated while peeping through it until it is correctly adjusted and the scale becomes clearly visible.

iii. If boundary line is coloured or unclear, the boundary line should be made clear by rotating the adjusting ring for colour elimination.

2.2.7. pH Value

The electrode of a Jenway 3015 pH meter was cleaned using distilled water and dried. The meter was switched on and the electrode inserted into a buffer solution

i.e. a solution whose pH is known to be 7.00, prepared by adding a weak acid to its salt.

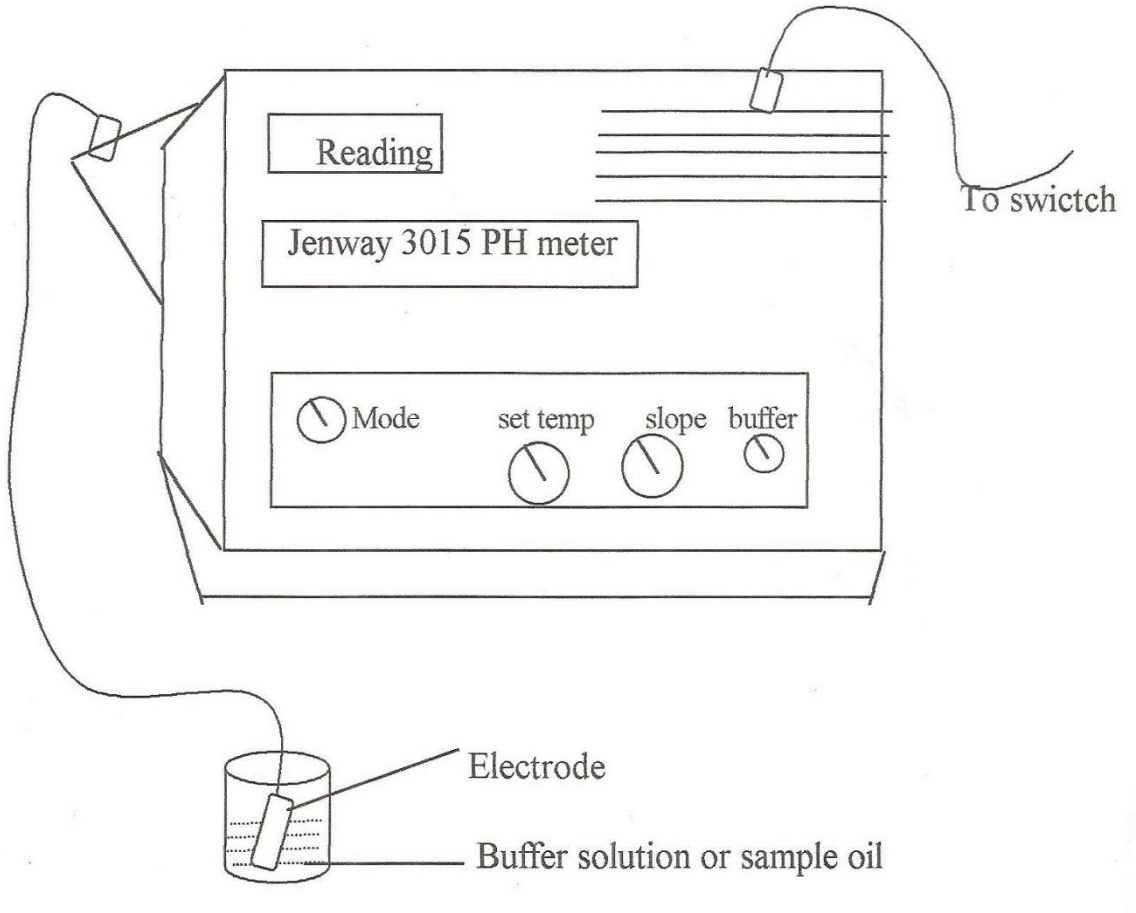


Fig.6: Jenway 3015 pH meter.

Adjustment was made till the pH reading for the buffer solution was at exactly 7.0. The electrode was then removed and inserted into a little of the sample oil in a beaker. The readings pulsated till it came to a constant value. This constant reading was taken as the pH of the sample oil.

2.2.8. Physical Stability

The physical stability of the oil was measured by exposing a sample of the oil to different extremes of temperature e.g. 100 ^{o}C, 50 ^{o}C and 0 ^{o}C. This was done to see whether the oil could still retain its physical properties under different ranges of temperature. The sample oil was also subjected to vibration and also to compression to see whether it is physically stable.

2.3. Measurement of Chemical Properties

2.3.1. Iodine Value

To 5 mls of 1 % sample oil in chloroform 5 mls Dams Iodine was added. The mixture was corked and placed in a dark cupboard for 5 minutes.

5 mls of 10 % potassium iodide solution was added followed by 20 mls of distilled water. The solution was thoroughly mixed after which it was titrated against 0.025 N standard sodium thiosulphate using freshly prepared 1 % starch solution added towards the end of the titration as indicator. End point was detected by colour change from blue to colourless. The same procedure was repeated for the blank with 5 mls chloroform ($CHCl_3$) instead of 1 % sample oil in chloroform. Iodine value was calculated using equation (2)

$$Iodine\ Value = 0.003175\ (B - S) \times 100 \qquad (2)$$

$where\ B\ is\ the\ titre\ for\ the\ blank\ and\ S\ is\ the\ titre\ for\ the\ test.$

1 ml 0.025 N $Na_2S_2O_3$ = 0.003175 g iodine

2.3.2. Acid Value

10 g of the sample oil was added to 2 mls of neutral ethyl alcohol, the mixture was gently heated until it began to boil. It was then titrated with 0.1 N KOH while still hot using 2 drops of phenolthalein indicator. The titration was stopped when permanent faint pink colour was obtained. The Acid value was obtained using equation (3)

$$Acid\ Value = 0.56\ X \frac{N}{W}\ mg \frac{KOH}{g}\ of\ sample\ oil \qquad (3)$$

Where X is Mililitres of KOH used. W,weight of sample oil used. N, normality of caustic alkali used. Ethyl alcohol is flammable so heating was carried out in a fume cupboard.

2.3.3. Free Fatty Acid (FFA)

The same procedure for acid value was followed except that 0.098 N NAOH was used instead of 0.1 N KOH. The formula for FFA given in equation (4) was used for the calculation.

$$FFA = X\ 28.2 \frac{N}{W} \qquad (4)$$

Where X is mililitres of NAOH used. N, Normality of NAOH used. W, weight of sample oil used.

2.3.4. Saponification Value

2.0 grams of sample oil was weighed into 250 mls flat bottomed flask after which 25 ml of 0.5 M alcoholic Potassium hydroxide was added. A few anti bumping granules were added and the flask connected to a reflux condenser. The solution was refluxed for about 30 minutes and then titrated while still hot against standard 0.1 M HCl using phenolthalein as indicator. The end point was detected by a colour change from pink to colourless. A blank test was carried out using 25 mls of alcoholic potassium hydroxide against the same acid. The saponification value was obtained using equation (5)

$$Saponification\ Value = 56.1\ M\ \frac{B - S}{W} \qquad (5)$$

Where M is Molarity of the acid. B, Volume of titre for blank. S, Volume of titre for test.

W, weight of sample oil used.

2.3.5. Peroxide Value

1 g of sample oil was weighed into a conical flask and 20 ml of solvent mixture (glacial acetic acid 2 % volume and 1 % volume of chloroform solution) was added after which 1 gram of potassium iodide was added. The mixture was boiled for 1 minute and the hot solution quickly transferred into a flask containing 20 ml of 10 % potassium iodide. Some drops of 1 % starch solution was then added and the mixture was titrated with 0.025 N $Na_2S_2O_3$ till the colour changed from blue to colourless. The peroxide value was calculated using equation (6)

$$Peroxide\ Value = S\ \times N \times \frac{1000}{W} \qquad (6)$$

Where S is mls $Na_2S_2\,O_3$ used or the average titre. N, normality of $Na_2S_2O_3$ used. W, weight of sample oil used.

2.3.6. Photostability

12 g of sample oil was exposed to strong sunlight for about 6 hours in order to see whether any chemical change could result under effect of solar intensity.

2.3.7. Biodegradability

0.2 g each of sample oil was poured into two different sample bottles. Micro-organisms (*streptococcus* and *staphylococcus*) obtained from Yola Specialist Hospital were introduced into each of the sample bottles and the bottles kept overnight in order to see whether the sample oil could decay under the action of the introduced micro-organisms.

2.3.8. Flash Point

The sample oil was poured into the sample cup of the Abbels closed-up apparatus. The cup was closed and heated with a continuous stirrer and thermometer inserted into it. The flash point was detected as the temperature for which the vapour escaping from the closed-cup mixed with a little volume of air was ignited in a spark chamber.

The Abbels closed-cup apparatus work by the principle that every liquid has a particular temperature for which it could be ignited if exposed to a spark, ignition source or an open flame. The flash point is helpful in determining that no hazardous amounts of solvents have been left in the solvent extracted oil. It helps classify products as flammable or non-flammable.

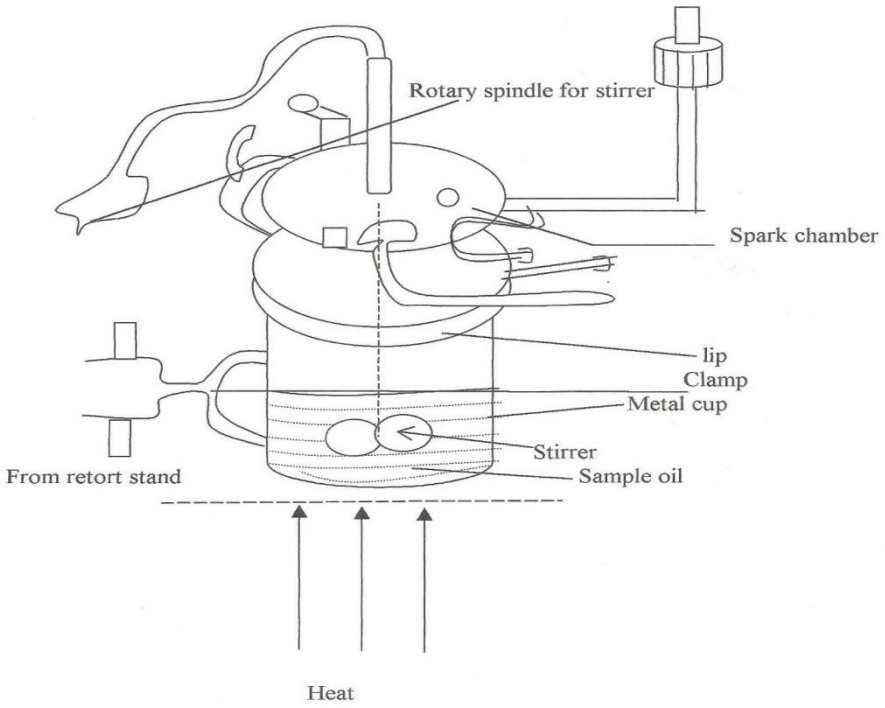


Fig. 7: Abbels Closed-Cup Apparatus

2.4. Determination of Mosquito Repellency Component

2.4.1. Infra-Red Spectrophotometry

A sample of the Neem Seed Kernel Extract or sample oil was bombarded with infra-red radiation. The wave band corresponding to the different functional groups were used to detect the functional groups.

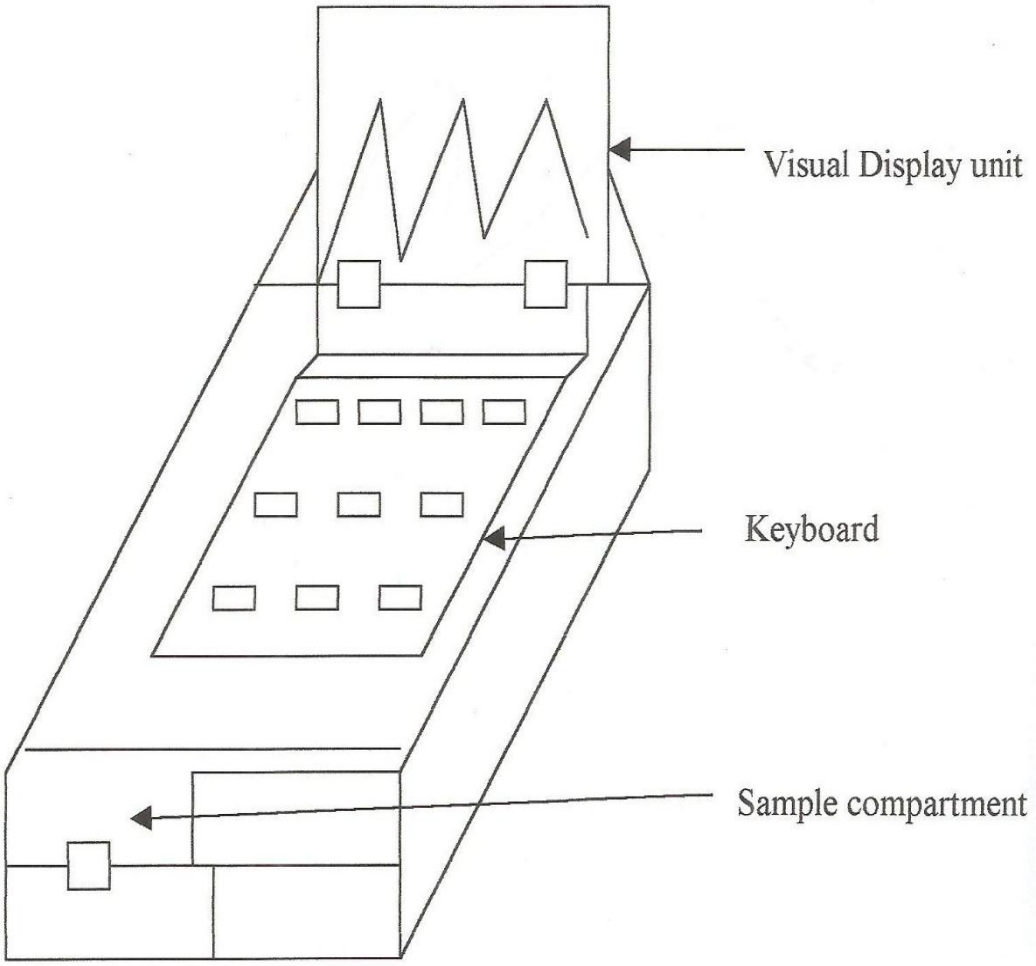


Fig.8: Ati Matson Infra red Spectrophotometer

2.4.2. Ultra Violet Spectrophotometry

The ultra violet spectrophotometry was used to confirm the functional groups detected by the infra-red spectrophotometry and to differentiate between them.

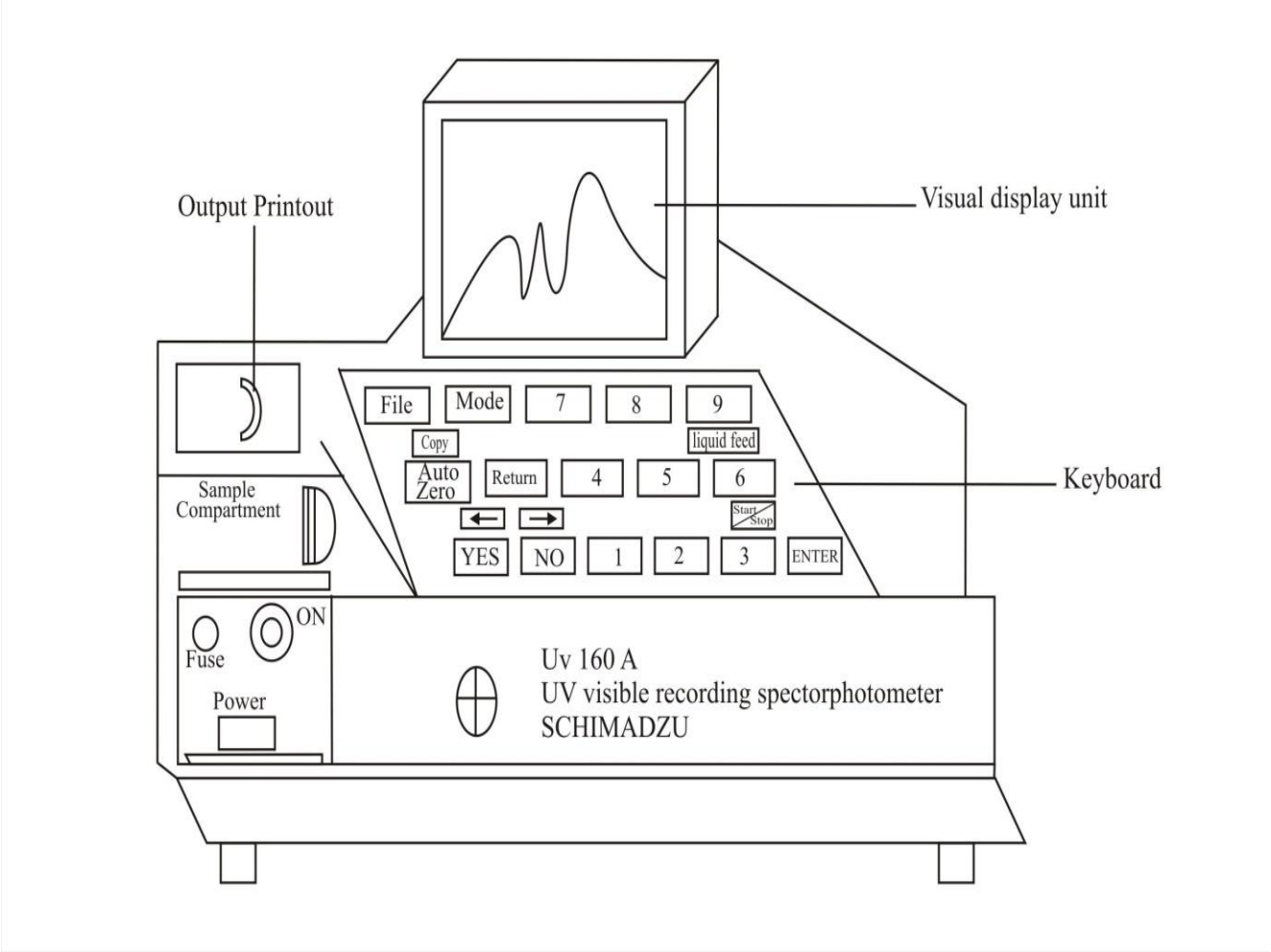


Fig.9: Schimadzu 160 A Ultra Violet Spectrophotometer

2.4.3. Nuclear Magnetic Resonance (NMR)

Besides proton NMR, the carbon – 13 NMR spectrophotometer was used for this analysis because it allows more detailed analysis of structural features of fairly large molecules. The extract was placed in a magnetic field and bombarded with electromagnetic radiation. The orientation of the magnetic nuclei changed as a result of resonance absorption of electromagnetic radiation. From the NMR spectrum, the intensity of each peak was obtained as the area under each peak in the NMR spectrum. The position and intensity of lines in the NMR spectrum was used to locate the position of the functional groups as they are attached to the parent atom.

2.4.4. Gas Chromatography / Mass Spectrophotometry (GC – MS)

Methylation:

Methylation was carried out in in order to make the extract volatile enough not to block the chromatographic column and damage the equipment. 5 mls of the

sample oil was added to 10 mls of petroleum ether in a round bottomed flask. A few drops of anti bumping granules were added after which 1 ml of sodium ethoxide and dry methanol (0.5 N) were added. The mixture was refluxed for 5 minutes at 40 ^{o}C in a water bath and 2 mls of $NaHSO_4$ added in order to neutralise excess sodium ethoxide. The mixture was allowed to settle and the upper layer taken. A little Na_2SO_4 was then added in order to absorb moisture.

Equipment:

The (GC – MS), a standard equipment, was used to detect the various components of the neem seed kernel extract. It has an in-built standard, so it can detect even the minutest components very efficiently. The interfacing of chromatographic columns to mass spectrometers permit instantaneous display of the spectrum of each species as it leaves the chromatographic column. The instrument is also interfaced with a computer so that each spectrum is digitalised and stored for later production. With a gas chromatograph alone, components with almost similar peak heights and retention times may be imbibed in the other components, hence the need to combine gas chromatograph with a mass spectrophotometer. A mass spectrophotometer bombards the substance under investigation with an electron beam and quantitatively records the result as a spectrum of positive fragments.

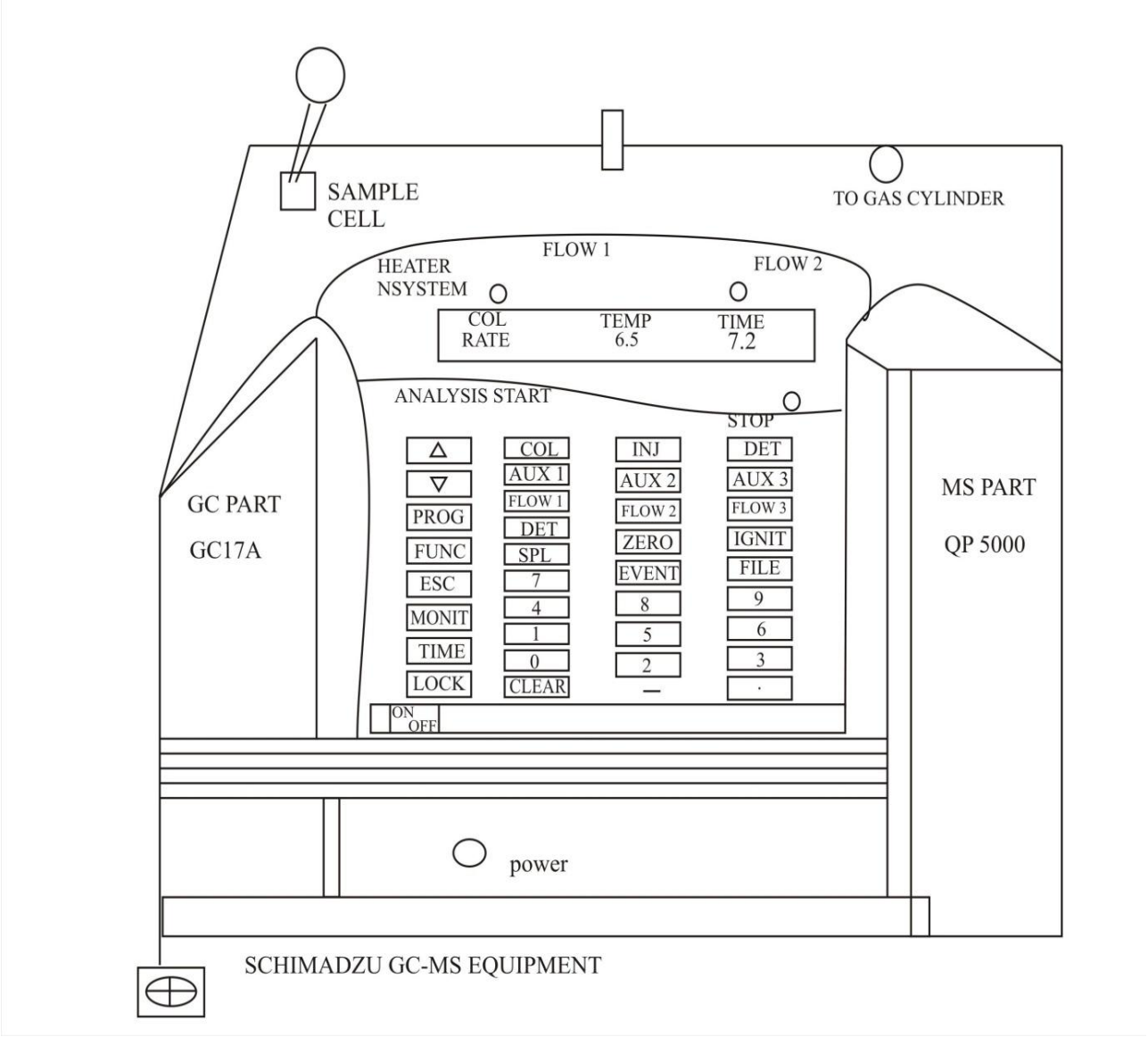


Fig.10: Schimadzu Gas Chromatograph/Mass Spectrophotometer

1 µL of the methylated extract was weighed and injected into a carrier (He) gas supplied from high-pressure cylinders at constant flow rate and pressure. The injected sample was heated to vapour and carried by the carrier gas to a hydrogen flame ionisation detector maintained at constant temperature and supplied with combustion air and hydrogen. A detector recorded retention times (time taken to elute the column) and a recorder recorded peak heights of each component. The retention time was used to identify the component but the peak height was used to determine the amount of each component.

3.0. Results

3.1. Physical Property Data

A physical property is a property determined without any induced chemical

Change in the course of the experiment.

3.1.1. Oil Content

$$\%\ oil\ content = \frac{weight\ of\ extract}{weight\ of\ sample} \times 100 \qquad (7)$$

$$= \frac{3.89\ g}{8.86\ g} \times \frac{100}{1} = 43.91\ \%$$

3.1.2. Colour of Extract

Before Bleaching

20 Yellow + 19.20 Red + 4.0 Blue

After Bleaching

20 Yellow + 9.00 Red + 0.0 Blue

$$\text{Percent bleaching} = \frac{Weight\ of\ bleaching\ earth}{Weight\ of\ oil} \times 100 \qquad (8)$$

$$= \frac{30.9\ g}{217.8\ g} \times 100$$

$$= 14.2\ \%$$

3.1.3. Viscosity

$$K = \frac{\boldsymbol{\mu}}{\boldsymbol{\rho} Q} \qquad (9)$$

Where K is the constant for the viscometer. μ, the viscosity for liquid of known viscosity. ρ, the density of the liquid. Q, the efflux time for the liquid.

$K = \frac{801}{1000\ x\ 3.20} = \frac{801}{3200} = 0.25$ from which μ for extract can be found below.

Where μ is the viscosity of the extract. K, the constant for the viscometer. ρ, the density of the extract. Q, the efflux time for the extract.

$$\mu = 0.25 \times 1070 \times 65 = 17387.5\ Poises$$

$$= 1738750\ Centipoise \quad (\rho \times 100)$$

$$= 17.39\ NSm^{-2} \text{ or } kgm^{-1}\ Sec^{-1}.\ (\rho \times 0.001)$$

$$= 6259500\ kghr^{-1}\ m^{-1}\ (C\rho \times 3.6)$$

Table 1: Summary of Physical Property Data

S/N	Property	Value
i.	Oil content	43. 91%
ii.	Extract colour	
	Before Bleaching	20 Yellow + 19.20 Red + 4.0 Blue
	After Bleaching	20 Yellow + 90.00 Red + 0.0 Blue
iii.	% Bleaching	14.20 %
iv.	Conductivity	8. 1 x 10^{-6} $mscm^{-1}$
v.	Resistivity	0.123μΩcm^{-1} or 123456.79 Ωcm
vi.	Viscosity	17.39 kgm^{-1} Sec^{-1} or NSM^{-2}
vii.	Refractive Index	1.463
viii.	pH value	4.63
ix.	Odour	Repulsive garlic
x.	Taste	A little bitter
xi.	Physical stability	Physically stable

3.2. Chemical Property Data

3.2.1. Iodine Value

$$0.003175\ (B - S)\ \times 100 \qquad (10)$$

Where B is average titre for blank sample and S the average titre with sample oil.

$$0.003175\ (58.81 - 38.10) \times 100 = 65.80\ mg\ iodine\ per\ 100\ g\ sample\ oil.$$

3.2.2. Acid Value

$$56.1 \times \frac{N}{W} \qquad (11)$$

Where X is the average titre. N, Normality of KOH used

W,Weight of sample oil used.

$= 56.1 \times 6.35 \times \frac{0.1}{10\ g} = 3.56$

= 3.56 mg KOH per gram of sample oil

3.2.3. Free Fatty Acid (FFA)

$$FFA = X\ 28.2\ \ \frac{N}{W} \qquad (12)$$

Where X is the average titre. N, Normality of NaOH used. W, weight of sample oil used.

$$9.60 \times 28.2 \times 0.098 \frac{N}{4.92} = 5.39\ \%\ FFA$$

3.2.4. Saponification Value

$$56.1\ M \frac{(B-S)}{W} \qquad (13)$$

Where M is the molarity of HCl used. B, the average titre for the blank. S, average titre for the sample. W, weight of sample used.

$$56.1 \times \frac{0.1(28.68-11.01)}{0.5} = 198.28\ mg \frac{KOH}{g}\ of\ sample\ oil.$$

3.2.5. Peroxide Value

$$Peroxide\ Value = SN \frac{1000}{W} \qquad (14)$$

Where S is the average titre. N, normality of NaS_2O_3 used.

W, weight of sample oil used.

$$3.5 \times 0.025 \times \frac{1000}{10} = 8750\ per\ kg$$

meq per kg = $8750/10^3 = 8.7$

= 8.70 meq of peroxide per kilogram of sample oil.

Table 2: Summary of Chemical Property Data

S/N	Property	Value
i.	Iodine Value	65. 80 mg 1/100 g S
ii.	Acid Value	3.56 MgKOH/g of Sample
iii.	Free Fatty Acid	5.39 %
iv.	Saponification	198.26 MgKOH/g of sample
v.	Peroxide Value	8.7 meq Peroxide /kg sample
vi.	Photostability	Photostable
vii.	Flash Point	260 ^{0}C
viii.	Biodegradability	Biodegradable

3.3. Chromatography/Spectrophotometry

3.3.1. U.V and I.R Spectrophotometry

Functional groups detected were;

i. CH_3
ii. C = C
iii. – COO
iv. CO_2 ME
v. AOC
vi. -OH
vii. -O
viii. C – C
ix. C – H

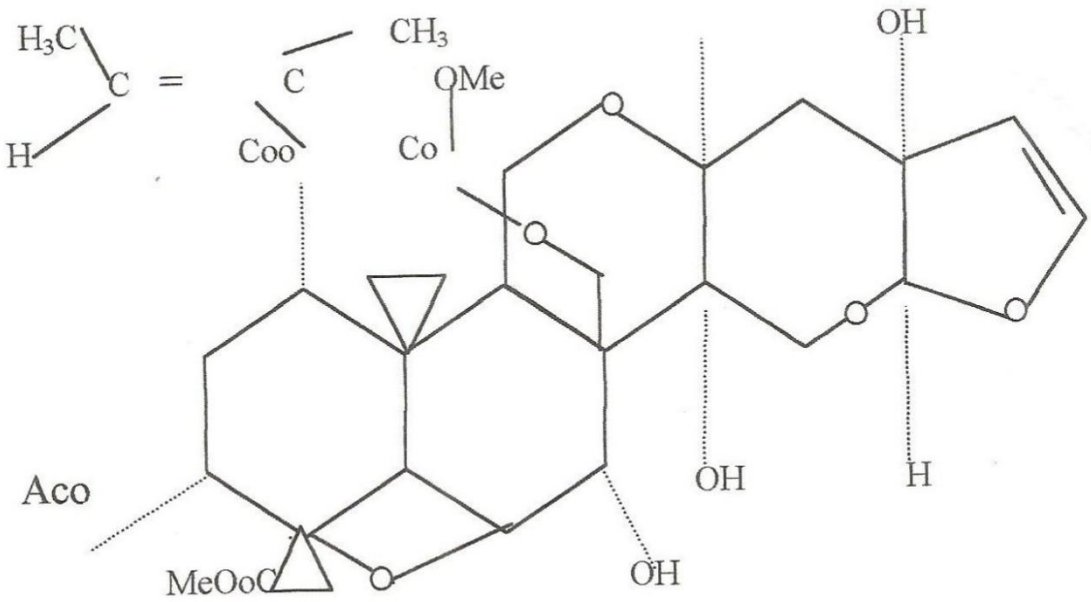
3.3.2. U.V. Spectrophotometry

According to Basler (1991) a peak range of 425 – 440 Nm show the presence of methyl ester. 441.0 Nm confirms methyl ester group.

3.3.3. Nuclear Magnetic Resonance (NMR) Spectrophotometry

The functional groups detected by the ultraviolet (U.V) and the infrared (I.R)

Spectrophotometry are attached at the following positions on the parent atom:



Chemical formula: $C_{35}H_{44}O_{16}$

Fig. 11: Positions of Functional groups in Parent atom as detected by NMR

3.3.4. Gas Chromatography- Mass Spectrophotometry (GC-MS)

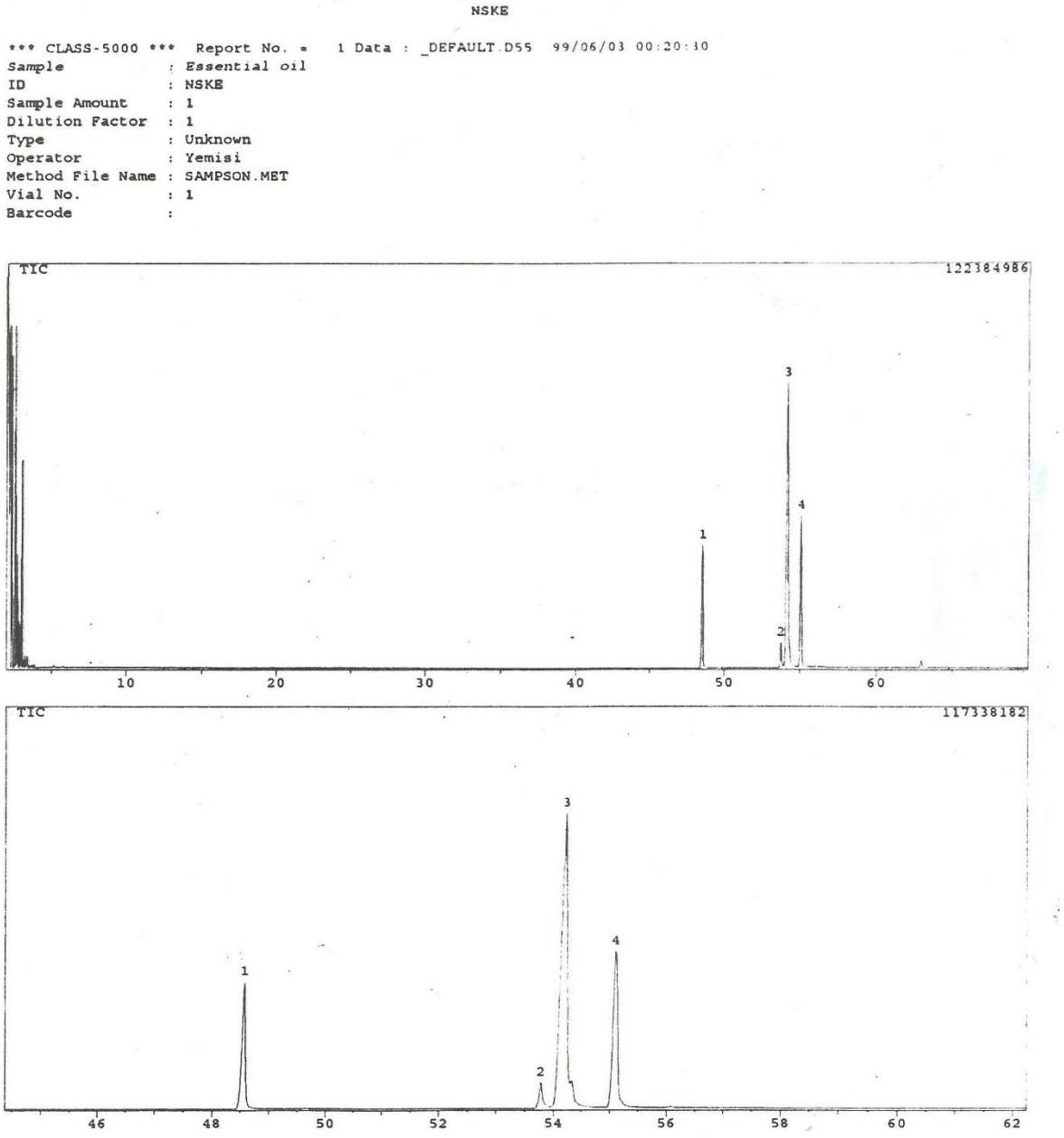
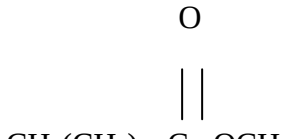


Fig.12: Gas Chromatography- Mass spectrophotometry Waveform

From the GC-MS analysis and GC-MS library information the major components of the Neem Seed Kernel Extract were as follows:

Peak 1: Tetradecanoic Acid (Methly Ester)

Structure:



Mol. Wt. 242 **Percentage**: 15.02 **Formula**: $C_{15}H_{30}O_2$

Peak 2: 11,14 –Eicosadienoic Acid (Methyl Ester)

Structure:

Mol.Wt. 322 **Percentage:** 2.95 **Formula**: $C_{21}H_{38}O_2$

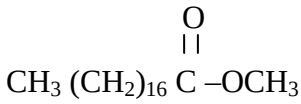
Peak 3: 10 – Octadecenoic Acid (Methyl Ester)

Structure

Mol.Wt. 296 **Percentage**:59.10 **Formula:** $C_{19}H_{36}O_2$

Peak 4: Octadecanoic Acid, (Methyl. Ester) or Stearic Acid

Structure



Mol.Wt. 298 **Percentage:** 22.92 **Formula**: $C_{19}H_{38}O_2$

Removal of one methyl group from each of the above methyl esters yield their corresponding free fatty acids which are Palmitic, Arachidonic and Oleic acids respectively.The fourth is stearic acid. According to Keneth & Hassal (1990) an insect repellent must have the following properties: A methoxy group, Ester linkage to a leaving group, hydrophobicitry and unsaturation. Another property that gives these methyl esters insect repellency property is that they are highly volatile, easily distilled and vaporised into the air and hence effective in repelling the target mosquitoes. It is

known that the presence of double bond show the level of unsaturation and hence reactivity, therefore among the four-methyl esters 11, 14 – Eicosadienoic Acid appears to be the most active followed by 10-Octadecenoic acid.

These methyl esters react with indogenuos groups and Oxygen to produce toxic metabolites, that is why it is more effective when burnt. The combustion reaction increases the toxicity of the repellent to the target mosquitoes. A further research to investigate the combustion products may require Air space chromatography which is not available in Nigeria.

4.0. Discussion

The oil content of 43.91 % shows that Neem Seed Kernel contains an appreciable quantity of oil and hence can be utilised for large-scale industrial production. The physical properties of the extract show that the extract posess the basic properties of a good insect pest repellent. A conductivity value of 8.1×10^{-6} $mscm^{-1}$ shows that the oil can be stable under conditions of proper handling, processing and storage. A viscosity of 17.39 kgm^{-1} sec^{-1} shows that a little wetting agent or spreader is necessary to make a formulation that can be easily burnt or sprayed. A refractive index of 1.463 falls within the acceptable range and confirms that there are no impurities in the extract. A pH value of 4.63 increased to 7.0 by addition of lime and filtering show that the extract cannot cause skin burns or irritation when rubbed on the skin to prevent mosquito bites.

A flash point of 260 ^0C shows that the extract is non flammable and there is no hazardous amount of solvent left in the solvent extracted oil.

Moreover, the extract has good storage property as could be seen in the values of most chemical properties viz: An iodine value of 65.80 mg/100 g shows that the oil is non-drying and capable of withstanding oxidative rancidity. The low iodine value is also an indication of the low degree of saturation of the oil. Being non-drying, the oil is good for soap, lubricants, grease but not good for paints and varnishes. A peroxide value of 8.7 meq/kg shows that the extract can withstand deterioration and is not prone to oxidative rancidity.

A free fatty acid level of 5.39 % is an indication that the oil is not prone to lipase action rancidity and hydrolytic action rancidity. A low acid value of 3.56 mg/g shows that the extract is not acidic for it to cause harm to the user. A saponification value of 198.26 mg/g shows that the oil can be utilised for production of soap.

Chromatographic and spectrophotometric analysis reveals that the Neem Seed Kernel Extract contains four methyl esters close to the structure of azadirachtin. This methyl esters are active, easily distilled and vaporized into the air; hence their effectiveness in

causing mosquito repellency action of the extract. 11, 14 – Eicodsadienoic Acid is the most active because of the presence of two double bonds.

5.0. Conclusion

It is concluded that:

Neem Seed Kernel Extract is best for use in the production of mosquito repellent considering that it is cheap and readily available, non-toxic to man and his domestic animals and contain an appreciable quantity of oil. Moreover, the extract can be stored for a reasonable long time without going rancid. The repellent is non-hazardous and non-toxic to man and his domestic animals but highly toxic to the target mosquitoes through the antifeeding or phagorepellent action of the active component (Azadirachtin), a complex methyl ester, an unstable compound which transforms itself into four simpler methyl esters as confirmed by GC – MS analysis. These methyl esters are active in one way or the other, but 11, 14 – Eicosadienoic Acid has structure closest to azadirachtin. It is the most active because of the presence of two double bonds.

6.0. Recommendations

Besides the production of mosquito repellent, Neem Seed Kernel Extract (NSKE) can also be used for soap production, tooth paste, cream, waxes, lubricants and fuel. Government should therefore encourage research in the use of NSKE for these products. A large scale production plant should be set up for the production of these products from NSKE.

7.0. Nomenclature

Abbreviation	Definition
GC	Gas Chromatography
GC – MS	Gas Chromatography and Mass Spectrophotometry
TLC	Thin Layer Chromatography
IR	Infra-Red
NMR	Nuclear Magnetic Resonance
NSKE	Neem Seed Kernel Extract
NSK	Neem Seed Kernel
UV	Ultra Violet
AC	Active Component

8.0. References

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