

# Determination of the chemical and nutritive constituents in oil extracts of whole edible portion of *Rhynchophorus phoenicis* and *Oryctes rhinoceros*

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## ABSTRACT

**Background:** *Oryctes rhinoceros* and *Rhynchophorus phoenicis* larvae are delicacies eaten by major tribes of the Niger Delta region of Nigeria.

**Aim:** Insects and their larvae are sources of micronutrients, proteins and biological active compounds. This study is aimed at characterizing the chemical constituents in oils extracted from these larvae.

**Methods:** The worms were macerated and extracted using 450 mL of n-hexane:dichloromethane (50:50). Extract was concentrated to 1 mL *in vacuo* and subjected to GC-MS analysis, while elemental analysis was carried out to assess the levels of mineral constituents in whole worms.

**Results:** GC-MS analyses of extracts were mainly, esters, fatty acids, sterols, ketones, aldehydes, alkanes and alcohols. Spectra showed the presences of 19 and 23 compounds in *Rhynchophorus phoenicis* and *Oryctes Rhinoceros* larvae respectively - of which these esters, Methyl hexadecanoate, Methyloctadecan-9,12-dienoate and Methyl stearate were found in both, while major difference is the presence of the tocopherols (Vitamin E) in *Rhynchophorus phoenicis* only. These compounds were confirmed from the NIST library. Amongst the mineral nutrients, Na content was the highest (207.41 and 216.55 mg/Kg in *R. phoenicis* and *O. Rhinoceros* respectively). The order was Na>Fe>Mn>Zn>K>P>Mg>Ca, while Al, Se, Cr and Pb were >0.001 mg/Kg.

**Conclusion:** Most of the compounds identified are known to exhibit bio-active and curative properties and could also boost the amelioration of different ailments. Therefore, both insect larvae species are likely to have high medicinal and nutraceutical potentials.

**Keywords:-** *Oryctes rhinoceros*, *Rhynchophorus phoenicis*, Elemental composition, physical parameters.

## 1.0 INTRODUCTION

Food safety, availability and affordability is presently creating a major challenge in several underdeveloped and developing countries, many people are now falling back on alternative food sources. Thus, there is the necessity to evaluate other sources of cheap and readily available nutrient to meet daily nutritional requirements. Edible insects as food are viewed by many to have good nutritive value and more sustainable solution to the challenge of food security [1-3]. Entomophagy, the consumption of insects as food, has been a long-established practice in many cultures and religions around the world – particularly in tropical and subtropical regions as a result of the humid climate [4-5]. Several countries in the continents of Africa, South America, and Asia see insect-eating as a way of life, probably for human consumption. Studies have revealed that insects are sources of vitamins and minerals, protein, fats and oils [4-7]. In addition, researchers and nutritionists have shown that certain edible insects have nutritional constituents that are comparable with that of fish and meat [8]. Notable among others are the palm

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beetles - *Rhynchophorus phoenicis* and *Oryctes rhinoceros*; variegated grasshopper – *Zonocerus verigatus*; cricket - *Acheta domesticus*; honeybee - *Apis mellifera*; mopane caterpillar - *Imbrasia belina*; yellow meal - worm *Tenebrio molitor*; domesticated silkworm - *Bombyx mori* and termites - *Macrotermes bellicosus*. Most of these insects are generally large in size and harvestable [7]. However, it is the immature stages (pupae and larvae) that are preferred, owing to their abundant amino acids, fatty acids with distinctive and captivating flavour, beside their nutritional value [7]. It is pertinent to mention that of all edible insect listed, only the larvae of *R. phoenicis*, *T. molitor* and *I. belina* are typically consumed [7]. *Rhynchophorus phoenicis* (Figure 1a) and *Oryctes rhinoceros* (Figure 1b) are commonly referred to as African red and coconut palm weevils respectively, which correspond to species of beetles belonging to the families Curculionidae and Scarabaeidae. Both species can reach a body length of about 25 mm. They are considered as serious pest in palm plantations, particularly damaging young palms of the species:- *Cocos nucifera* (Coconut Palm), *Phoenix Dactylifera* (Date Palm), Metroxylon sago (Sago palm), *Elaeis Guineensis* (Oil Palm), *Roystonea Regia* (Royal Palm). They can be eaten raw, fried, roasted, steamed, toasted, barbequed, cooked and deep-fried with the larvae stage being more delicious. The larvae are found in trunks of fell or dead palm trees that are slit or cut open. They are also known to have higher fat or lipid content than the adult stage [7, 9-10]. The level of fat content reported in both species ranged from 28.90 to 62.13% (w/w) [11-14]. According to Elemo *et al.*, (2011), [12] essential fatty acids are found in the larvae oil of red palm weevil; thus making it suitable for pharmaceutical use as a flavouring agents, animal feed and fertilizer. It also improves soil fertility, antimicrobial activities. The high dietary fibre found in the red palm grubs, could serve as great source of dietary roughage that may aid easy digestion of food and help prevent constipation and flatulence. In recent times, the evaluation of oils extracted from edible larvae as potential sources of biological active compounds have been reported [15-17]. The presence of chemical components in some edible larvae has clearly demonstrated their potency in anti-inflammatory, antioxidant, anti-angiogenic, anti-hypertensive, anti-diabetic, or anti-lipidemic properties [15-19]. In addition, many of these bioactivities are associated with the prevention and/or reduction of the likely emergence of multiple pathologies that could be fatal. For instance,  $\alpha$ -Linolenic acid, a potential nutraceutical for protection of the brain from stroke has been found in edible larvae [20]. Also, they are precursors for the synthesis of thromboxane leukotriene and prostaglandin, which are essential in the maintenance of normal physiological function in the body. The aforementioned hormones are known to lower gastric secretions, mediate inflammation, elicit uterine contractions, reduce blood pressure, influence blood clotting and cause asthma-like allergic response. The inadequate intake of  $\alpha$ -Linolenic acid can result to growth retardation, reproductive disorders, dermatological diseases (rash, etc.) as well as renal and neurological diseases. *Rhynchophorus specie* is a good source of vitamin E [21] and essential micronutrients such as iron, magnesium, manganese, phosphorous, potassium, selenium, sodium and zinc [22]. This study therefore is aimed at determining the chemical and nutritive constituents in oil extract and whole edible portion respectively of *R. phoenicis* and *O. rhinoceros* harvested from raphia palm trunk in the Niger-Delta swamps of Nigeria.

## 2.0 MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Reagents and Chemicals

Reagents used for the analysis were of analytical grade. Dichloromethane (DCM, 99%) and n- hexane (99%) used were of analytical grade manufactured by Merck Germany. Whatman filter paper No.41 was used for filtration.

#### 2.1.2 Apparatus and Equipment

Analytical balance (Ohaus model), Atomic Absorption Spectrophotometer (AAS) - Agilent technologies 200 Series Model, GC-MS QP2010SE Shimadzu, Japan Viscometer NDJ-5S, UV Spectrometer (Spectrumlab752pro Model), Abbe refractometer. The elemental analysis was done using Agilent technologies 200 Series AA, while for infrared spectroscopy, analysis was by Agilent Cary 630 FTIR using ATR accessory.

### 2.2 Methods

#### 2.2.1 Sample collection

The samples were collected from the wild, in the rafia palm forest in Peretorugbene, Ekeremor Local Government Area, Bayelsa State, Nigeria in October, 2021. The live species of the larvae were washed with distilled water, packed together in midst of moistened raphia palm (on which they feed) in a well-ventilated plastic containers and transported to the Department of Pharmaceutical and Medicinal Chemistry Laboratory, Niger Delta University, Amassoma. Both larvae were identified by an Entomologist, Mr. Tonsintei Table in the Department of Biological Science, Faculty of Sciences, Niger Delta University, Amassoma.





Fig 1a: African palm larvae (*R. phoenicis*)  
Family: Curculionidae



Fig. 1b: Coconut beetle larvae (*O. rhinoceros*)  
Family: Scarabaeidae

### 2.2.2 Pre-extraction treatment of sample

The larvae samples were washed gently with distilled water to remove raphia palm and other extraneous substances. The water was mopped from samples to facilitate drying. The larvae were cut from their tail to remove the intestinal waste. These were then commuted and blended to homogenize using a Kenwood blending machine.

### 2.2.3 Extraction of larvae tissue

Homogenized whole tissue samples of 87.743 and 563.62 g of *R. phoenicis* and *O. rhinoceros* larvae respectively, were macerated separately in 300 and 1200 mL of 50:50 ratio of n-hexane and dichloromethane for three days. The n-hexane:dichloromethane extract was then filtered with the aid of a whatman filter paper no 41 and concentrated *in vacuo* using a rotary evaporator. The residue oily extract was further exposed to a gentle stream of nitrogen gas to eliminate solvent mixture completely. The extracted oils were weighed and stored in an air-tight specimen bottles.

### 2.2.4 Physicochemical Analysis

#### 2.2.4.1 Relative Density Measurement

The relative density was determined using relative density bottle and the specific gravity was calculated using the formula in equation 3.1 below. 1 [23-25].

$$\text{Specific gravity} = \frac{W_3 - W_1}{W_2 - W_1} \quad \text{Equation 3.1}$$

Where  $W_1$ = weight of empty density bottle,  $W_2$ = weight of density bottle + distilled water, and  $W_3$ = weight of density bottle + Oily sample

#### 2.2.4.2 Refractive Index Measurement

The refractive indices of the oils were determined at 20°C using the Abbe refractometer [24]

#### 2.2.4.3 Viscosity Measurement

The viscosities of the samples were determined at four rotation amplitudes; 6, 12, 30 and 60 rpm (revolutions per minutes) at 20°C using NDJ-5S Viscometer with a n°3 spindle. [26-28].

### 2.2.5 Elemental analysis

A modified method by Helaludin *et al.*, (2016) was adopted. Whole samples were digested and the levels of elements were determined using AAS model Agilent technologies 200 Series [29].

### 2.2.6 Infrared spectroscopy

The infrared (IR) analyses of extract from both larva species were determined using Agilent Cary 630 FTIR using ATR accessory.

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## 2.2.7 GC-MS Analysis

The extracts from larvae were analyzed using GCMS-QP2010SE (Shimadzu, Japan) with a DB-5 MS (0.25  $\mu\text{m} \times 30 \text{ m} \times 0.25 \text{ mm}$ ) column. Helium was the carrier gas, with a flow rate of 0.9  $\text{mLmin}^{-1}$ . The injection temperature and injector volume were at 250°C and 1.0  $\mu\text{L}$  respectively, while the ion source temperature at 200°C. The interface and initial oven temperatures were at 250°C and 60°C respectively, and the latter was held for 2 minutes, followed by a 15°C $\text{min}^{-1}$  increase to 120°C and ending with 300°C (15°C $\text{min}^{-1}$ ). Mass spectrometer was adjusted to operate in electron ionization mode with an ionizing energy of 70eV as acquisition mass range from 45-700 a.m.u. Total running time was about 30 min. Obtained mass spectra were identified by comparison with those in the National Institute of Standards and Technology (NIST) database or library stored in the equipment.

## 2.3 Data Analysis

The data obtained from viscosity were analysed using Origin 80 software, while graphical plots were constructed with Excel and Origin8 Pro SR4 software (2018, model) package.

## 3.0 RESULTS

Table 1: Physical properties of samples of oils

Sample type	Wt. of sample taken (g)	Amount of oil (g)	Percentage yield (%) (w/w)	Relative density	Refractive index
<i>R. phoenicis</i>		105.108	11.94%	0.907 $\pm$ 0.003	1.453 $\pm$ 0.001
<i>O. rhinoceros</i>		10.000	1.12%	0.871 $\pm$ 0.001	1.470 $\pm$ 0.002

RD = Relative Density, RI = Refractive index

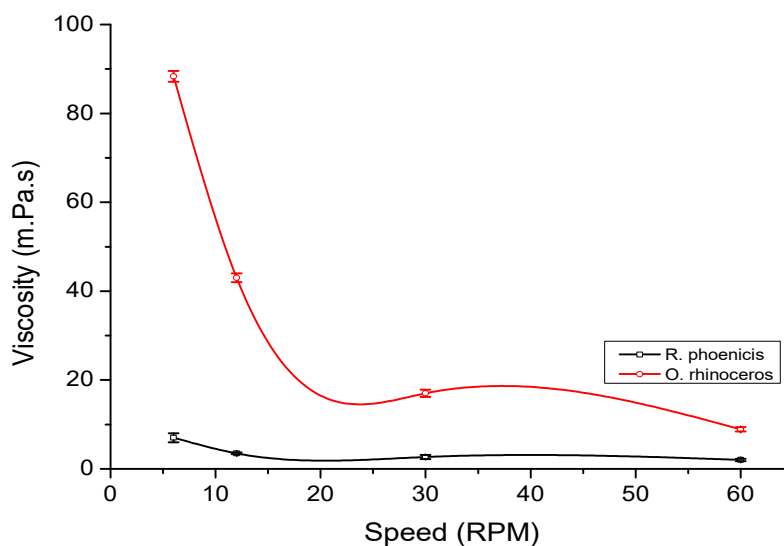
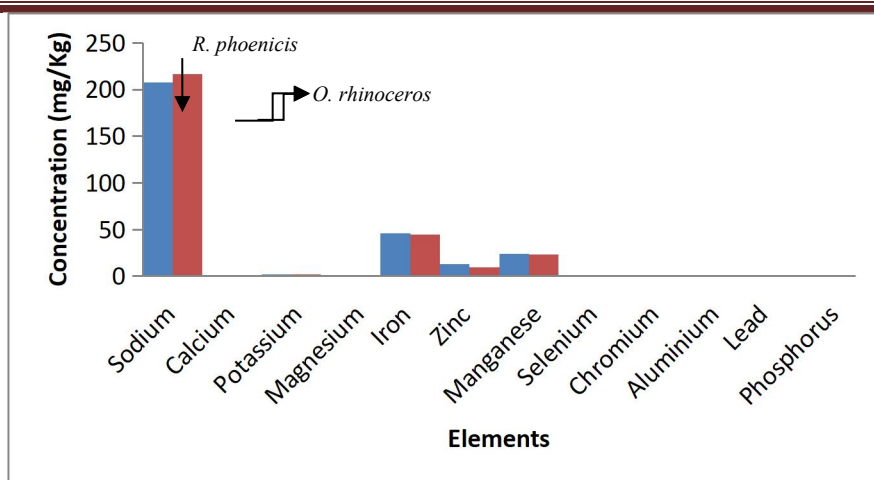
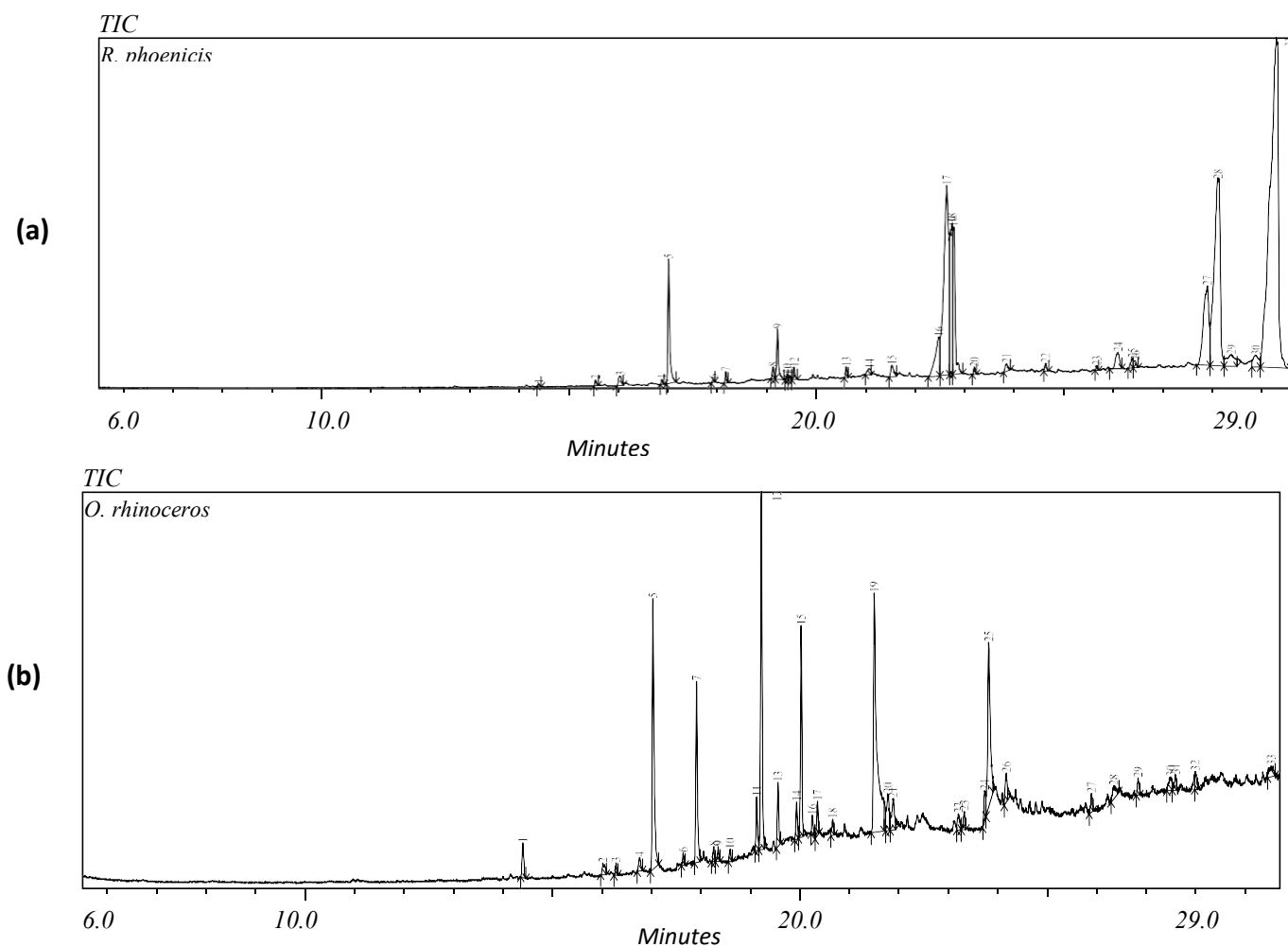


Figure 2: Viscosity profile of oils extracted from *Rhynchophorus phoenicis* and *Oryctes rhinoceros*



**Figure 3:** Micro nutrient metal profile in *Rhynchophorus phoenicis* and *Ocytes rhinoceros*



**Figure 4:** GC-MS chromatogram of oil extracted from (a) *R. phoenicis* (b) *O. rhinoceros*

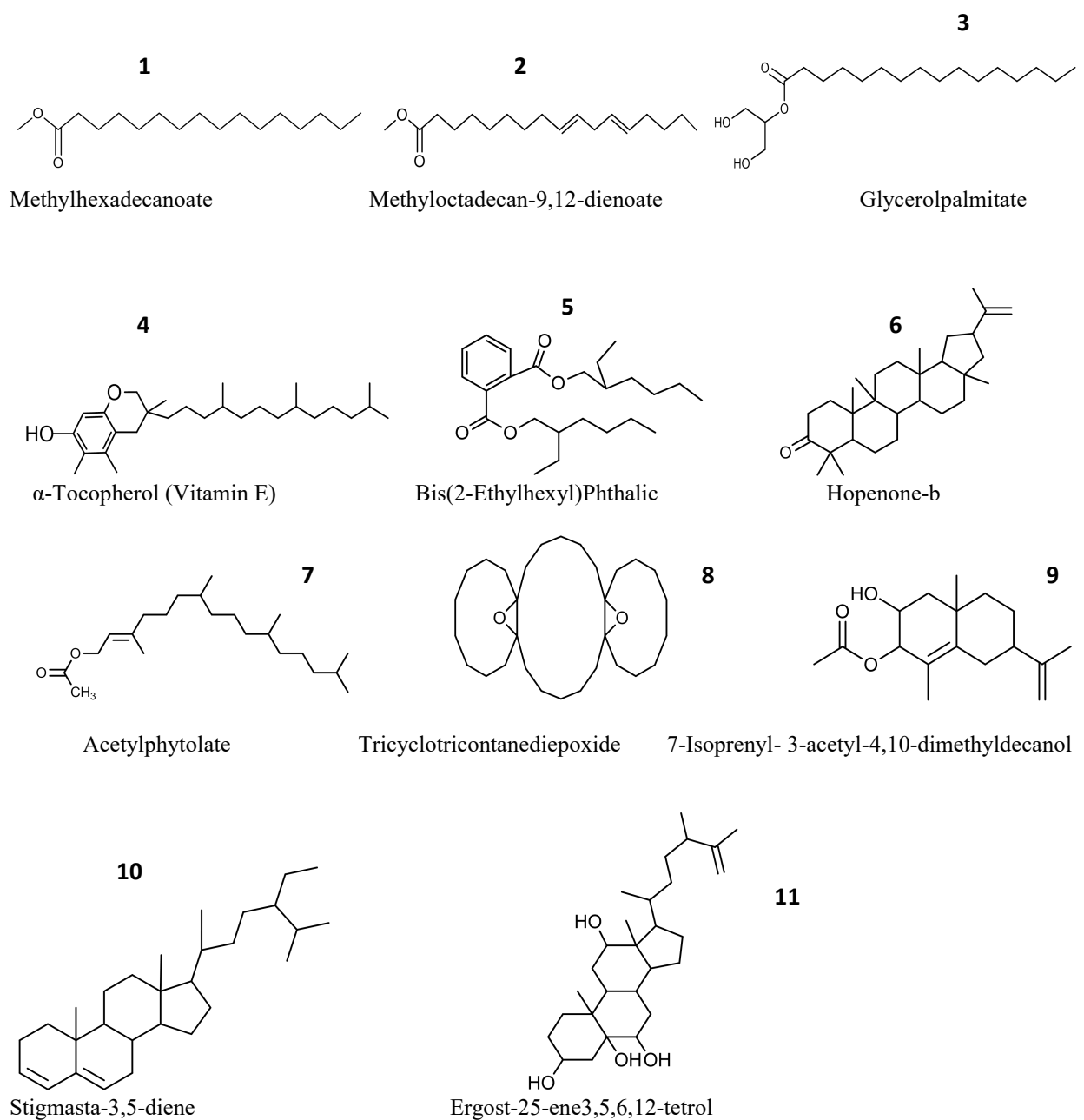
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**Table 2: Compounds identified by GC-MS in oil extracted from *R. phoenicis***

S/N	RT	Name of compound	M. formula	Mw.t	Peak Area (%)
1	16.035	ButylundecylPhthalate	C <sub>23</sub> H <sub>36</sub> O <sub>4</sub>	376.51	0.44
2	17.009	Methylhexadecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.17	3.37
3	19.133	Methyloctadecan-9,12-dienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.19	1.60
4	19.543	Methylstearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298.19	0.29
5	20.596	10-Methyleicosane	C <sub>21</sub> H <sub>44</sub>	296.21	0.24
6	21.068	Stigmasta-3,5-diene	C <sub>29</sub> H <sub>48</sub>	396.29	0.31
7	21.516	Glycerolpalmitate	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330.19	0.55
8	21.516	α-Tocopherol (Vitamin E)	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430.29	0.55
9	22.465	Tocopherol	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430.29	2.53
10	22.626	γ- Tocopherol	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430.29	14.04
11	22.735	dl-alpha-Tocopherol	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430.29	5.26
12	23.831	9-Hexadecenal	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>	338.22	0.29
13	26.086	Bis(2-Ethylhexyl)Phthalic	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.24	0.90
14	26.375	Ergost-25-ene3,5,6,12-tetrol	C <sub>28</sub> H <sub>48</sub> O <sub>4</sub>	448.28	0.32
15	26.430	2-ethyltetradecyloxalic acid	C <sub>24</sub> H <sub>46</sub> O <sub>4</sub>	398.24	0.27
16	27.887	Hopenone-b	C <sub>30</sub> H <sub>48</sub> O	424.30	6.31
17	28.369	Acetylphytolate	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>	338.22	1.37
18	28.865	Tricyclotricontanediepoide	C <sub>30</sub> H <sub>52</sub> O <sub>2</sub>	444.30	0.96
19	29.285	7-Isoprenyl- 3-acetyl-4,10-dimethyldecanol	C <sub>17</sub> H <sub>26</sub> O <sub>3</sub>	278.17	38.75

**Table 3: Compounds identified by GC-MS in oil extracted from *O. rhinoceros***

S/N	RT	Name of compound	M. formula	Mw.t	Peak Area (%)
1	14.380	1,5diisopropyl-2,3-dimethylcyclohexane	C <sub>14</sub> H <sub>28</sub>	196.14	1.42
2	15.996	2-Pentadecanone	C <sub>18</sub> H <sub>36</sub> O	268.18	0.77
3	16.740	Methyl-9-octadecenoic acid	C <sub>19</sub> H <sub>36</sub> O	280.19	0.75
4	17.006	Methyl-8- hexadecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.17	12.3
5	17.619	Ethyl-9-hexanoate	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	284.18	0.45
6	17.888	Ethylpalmitate	C <sub>18</sub> H <sub>36</sub> O <sub>3</sub>	300.18	7.3
7	18.328	Octadecanal	C <sub>18</sub> H <sub>36</sub>	252.18	0.53
8	18.567	Diisobutyldecadiene	C <sub>20</sub> H <sub>38</sub>	278.20	0.45
9	19.104	Methyl 9,12-Octadecadienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.19	2.07
10	19.199	Methyl, 11-octadecenoate	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.19	13.66
11	19.534	Methyl stearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	296.19	2.41
12	19.911	Ethyl linoleate	C <sub>20</sub> H <sub>36</sub> O <sub>6</sub>	372.19	1.43
13	20.004	Ethyl-9-octadecenoate	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	308.20	7.51
14	20.334	Ethylloctadecanoate	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312.20	1.31
15	20.640	Phytylacetate	C <sub>22</sub> H <sub>40</sub> O <sub>2</sub>	336.22	0.65
16	21.481	Eicosanoic acid	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312.20	19.81
17	21.761	15-Heptadecenal	C <sub>17</sub> H <sub>32</sub> O	252.17	2.27
18	21.860	10-Methyleicosane	C <sub>21</sub> H <sub>44</sub>	296.21	2.18
19	23.183	Glycerylmonoleate	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	356.21	0.93
20	23.300	6-Propyltridecane	C <sub>16</sub> H <sub>34</sub>	226.16	0.68
21	26.325	n-Nonacosane	C <sub>29</sub> H <sub>60</sub>	408.29	1.14
22	27.970	Olean-12-ene-3,28-diol	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>	442.30	0.70
23	29.50	Luciferin aldehyde	C <sub>14</sub> H <sub>24</sub> O	208.14	1.02



**Figure 5:** Chemical Structures of major constituents identified in hexane:dichloromethane extracts of *Rhyncophorus phoenicis* larvae tissues using GC-MS.



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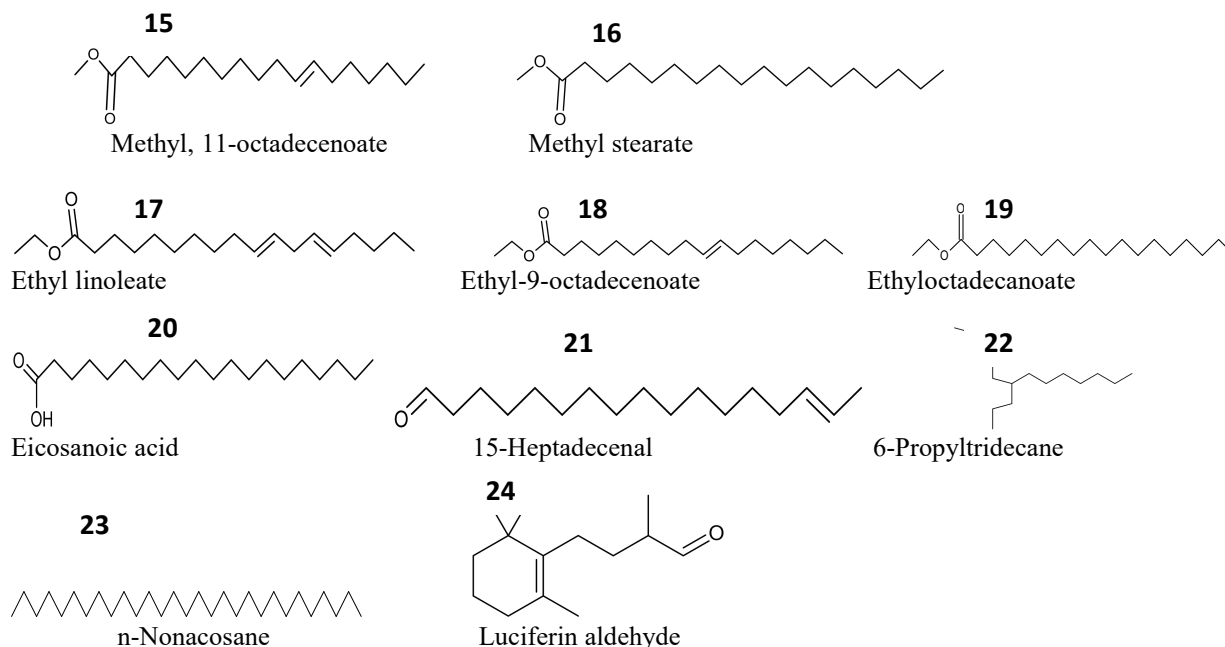


Figure 6: Chemical Structures of major constituents identified in hexane:dichloromethane extracts of *Oryctes rhinoceros* larvae tissues using GC-MS.

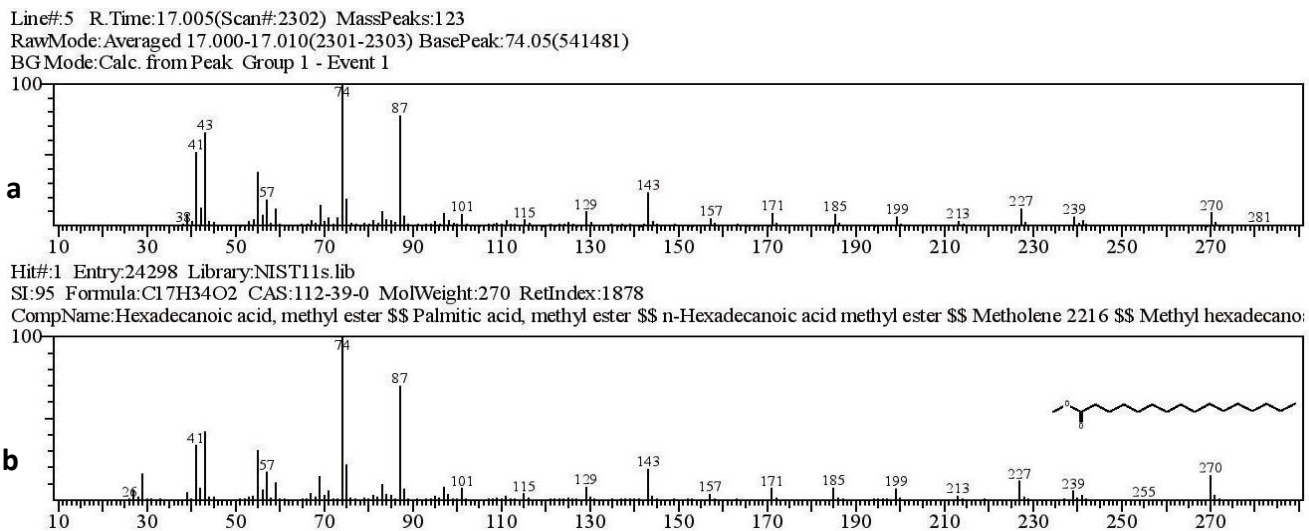


Figure 7: Mass spectra of Methyl-hexadecanoate (a) in oil extracted from *O. rhinoceros* (b) NIST library



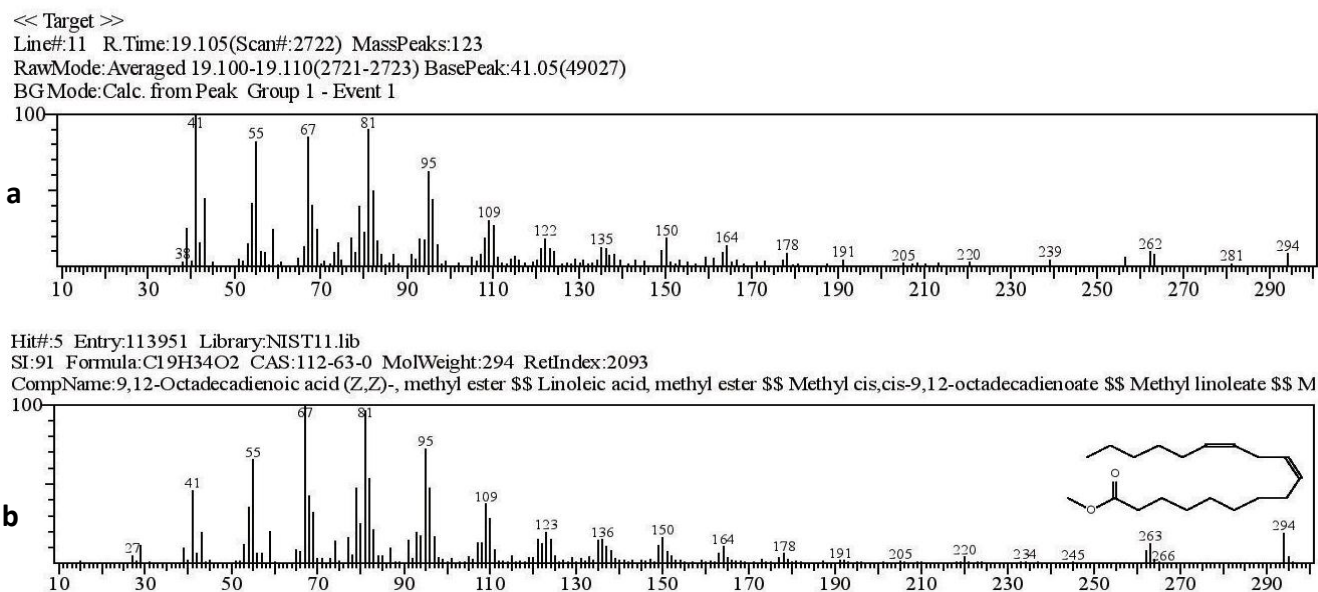


Figure 8: Mass spectra of Methyl 9,12-Octadecadienoate (a) in oil extracted from *O. rhinoceros* (b) NIST library

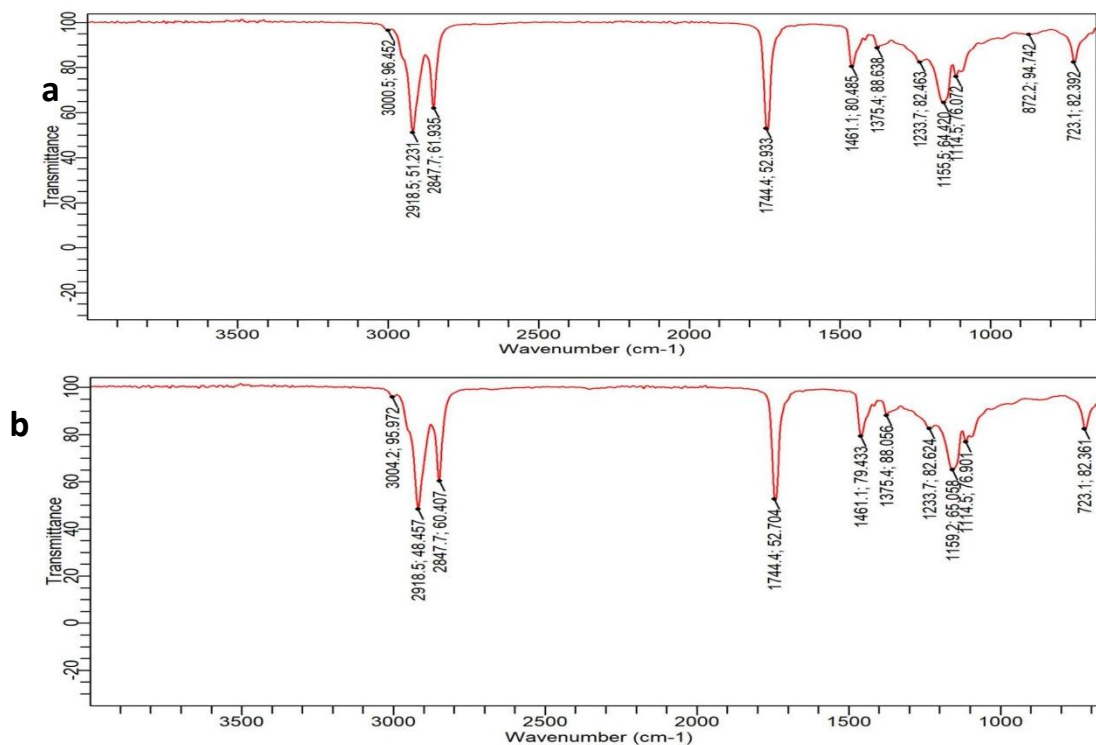


Figure 9: Infrared spectra of oil extracted from the larva of (a) *R. phoenicis* (b) *O. rhinoceros*

#### 4.0 DISCUSSION

*Physical properties – percentage yield, relative density, refractive index and viscosity*

Table 1, shows results obtained for the physical properties of oils found in both samples.

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The percentage yield of extracted oil from *Rhynchophorus phoenicis* and *Oryctes rhinoceros* were 11.19 % (w/w) and 1.13% (w/w) respectively. This shows that *R. phoenicis* had a higher yield compared to *O. rhinoceros*. These values were significantly lower when compared to previous studies [11]. The low percentage yield may be due to the time of sample collection or their innate chemical composition and sampling location. The relative densities of the oils were  $0.907 \pm 0.003$  and  $0.871 \pm 0.001$  for *R. phoenicis* and *O. rhinoceros* respectively. These values were within the stipulated range of 0.840 – 0.960 for fixed oils [30]. The refractive indices of the oil samples ranged from  $1.453 \pm 0.001$  to  $1.470 \pm 0.002$ . This implies that the values obtained are satisfactory with respect to SON's stipulated standard for edible oils [31]. The viscosities of the oils at 6, 12, 30 and 60 rpm exhibited different flow pattern due to remarkable different chemical composition of the oils (Figure 2). Viscosity measures the internal frictions of the oil molecules as well as its resistance to flow and both are dependent on the chemical composition of the oils; the more viscous an oil, the better its lubricating properties [32-34]. In addition, oils with low viscosities are usually light, free flowing and are mostly unsaturated [33]. The oil extracted from *O. rhinoceros* showed greater viscosity than that from *R. phoenicis*. This further corroborates the liquid and semisolid nature of the oils from *R. phoenicis* and *O. rhinoceros* larvae respectively at room temperature and the phase differential could be due to the remarkable difference in their chemical constituents and composition [32, 33, 35].

### Elemental analysis

Figure 3, shows the results obtained for elemental analysis of both samples. The following minerals - potassium, sodium, calcium, zinc, magnesium, iron, manganese and phosphorus were found present in both samples. The mean  $\text{Na}^+$  content in *R. phoenicis* and *O. rhinoceros* were 207.41 mg/Kg and 216.55 mg/Kg respectively. These values were comparable with those reported for *R. phoenicis* by Okaraonye and Ikewuchi (2008) [36]. Sodium is a macro-nutrient in edible worm and could serve as a source of  $\text{Na}^+$  in man. Sodium ion is an important electrolytes needed for the regulation of blood, present in extracellular fluid (ECF) [37]. It plays pivotal roles in muscle contraction, enzyme operations, fluid maintenance and osmoregulation within the body [38]. In addition, it helps to improve the performance of the heart, glucose absorption and nervous system. The level of  $\text{Na}^+$  found in the worms were significantly lower than the daily dietary amount of  $\leq 5$  g/daily for adult [39]. The  $\text{Ca}^{2+}$  concentration in *R. phoenicis* and *O. rhinoceros* were 0.18 and 0.27 mg/Kg respectively. The values obtained in this study were comparable with those of previous studies for *R. phoenicis* harvested from Oruma and Yenegwe-Epie and Edepie in Bayelsa State [11]. The WHO/FAO recommended daily intake of  $\text{Ca}^{2+}$  ion, is dependent on age, gender and life stages (pregnancy and lactation) – daily intake ranges from 300 (in infants) to 700 mg (children of 8 years); and to 1300 mg in children (9 - 18 years) and older adults [40]. Low intake and deficiency of calcium mineral often leads to nutritional rickets in children and osteomalacia in adult, while adequate intake results in healthy bone mass and prevention of bone loss and fractures in different age brackets [40-42]. The concentrations of potassium were 1.93 and 1.65 mg/Kg in *R. phoenicis* and *O. rhinoceros* respectively. These levels were significantly lower than values found in *R. phoenicis* by previous study [11]. However, they were comparable values reported for grab worm found in Oyiabo town, Rivers State [36]. Potassium mineral intake is reported to have strong links with several health conditions – especially cardiovascular endpoints and amongst adults in European countries, potassium intake of 3,500 mg (90 mmol)/day has been reported to have beneficial effects on blood pressure, while intake below this is associated with higher risk of stroke [43]. The content of iron in *R. phoenicis* and *O. rhinoceros* were 45.71 and 44.91 mg/Kg respectively - there is no remarkable difference in the levels of mineral iron in both larvae species. The values obtained in this study were significantly lower than values reported by Okoli *et al.*, (2019) [11]. Iron is known to function as a vital component in numerous proteins, including enzymes and haemoglobin, in which the latter plays key role in the transport of oxygen to tissues throughout the body for metabolism [42]. It has been reported that about two-thirds of iron in man is found in the haemoglobin. The median dietary intake of iron ranges from 16 to 18 mg/day for men and 12 mg/day for women, while 45 mg/day is the Tolerable Upper Intake Level (UL) for adults [42]. The level of iron in both larvae was adjudged satisfactory and its consumption would be a good source of mineral iron. The values obtained in this study will get to the UL, only if one kilogramme weight (1 Kg) of the larvae is consumed. The amount of magnesium found in *R. phoenicis* and *O. rhinoceros* were 0.35 and 0.48 mg/Kg respectively. These values were comparable with previous study of both species harvested from raphia palms in Rivers State [36, 44], however, they were significantly low in the study by Okoli *et al.*, 2019 [11]. Soft tissue magnesium functions as a co-factor of many enzymes [45, 46] while low plasma calcium has been associated with decline of magnesium in the human body. Magnesium content in samples were significantly lower than the stipulated FAO/WHO tolerable upper limits of 65 – 110 mg/day (children) and 350 mg for adolescents and adults [42]. Therefore these larvae could serve as dietary supplement for magnesium. Manganese concentrations in *R. phoenicis* and *O. rhinoceros* were 23.98 and 23.16 mg/Kg respectively – with marginal difference in concentration between both organisms. The tolerable maximum limit per day for manganese is 11 mg [42] and to exceed this limit,



over 500 g of both worms would have to be consumed. The obtained values are considered satisfactory. Soft tissue mineral manganese may function as activator for several enzymes in man [46]. Manganese is reported to be ubiquity in foods; hence its deficiency is not likely to occur if appropriate diet regime is kept [47]. The stipulated AI values by IOM for manganese for males and females within the age range of 31–50 years are 2.3 and 1.8 mg/day respectively [42]. Zinc levels in *R. phoenicis* and *O. rhinoceros* were 23.98 and 23.16 mg/Kg respectively – with the former having a marginal higher concentration of 3.42% (w/w) over the latter. These concentrations were significantly lower when compared with *R. phoenicis* harvested in the palm groves of Notse, Togo [48], however, they were higher than values reported by Okoli *et al.*, (2019) [11]. The recommended daily allowance (RDA) values for females and males are 8 and 11 mg/day, respectively for adults within the range of 31–50 years [42]. The concentrations of zinc in both larvae were considered satisfactory and could adequately and readily be a diet Zn supplement source. Phosphorus is reported to play predominant roles in bones and teeth formation and how carbohydrates and fats are utilized in the human body [49,50]. In addition, it is required for the synthesis of proteins for the growth, maintenance, and repair of damaged cells and tissues in man. However, the presence of excessive phosphorus in blood may leach calcium from the bone, thus leaving them feeble or may combine with it to form deposits in the soft tissues of the body and leading to high risk of heart attack, stroke or death [49,50,51]. Chang and Anderson (2017) reported that high ingestion of phosphorus can lead to calcium build up (calcification) in vascular and renal systems, renal tubular damage, and premature death in several animal models [51]. Other micro-nutrients - selenium, chromium, lead and aluminium were  $\leq 0.001$  mg/Kg (below the detection limit of the instrument).

#### GC-MS Analysis

Figures 4a and 4b shows the GC-MS spectra of hexane:DCM oil extracts of *R. phoenicis* and *O. rhinoceros* respectively, while the chemical compounds in extracts, their retention times, molecular weight, molecular formula and percentage peak areas (or percentage composition) are presented in **Tables 2** and **3**. The identification of chemical constituents was based on the National Institute of Standards and Technology (NIST) library similarity and probability index. The GC-MS analysis of oils obtained from *R. phoenicis* and *O. rhinoceros* showed the presence of 19 and 23 compounds respectively. These compounds were mostly esters, fatty acids, sterols, ketones, aldehydes and alcohols.

#### Chemical composition of oil found in *Rhynchophorus phoenicis*

Amongst the 19 compounds identified in the oil extracted from *Rhynchophorus phoenicis*, 12 (Twelve) of them were predominant. They are:- 7-Isoprenyl-3-acetyl-4,10-dimethyldecanol (38.75%),  $\gamma$ - Tocopherol (14.04%), Hopeneone-b (6.31%), dl-alpha-Tocopherol (5.26%), Methylhexadecanoate (3.37%),  $\beta$ -Tocopherol (2.53%), Methyloctadecan-9,12-dienoate (1.60%), Acetylphytolate (1.37%), Tricyclotricontanediepoxyde (0.96%), Bis(2-Ethylhexyl)Phthalate (0.90%),  $\alpha$ -Tocopherol (Vitamin E) (0.55%) and Glycerolpalmitate (0.55%) - of which the tocopherols (vitamin E, made of  $\alpha$ -,  $\beta$ -,  $\lambda$ - and racemic dl- $\alpha$ - isomers) constituted a total of 22.38 %. The structures of these compounds are presented in Figure 5. Most of these chemical constituents have been found to be bio-active against some pathogens and infirmities. For instance, vitamin E (tocopherol) which is about 22.38 % in *R. phoenicis* is a fat-soluble vitamin which exist in several forms (tocopherol and tocotrienols), however, only the alpha-tocopherol isomer that is used by the human body. It is a useful antioxidant - scavenging free radicals that can damage cells and tissues [52]. Although vitamin E deficiency is rare, its' deficiency has been reported to cause neurological problems as a result of weak nerve conduction – which consist of neuromuscular problems such as spinocerebellar and myopathies [53]. In addition, deficiency may cause anemia, due to oxidative injury to red blood cells [54]. The compound, 7-Isoprenyl-3-acetyl-4,10-dimethyldecanol, contains the isoprenyl moiety, which has been associated with broad spectrum of bio-activities [55-56]. The prenylation of organic compounds tend to enhance the activity of such compounds [57]. Chen *et al.*, (2014) reported that the prenylation of flavonoids led to their antibacterial, anti-inflammatory, antioxidant, cytotoxicity, larvicidal and estrogenic activity enhancement [57]. It is pertinent to mention that pharmacologically active essential oils of terpene origin are mainly constituted by isoprene units as backbone [58, 59]. The compound hopeneone -B, a phytosterol also named moretenone has been reported to exhibit analgesic and anti-inflammatory activities - with the analgesic properties much higher than that of aspirin and paracetamol [60, 61, 62, 63]. In addition, it is known to possess antioxidant and hypocholesterolemic properties [64]. Also, the ester, methylhexadecanoate (methyl palmitate) has shown very high antimicrobial effect against clinical pathogenic bacteria – a multidrug resistant (MDR) bacteria [65, 66] while Hema *et al.*, (2011) and Asghar and Choudahry (2011) [67,68] showed that this compound do have the ability to reduce blood cholesterol and also inhibits the cyclooxygenase II enzymes - thus, it can elicit a selective anti-inflammatory action [68]. Acetylphytolate (Phytol acetate) – a derivative of phytol is a diterpenoid used for cancer-prevention, antimicrobial, anti-inflammatory and diureatic [69, 70] [71] and also been used as a fragrance, with faint-floral balsamic odour [72]. Methyl 9, 12-



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Octadecadienoate, an essential polyunsaturated methyl ester found in mammalian nutrition is known to exert anti-microbial, analgesic, anti-inflammatory and ulcerogenic effects on health [73]. In addition, it plays key role in the biosynthesis of prostaglandins and cell membranes [74, 75]. The compound Bis(2-Ethylhexyl)Phthalate also named Di-(2-ethylhexyl) Phthalate (DEHP) was reportedly isolated from fungus *Aspergillus awamori* and has shown antimicrobial activity against Gram-positive bacteria and fungi, and in addition to exhibiting potent cytotoxic effect against some human carcinoma cells [76]. No pharmacological activity has been reported for tricyclo-tricontanediepoide. However, some steroidal compounds such as Stigmasta-3,5-diene (0.31%) and Ergost-25-ene 3,5,6,12-tetrol (0.32%) with were found in the extract. Stigmasta-3,5-diene and Ergost-25-ene 3,5,6,12-tetrol have been reported to exhibit free radical scavenging, antidiabetic, anticancer, anti-inflammatory activity and contributes to human reproductive regulation as well as cell membrane stability [77,78]. The compound glycerolpalmitate is an ester of palmitic acid that is known to protect the human skin from external damaging factors, and provide a moisturizing effect on the skin when the oil is used topically [79]

### *Chemical composition of oil found in Oryctes rhinoceros*

Figure 6, shows 14 chemical compounds that were predominant amongst the 23 compounds identified in the *O. rhinoceros* oil extract (Table 3), amongst which two compounds - Methyl 9,12-Octadecadienoate (2.07%), and methyl-8-hexadecanoate (12.30%) were also found in *R. phoenicis* extract and have been discussed. However, the other compounds found were:- Eicosanoic acid (19.81%), Methyl, 11-octadecenoate (13.66%), Ethyl-9-octadecenoate (7.51%), Ethylpalmitate (7.3%), Methyl stearate (2.41%), 15-Heptadecenal (2.27%), 10-Methyleicosane (2.18%), Ethyl linoleate (1.43%), 1,5-diisopropyl-2,3-dimethylcyclohexane (1.42%), Ethyloctadecenoate (1.31%), n-Nonacosane (1.14%) and Luciferin aldehyde (1.02%). The aforementioned compounds are predominately esters, fatty acids and aldehydes. Eicoisanoic acid also known as arachidic acid is a saturated fatty acid (SFA) with 20-carbon chain and has been reported having anti-cancer and anti-inflammatory potential [64]. It forms the parent backbone for the group of compounds referred to as “eicosanoids” which are also known for various homeostatic and anti-inflammatory processes. For example, arachidonic acid a polyunsaturated fatty acid (PUFA) is dehydrogenated eicoisanoic acid (lose 8 H-atoms), and majority of eicosanoids are biosynthesized in human cells from the oxidation of arachidonic acid and other PUFAs by enzymes (such as cyclooxygenase - COX; cytochrome P450 - CYP) or via free radicals [80]. Studies have shown that natural occurring esters of palmitic acid – methylhexadecanoate (Methyl palmitate) and ethylhexadecanoate (ethyl palmitate) do exhibit anti-inflammatory and antioxidant activities [81, 82, 83]. In addition, Kizilay & Cetin, (2018) [82] reported that oral methyl palmitate decreases the formation of epidural fibrosis, while both esters retarded carrageenan-induced planar inflammation by diminishing prostaglandin E2 (PGE2) levels in exudates [83]. Ethyl palmitate is also used as flavouring and seasoning agents [84] acaricide [85] nematicide, pesticide and 5-alpha reductase inhibitor [86]. Ethyl linoleate (ELA) or Linoleic acid ethyl ester is an unsaturated fatty acid often used in the formulation of many cosmetics, due to its antibacterial and anti-inflammatory properties [87, 88, 89] and anti-acne property [90]. In addition, studies have shown that it inhibits melanogenesis through Akt/GSK3 $\beta$ / $\beta$ -catenin signal pathway [91]. This implies that the biosynthesis of melanin pigments is retarded by the presence of ELA. Ethyl-9-octadecenoate has been found to have anti-inflammatory activity [92]. Xie *et al.*, (2022) showed that Ethyl-9-octadecenoate suppresses the production of nitric oxide (NO), prostaglandin E2 (PGE2), and tumour necrosis factor- $\alpha$  (TNF $\alpha$ ) to elicit its anti-inflammatory action[92]. Ethyl Octadecanoate (EOD) or Ethyl stearate a saturated ester has been found to mediate protective effects on dopaminergic neurons [93]. The aforementioned is due to the ability of COD to produce and upregulate Tyrosine hydroxylase (TH) enzyme [94], which is a biomarker of dopaminergic neurons [95-96]. This is a vital protease that converts tyrosine into L-DOPA (L-dihydroxyphenylalanine), the dopamine precursor of melanisation [97]. The chemical constituent 10-Methyleicosane is an alkane and has a strong antioxidant property [98], while 15-heptadecenal is reported to exhibit anti-inflammatory, antibacterial, antifungal, anticancerous and antioxidant properties [99-101]. Kanimozhi and Bai (2012) and Yayli *et al.*, (2006) have reported the antimicrobial capacity of n-nonacosane a saturated aliphatic alkane [102-103].

### *GC-MS identification of chemical constituents in R. phoenicis and O. rhinoceros:*

The MS fragmentation patterns of prominent chemical compounds in extracts were identified using the NIST library. Below are the MS for some selected constituents (Methyl-8- hexadecanoate and Methyl 9,12-Octadecadienoate) in oils extracted from *R. phoenicis* and *O. rhinoceros* - these were matched with the NIST library (Figures 7 and 8). In addition the retention times (RTs) of these constituents were significantly the same (Tables 4 and 5).

### *IR spectroscopic analysis:*

Figures 9a and 9b shows the IR spectra of oils extracted from *R. phoenicis* and *O. rhinoceros* respectively. The spectra pattern for both extracts were found to be similar and showed distinct peaks that are characteristic for





functional groups of identified compounds; such as esters, alkane, alkene, ketones, aldehydes, alcohols, ethers and carboxylic acids. The peaks at  $3000\text{ cm}^{-1}$  (sh) and  $3004\text{ cm}^{-1}$  (sh) found in oils extracted from *R. phoenicis* and *O. rhinoceros* respectively are due to C-H stretch in alkanes [104,105, 106];  $2918\text{ cm}^{-1}$  (vs) is due to C-H stretch of alkane and alkenes, while the  $2847\text{ cm}^{-1}$  (vs) peak corresponds to C-H stretch of aldehydes [104]. These aforementioned functional groups are corroborated by the presences of the constituents identified in the GC-MS profile of the extracts from both larvae (Tables 3 and 4 and Figures 4 and 5). The peak at  $1744\text{ cm}^{-1}$  (vs) corresponds to the C=O stretch of esters and aldehydes [104, 106]. The constituents in the oils were predominately esters. The peak at  $1461\text{ cm}^{-1}$  was due to C-H bending vibration of C=C and aromatic groups, while peak at  $1375\text{ cm}^{-1}$  (sh) corresponds to C-H deformation of methyl group [107, 108, 109]. The spectra peaks at  $1114\text{ cm}^{-1}$  and  $1155\text{ cm}^{-1}$  are associated with C-O stretch of esters, carboxylic acids, ketones and aldehydes [104-106]. The strong peak at  $723\text{ cm}^{-1}$  corresponds to C-H rocking for long chained alkyl groups [106].

## 5.0 CONCLUSION

The results obtained from the experiment showed variation in the physical properties of the samples. These variations may arise from the fact that these two species live in different habitats and possessing different chemical compositions. This was further confirmed by the GC-MS analysis - that *Rhynchophorus phoenicis* differ in terms of chemical composition and rich in vitamin E and steroidal composition than *Oryctes rhinoceros* and may be used for its nutraceutical value. *Oryctes rhinoceros* which contain more of fatty acids, are nutritious and medicinal thus, recommended for human consumption.

**Acknowledgments:** We are grateful to Mr. Raji O. Rafiu and Prof. Ere Diepreye of the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria, for their technical contribution.

**Funding:** Nil

**Conflict of interest:** We declare no conflict of interest.

**Contribution of the Authors:** All authors design and implement the Study. Vaikosen, E.N and Owaba ADC, supervised the experiments interpreted results, writing and editing of the manuscript and while Eboh, AS and Peibulu, M carried out the experiments.

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