CHEMICAL AND TOXICOLOGICAL EVALUATIONS OF BOILED *Hemidactylus frenatus* AND *Scolopendridae cataracta* SOLUTIONS IN ALBINO RATS

BY

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CERTIFICATION

CERTIFICATION

This is to certify that this study, **"Toxicological and Chemical Evaluation of *Hemidactylus frenatus* and *Scolopendridae cataracta* Boiled Solutions in Albino Rats"** by **ESSEH, JANE NNENNA (20204254338)** was formally approved for the award of Master of Science in Nutritional Biochemistry of Federal University of Technology Owerri.

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DEDICATION

This work is dedicated to God Almighty.

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ABSTRACT

This study evaluated the chemical profile and toxicological potential of *Hemidacylus frenatus* (wall gecko) *and Scolopendridae cataracta* (centipede) boiled solution in albino rats. Thirty-five (35) male albino rats that weighed 96.00 ± 0.06 g and aged 9 to 10 weeks were divided into seven groups: group 1 received feed and water only and served as the control group, group 2 received 10 ml/kg body weight (b.w) of boiled degutted wall gecko solution (BDWGS), group 3 received 20 ml/kg b.w of BDWGS, group 4 received 10 ml/kg b.w of boiled whole wall gecko solution (BWWGS), group 5 received 20 ml/kg b.w BWWGS, group 6 received 10 ml/kg b.w of boiled centipede solution (BCS) and group 7 received 20 ml/kg b.w BCS; for 28 days through oral route. The GC-MS analyses of the boiled solutions revealed the presence of benzenamine, methenamine, 1H-imidazole, halcinonide, acetamide, cyclohexane, colchiceine, ethyl oxamate and N-(4-Aminobutyl) aziridine in them. The results of the acute toxicity (LD_{50}) of the boiled centipede and wall gecko solutions showed no mortality at the study level. There were significant ($p<0.05$) increases in blood urea, creatinine, sodium ion, chloride ion concentrations and ALP, ALT and AST activities and a decrease in bicarbonate ion concentration following administration of BWWGS and BCS. Total and direct bilirubin concentrations increased in all treatment groups while total protein, albumin and globulin concentrations reduced in BWWGS and BCS groups when compared to control group. Alterations in renal and hepatic functional indices were confirmed in histopathological study of liver and kidney tissues; ostensibly caused by the toxic chemical compositions of the treatment solutions. In conclusion, the boiled wall gecko and centipede solutions were toxic to the liver and kidneys of the rats.

Keywords: Boiled centipede and wall gecko solutions, chemical compositions of hepatic and renal effects, wistar rats.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Lizards (wall gecko) and centipedes are versatile and as diverse as their dwellings. They have been around for a very long time with their ancestors having roamed the earth long before man.

Lizards belong to Order *Squamata*; Class *Reptilia*; Phylum *Chordata* of Kingdom *Animalia* (Uetz, 2010). Worldwide only two lizards of family *Helodermatidae* are known to be capable of delivering a venomous bite that may be serious to humans; *Heloderma suspectum* and *Heloderma horridum* (Beaman *et al*., 2006). They are found in North America, specifically in south-west part of United States and Mexico. Additionally, recent studies mention a venom system at work in two additional lizard lineages hitherto unreported: Monitor Lizards and Iguania (Fry *et al*., 2009).

The wall or house gecko (*Hemidactylus*) belongs to the second most species-rich lizard family in the world, *Gekkonidae*, of suborder ―*Gekkotal* (Uetz, 2010). Since prehistoric times, numerous myths have been associated with geckos. In many parts of the world lizards are considered to be venomous animals, capable of inflicting poisonous bites, causing diseases such as leprosy, vitiligo and rendering food poisonous (Sety and Hansen, 2008). Ancient Egyptians believed geckos to be hazardous to human health (Sety and Hansen, 2008), whereas in South Africa *Chondrodactylus anguilifer* and *Pachydactylus maculatus* are believed to be venomous. In Madagascar, geckos of the genus *Uroplatus* are regarded with such dread that buildings where they are found have occasionally been burned. In Mexico, *Coleonyx* species are believed to have a poisonous skin. In Malaysia, Dyak natives believe that a bite from *Crytodactylus* spp. is poisonous, and in Pakistan, *Eublepharis macularius* is believed to be even more poisonous than the cobra (Evans, 2002).

Though dominant across the African continent, *Hemidactylus* geckos remain poorly known, with various myths and disbeliefs associated with them. If lizard drops down in food it makes it poisonous, is perhaps the most common myth prevalent across all sections of Nigeria society. To augment this myth there have been several sporadic incidents of people falling ill after consuming food having lizard found in it (ANI, 2006; TNN, 2011). Across all the states of India, lizards have occasionally been found in food served at schools, hostels, and restaurants, and subsequently persons who consumed that food got admitted in hospital with complaints of nausea, vomiting and retching. People believe that breath, urine and faecal pellets of common wall gecko are poisonous, while some others attribute their skin as the culprit (ANI, 2006).

Centipedes are venom-injecting predator animals belong to phylum: *Arthropod*; Class: *Chilopoda*. There are approximately 3500 species of centipedes identified round the globe (Dugon and Arthur, 2012). These are ancient venomous animals whose first pair of front legs modified into large, poisonous fangs connected to venomous glands. Leg based fangs are used to inject venom which causes severe pain in humans (Radis and Konno, 2020). The venom gland covered by thick cuticle and epidermis, consisting of numerous epithelial secretory units each with its own unique valve-like excretory system (Undheim *et al*., 2016). Their venoms contain various components with different biomedical and pharmacological properties. *Scolopendra* attack and predate over small mammals, bats, and amphibians (Ross *et al*., 2022). Chinese redheaded centipede *Scolopendra subspinipes mutilans*, is a venomous centipede found in East Asia and Australia (Chen *et al*., 2014). The Vietnamese centipede (*Scolopendra subspinipes*) is one of the largest and most aggressive tropical centipedes (Bouchard *et al*., 2004).

Centipede venom contains a large number of components with different biochemical and pharmacological properties (Liu *et al*., 2012). Centipede envenomation causes physiological

problems such as acute hypertension, myocardial ischemia and infarction, hematuria, hemoglobinuria, rhabdomyolysis, hemorrhage, pruritus, eosinophilic cellulitis, and anaphylaxis reactions, in rare cases, death. Its venom also causes pain, paresthesia, lethargy, localized necrosis, headache, dizziness and nausea (Liu *et al*., 2012). Centipede venom also contains 'neurotoxic components that likely combine to cause rapid death. These neurotoxins target voltage-gated sodium and calcium channels as well as potassium channels (Luo *et al*., 2018). Centipede venoms possess chemical components which are used as an arsenal for defense, marking and killing prey (Ombati *et al*., 2018).

There has been reports of food poisoning as a result of centipede dropping into cooked food. This could be as a result of the venom of centipede or result from pathogenic bacteria which the centipede host. Angiostrongyliasis is an important food-borne parasitic and zoonotic disease (Kwon *et al.,* 2013). There is confirmed report of angiostrongyliasis due to eating centipedes (Nalini *et al.,* 2013).

In this present study, the effect of boiled solution of wall gecko and centipede on liver and kidney function indices were examined.

1.2 Statement of the problem

There have been reported incidences of families losing their lives mysteriously as a result of consumption of foods that had contact with wall gecko (*Hemidactylus frenatus)* and centipede (*Scolopendridae cataracta)* in Mowe Community of Obafemi Owode LGA, Ogun State and in Eziukwu Ukpa Community, Afikpo North Local Government Area, Ebonyi State respectively as reported in https://theeagleonline.com.ng/Retrived Dec. 2, 2022. So this work will give an insight to know if the wall gecko (*Hemidactylus frenatus)* and centipede (*Scolopendridae cataracta)* was the causative organisms or had an active

substances responsible for the deaths and to raise awareness of the risk of accidental or unintentional consumption of centipede and wall gecko.

1.3 Justification of study

The research will provide more information on the associations of wall gecko and centipede with foods. This will either support the myths and beliefs associated with these organisms or allay them. The society needs such information as it will help it know how to handle these organisms. A society that lives on myths and beliefs will someday run itself aground. That is where scientific research and this study take the central stage.

1.4 Aim of Study

This study was carried out to evaluate the chemical profile and toxicological potentials of *Hemidacylus frenatus and Scolopendridae cataracta* boiled solutions in albino rats.

1.4.1 Objectives of study

The objectives of this study include:

(a) To determine the chemical compositions of *Hemidactylus frenatus* and *Scolopendridae cataracta* boiled solutions.

(b) To determine the acute toxicity level (LD_{50}) of boiled solutions of *Hemidactylus frenatus* and *Scolopendridae cataracta in rats.*

(c) To determine the effects of the boiled solutions of *Hemidactylus frenatus* and *Scolopendridae cataracta* on kidney function parameters.

(d) To determine the effects of the boiled solutions of *Hemidactylus frenatus* and *Scolopendridae cataracta* on liver function parameters.

(e) To determine the effects of the boiled solutions of *Hemidactylus frenatus* and *Scolopendridae cataracta* on relative organ weight ratio of albino rats.

(f) To determine the effects of the boiled solutions of *Hemidactylus frenatus* and *Scolopendridae cataracta* on the histological of albino rat's organs.

CHAPTER TWO

2.0 LITERATUE REVIEW

2.1 Centipede

It is known that centipedes, among other insects, can cause not only local but also systematic clinical effects such as anaphylaxis or even hypotension and myocardial ischemia (from vasospasm and myocardial toxic effects of the venom) (Senthilkumaran *et al*., 2011). The toxins released with envenomation cause vagal activity which could result in circulatory symptoms. Histamine and Toxin S are considered as mediators for myocardial injury (Yildiz *et al*., 2006). Cases of acute myocardial ischemia have been described following a centipede bite, either as presenting manifestation (Uppal *et al*., 2000) or following local symptoms (Senthilkumaran *et al*., 2011). Furthermore, sensitivity reactions are not uncommon, although late hypersensitivity reaction is described less often (Fung *et al*., 2011). Immediate type allergic reaction against centipede venom has been reported, with dyspnea, general fatigue and urticaria and positive prick test. Some relationship between centipede allergy and bee allergy has been supported (Harada *et al*., 2005). An immune complex deposition syndrome (type III hypersensitivity reaction) manifesting as recurrence of swelling associated with pruritus at the venom exposure sites, has been further suggested (Bush *et al*., 2001). Proteinuria, usually in the context of nephritic syndrome (Hassan and Hassan, 2005), as well as extensive myonecrosis with subsequent compartment syndrome, rhabdomyolysis and acute renal failure may rarely occur due to centipede venom (Logan and Ogden, 2005). Other very rare clinical consequences following centipede venom ingestion include psychological disturbances and Korsakoff syndrome (Japanese Scolopendra species), Wells' syndrome (eosinophilic cellulitis) (Friedman *et al*., 2008) and pericoronitis (Gelbier and Kopkin, 2002). The patient may rarely feel fine (almost ''euphoric'') a few hours after the bite (Bush *et al*., 2001). Irritability and uncontrollable cry (beside local symptoms) have been reported in

neonates (Rodriguez-Acosta *et al*., 2000) and systemic side effects (due to systemic absorption of the venom) in infants, requiring, though, no active intervention (Barnett, 2001). Remarkably, a fatality has been reported following a sting by a large specimen of *S. subspinipes* to the head of a small Filipino child (Bush *et al*., 2001). Finally, there is also a report of postmortem injury (subcutaneous cavity on the victim's forearm) caused by a centipede venom (Harada *et al*., 2009).

2.1.1 Taxonomy of centipede

Centipedes are invertebrate animals belonging to phylum *Arthropoda*, class *Chilopoda* and subphylum *Myriapoda* (Bush *et al*., 2001). Although over 8000 species are estimated to exist worldwide, only 3000 have been described, in habitats varying from deserts to the Arctic (Adis and Harvey, 2000). There are four major orders (all venomous) of centipedes: *Geophilomorpha* (innocuous soil dwellers, small), Scolopendramorpha (giant or tropical *centipedes* known stingers), *Scutigeramorpha* (fast but delicate house centipedes) and *Lithobiomorpha* (garden or rock centipedes-resemble small *scolopendramorpha* in appearance with many anecdotal reports of stings) (Bush *et al*., 2001).

2.1.2 Morphology of centipede

Centipedes are distinguishable from insects by their many (from less than 20 to more than 300) legs (at least nine pairs) and by their largely uniform bodies (not divided into head, thorax and abdomen) (Walters, 2012). They are slender, multi-segmented arthropods characterized by one pair of legs on each body trunk segment (Bush *et al*., 2001) and one pair of antennae. Their coloration may range from bright yellow to brown-black (Plate 2.1). They have two sharp stinging structures connected to muscular venom glands and these structures constitute actually the modified first pair of centipede legs (Bush *et al*., 2001). These venomous fangs, their key characteristic, are their hunting and defensive equipment.

Centipedes range in length between 0.05-30 cm (Walters, 2012) and the common centipede is usually 2-3 cm long (Walters, 2012). The *Scolopendra* are the largest centipedes, probably the most venomous and therefore the most dangerous. They range between 8-15 cm in length (Figure 1), and *Scolopendra heros* can achieve lengths of more than 20 cm (Bush *et al*., 2001). Giant centipedes are found in a few places including Africa (*Scolopendra* spp.) (Walters, 2012), New Guinea (*Scolopendra morsitans* and *subspinipes*) (Haneveld, 2006) and Philippine Islands (*Scolopendra subspinipes*) (Remington, 2000). *Scolopendra* usually have a yellow-brown body with orange and blue cephalic/caudal parts (Bush *et al*., 2001).

Plate 2.1a: *Scolopendra cataracta*: Superior surface (Bush *et al*., 2001).

Plate 2.1b: *Scolopendra cataracta*: Inferior surface (Bush *et al*., 2001).

2.1.3 Habitation and distribution of centipede

Centipedes have a worldwide distribution and are well represented in Europe and Africa (Walters, 2012). They are found in wild habitats, warm temperate and tropical climates, but also in gardens (Walters, 2012). They are found mainly in soil and litter and prefer dark damp environments, such as below rocks and logs (Dejean and Lachaud, 2011). Common centipede hides by day in crevices, such as under loose bark, leaves or stones (Walters, 2012). Centipedes can sometimes be found inside sheds or even enter houses, especially in wet weather (rainy days) (Walters, 2012), thus constituting common household arthropods (Barnett, 2001). Centipedes are nocturnal carnivores with a wide range of prey (Dejean and Lachaud, 2011). Centipedes emerge at night to hunt primarily for insect larvae (crickets, cockroaches) and occasionally for slugs, worms, small snakes and even small mammals, catching them using their powerful venomous fangs (Walters, 2012). These fast-moving arthropods use their venom to paralyze prey prior to eating (Bush *et al*., 2001). Ants and termites are, among other animals, their natural predator (Dejean and Lachaud, 2011).

2.1.4 Centipede venom glands

The modification of the legs of the first trunk segment into maxillipeds (forcipules) with its characteristic poison claws is the most prominent autapomorphy of the Chilopoda. Each maxilliped contains a large poison gland that surrounds the proximal and median part of the common poison duct and opens on the inner side of the tarsungulum. Length and shape of the poison ducts varied among species (Chao and Chang, 2006). Light and first electronmicroscopical studies described the glands as unicellular, recent studies reveal that the poison gland of *Lithobius forficatus* is composed of hundreds of multicellular epidermal gland units. Each of them consists of three different cell types, a secretory cell, an intermediary cell, and two different canal cells (4-cell epidermal gland) (Rosenberg and Hilken, 2006).

Centipede forcipules are shaped like a set of piercing forceps, each consisting of four or five segments: a large trochanteroprefemur, two short segments (femur and tibia), and an apical claw. While the apical claw is made up of two segments in *Scutigeromorpha*, the tarsus and ungulum, these are fused in all other centipedes and hence referred to as the tarsungulum (Bonato *et al*., 2010). The outer surface of each claw contains at least three types of sensilla ceoloconica-type chemoreceptors, which may be used for tasting prey, stimulating the secretion of venom by sensing penetration by the apical claw, or both (Ernst and Rosenberg, 2003). Interestingly, the evolutionary progression from walking appendages to highly specialised venom delivery systems can be traced by comparison of forcipules from extant centipede orders (Haug *et al*., 2014). This reveals a gradual transformation of the plesiomorphic, slender forcipules found in *Scutigeromorpha* to the highly modified forcipules found in *Geophilomorpha*.

The venom glands of most centipedes are pear-shaped, with the exception of scolopendrid centipedes where they are elongated and kidney-shaped. The proximal segments of the forcipules usually contain the venom gland, which line the cuticle along the outer curvature of the appendage and terminate near the base of the forcipule. There are, however, some interesting exceptions. Within the genus *Cryptops* (*Cryptopidae, Scolopendromorpha*), for example, glands can vary from pear-shaped organs occupying a significant volume of the forcipule to just a few glandular cells (Antoniazzi *et al*., 2009). Gland size also varies within the *Scolopendridae*, such as in *Asanada socotrana* and *Arthrorhabdus formosus* where they extend into the posterior part of the forcipular coxosternite (Edgecombe and Koch, 2008). The most extreme variation, however, can be found among geophilomorph centipedes. In *Henia vesuviana* (Dignathodontidae), the venom glands are located in the trunk, between the 12th and 18th segments, while in *Aphilodon angustatus* (*Aphilodontidae*) these are placed even further back into the trunk, between the 15th and 23rd segments (Undheim and

King, 2011). In the latter case, each gland is placed in front of the other and even occupies most of the volume of the three segments it spans (Pereira *et al*., 2007).

While the forcipules are modified walking appendages, the venom gland is thought to have evolved through invagination of the cuticle and weaponization of the cuticular dermal glands (Undheim and King, 2011). This is evident from the chitinous duct, and the observation that the venom gland is actually a composite glandular epidermis composed of discrete sub-glands, or secretory units. Each secretory unit includes a distal and a proximal canal cell, one or more secretory cells, and an intermediate cell that line an extracellular storage space. These secretory units are individually connected to the lumen through a one-way valve formed by the distal canal cell that penetrates the chitinous duct though a pore. Venom is then expelled from the porous region of the duct, known as the calyx, and through the distal non-porous duct that terminates as a pore ("meatus") located on the outer curvature near the tip of each claw (Bonato *et al*., 2010).

2.1.5 Centipede venom

The complete mechanism of centipede venom toxicity is not yet completely understood (Haug *et al*., 2014) and this venom, as well as the venom apparatus of centipedes, remains largely unexplored (Antoniazzi *et al*., 2009). It contains several different enzymes, but is different to most other arthropods in that metalloproteases appear to be important. Cardiotoxic, myotoxic and neurotoxic activities have been described, with the latter being unusual in the fact of involvement of G-protein coupled receptors, directly as neurotoxin-targets or indirectly by activation of endogenous agonists (Duboscq, 2008). Noteworthy, the contents of approximately 1000 venom glands are required for a fatal sting in an average adult (Undheim and King, 2011).

More specifically, the venom from *Scolopendra* species is a lipid-toxin complex, similar to that of scorpion, which facilitates local cellular penetration and absorption (Undheim and King, 2011). Its compounds include 5-hydroxytryptamine (serotonin), histamine and cardiodepressant Toxin-S, mediating significant cardiovascular effects, a smooth muscle contractile agent with muscarinic activity, proteinases and lipoproteins (Undheim and King, 2001). *Scolopendra heros* contains also a cytolysin potentiating both its local and systemic effects.

The Chinese red-headed centipede (*Scolopendra subspinipes: mutilans*) is a venomous centipede found in East Asia and Australia (Lewis, 2002) and its venom contains 26 neurotoxin-like peptides (Antoniazzi *et al.*, 2009). Several of these neurotoxins contain potential insecticidal abilities, and they act on voltage-gated Na^+, K^+, and Ca^{2+} channels (Lewis, 2002). Among them, neurotoxin SsmTx-I blocks voltage-gated K^+ channels in dorsal root ganglion neurons, but has no effect on voltage-gated Na^+ channels (Lewis, 2002). Moreover, peptide μ-SLPTX-Ssm6a inhibits voltage-gated Na^+ channels and is more potent analgesic than morphine in rodents (Edgecombe and Koch, 2008). Finally, toxin RhTx induces pain and targets the heat activation machinery (potently activates the capsaicin receptor TRPV1) to produce excruciating pain (Pereira *et al*., 2007).

2.1.6 Components of centipede venom

2.1.6.1 Enzymes

Mohamed and co-workers (Mohamed *et al*., 2003) were the first to show enzymatic activity in centipede venom, namely phosphatase and esterase activity from the venom of *Scolopendra morsitans*. Since then, 11 types of enzymes have been described from the venoms of *Scolopendromorpha* and *Scutigeromorpha*. Some of these have been shown by proteomic analyses to be abundant venom components, indicating that enzymes generally form an important component of centipede venoms (Liu *et al*., 2012). Although most

centipedes have well developed mandibles that are used for mastication of solid food prior to ingestion (Cohen, 2005), the substantial enzymatic component of their venom suggest that it may contribute to extra-oral digestion of prey.

2.1.6.1.1 Metalloproteases

Both activity- and sequence-based investigations have revealed that metalloproteases are important components of centipede venoms (Undheim *et al*., 2014). Transcriptomic and proteomic analyses of the venom proteome of *Thereuopoda longicornis* (*Scutigeromorpha, Scutigeridae*) revealed that astacin-like metalloendoproteases (MEROPS family M12, subfamily A) accounted for ~10% of venom proteins identified (Undheim *et al*., 2014). Similarly, analysis of venom by 2D PAGE revealed that proteins with weak sequence homology to blastula protease 10, an M12A member from sea urchin (UniProt: P42674, *E*-value 0.001), were abundant in *scolopendrid* species included in the same study. This suggests that metalloproteases in *scolopendrid* venoms could be derived members of the M12A subfamily, although proteolytic activity should be verified to confirm this. While no putative metalloproteases were reported from the venoms of *Scolopendra viridis* or *Scolopendra subspinipes dehaani* (Gonzalez-Morales *et al*., 2014), this may be due to the limitations of the analytical approaches taken. For example, a search against the full set of published centipede-venom protein sequences reveals an EST (NCBI accession number JZ574148) that is highly similar to members of the *scolopendrid* putative M12A family (lowest *E*-value 3 × 10−72, to GASH01000091). Moreover, conducting the same search using the tryptic fragments from spot 2 from the 2D-PAGE of *S. viridis* reveals that this protein is actually a member of the same protein family. Hence, M12A proteases are probably a plesiotypic characteristic of centipede venoms (Undheim *et al*., 2014).

Although the M12 family has been recruited into the venoms of most groups of venomous animals (Brust *et al*., 2013), the majority of these are members of subfamily M12B. Exceptions include metalloproteases in the venom from spiders of the genus *Loxosceles*, and from the nematocysts of the sea anemone *Nematostella vectensis*, which belong to M12A (Moran *et al*., 2013). Many members of the M12A subfamily cleave matrix proteins and could thereby facilitate the spread of other centipede-venom components (Bond and Beynon, 2005), a function that has also been suggested for spider-venom proteases (King and Hardy, 2013). In addition, venom metalloproteases are often involved in skin damage, oedema, blister formation, myonecrosis and inflammation, and this is consistent with several of the recurrent symptoms associated with centipede stings (Undheim and King, 2011).

2.1.6.1.2 Serine proteases

In addition to metalloproteases, serine protease activity has been demonstrated from *scolopendrid* centipede venom (Malta *et al*., 2008). Supporting this, both S1 and S8 type protease transcripts and venom proteins have been identified from both subfamilies of *Scolopendridae* (*Otostigminae* and *Scolopendrinae*) (Undheim *et al*., 2014). While venom S8 proteases appear to be unique to centipedes, S1 proteases have been widely recruited into animal venoms where they are involved in a range of functions, including vasodilation, smooth muscle contraction, anticoagulation and immunosuppression (Womg *et al*., 2012).

However, as evident from proteomic analyses, S1 and S8 proteases are not particularly abundant in centipede venoms, and proteolytic activity can be virtually abolished by incubating venom with the metal chelator 1,10-phenanthroline (Liu *et al*., 2012). Metalloproteases therefore appear to be the dominant form of proteases in centipede venom, and serine proteases may instead play a role in toxin processing (Knapp *et al*., 2010). This

suggests that toxins are activated during storage subsequent to release into the extracellular space, upon venom expulsion, or even both. Consistent with this hypothesis, Undheim and co-workers found that mature toxins encoded by multi-toxin transcripts are present in the venom gland (Unheim *et al*., 2014). It also raises the possibility that venom obtained by electrostimulation may contain unprocessed or partially processed toxins due to the involuntary secretion of venom, perhaps explaining the finding by Rates and co-workers (Rates *et al*., 2007) that the same toxin was present with and without a 10-residue *N*-terminal tail.

2.1.6.1.3 γ-glutamyl transpeptidase

γ-Glutamyl transpeptidases (GGTs) are enzymes involved in regulation of oxidative stress and xenobiotic detoxification (Couray *et al*., 2002). GGT was previously reported from the venom of parasitoid wasps, where it is proposed to induce apoptosis of host ovaries via oxidative stress (De Graaf *et al*., 2010). Although it appears not to be present in other centipede venoms, transcriptomic and proteomic analyses show that GGT is both highly expressed and abundant in *scolopendrine* (*Scolopendridae*) venoms (Undheim *et al*., 2014). Centipede-venom GGT induces aggregation of human platelets and hemolysis of red blood cells from mice and rabbits but not humans (Liu *et al*., 2012). However, targeting vertebrate hemostasis is unlikely to be the primary function of centipede-venom GGT due to the small body size of many *scolopendrid* species in which GGT forms a major venom component (e.g., *Cormocephalus*) (Undheim *et al*., 2014). Nevertheless, the abundance of GGT suggests that it is an important constituent of *scolopendrine* venoms that was probably recruited into the venom subsequent to the split between the two *scolopendrid* subfamilies approximately 230 mya (Joshi and Karanth, 2011).

2.1.6.1.4 Glycoside hydrolases

Members of the glycoside hydrolase (GH) superfamily hydrolyze the glycosidic bond between carbohydrates or between a carbohydrate and a non-carbohydrate moiety. Three glycoside hydrolase families have been found in venoms from *Scolopendridae*, namely chitinase (GH family 18; *Cormocephalus*), lysozyme (GH family 22; *Scolopendra*), and hyaluronidase (GH family 56; *Scolopendra*, *Ethmostigmus*, and *Otostigmus*) (Gonzalez-Morales *et al*., 2014). While chitinases are found in several venoms and could perhaps aid in digestion of arthropod prey (Balasubramanian *et al*., 2012), lysozyme hydrolyses β-1,4-links between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine in the peptidoglycan of bacterial cell walls and could therefore act as an antibacterial agent (Undheim, *et al.,* 2014). Hyaluronidases hydrolyse non-sulfated glycosaminoglycans that are widely distributed in connective, epithelial, and neural tissues as well as extracellular matrix, and hence are often regarded as "spreading factors" that increase the pathological impact of other venom components (Violette *et al*., 2012).

2.1.6.1.5 Phospholipase A2 (PLA2)

The PLA2 are found in a very wide range of animal venoms, where they display a diverse array of catalytic and derived non-catalytic activities (Fry *et al*., 2009). In centipedes, however, PLA2 activity has so far been found only in *Scolopendrid* venoms (Liu *et al*., 2012). Phylogenetic analysis of centipede-venom PLA_2 revealed that they form a monophyletic group and thus originate from a single recruitment event (Undheim *et al*., 2014). According to the available data, this probably occurred prior to the split between the two *Scolopendrid* subfamilies approximately 230 mya but subsequent to the split from *Cryptopidae* ~200 mya (Fernandez *et al*., 2014). Centipede-venom PLA_2 are also unique in

that they form a sister-clade to Group X-related PLA_2, unlike any venom or invertebrate PLA2 described to date (Undheim *et al*., 2014).

Although PLA_2 was recruited into the venom of a *Scolopendrid* ancestor, not all centipede venoms have PLA_2 activity. PLA_2 hydrolyse glycerophospholipids at the *sn*-2 position to release lysophospholipids and fatty acids such as arachidonic acid. However, neofunctionalisation of snake-venom PLA_2 often removes the ability to catalyse this reaction (Fry *et al*., 2009), and this may also be the case for PLA_2 in *scolopendrid* venoms. For example, ScolPLA from the venom of *Scolopendra viridi* has a high level of PLA2 activity, but no PLA2 activity was detected in venom from a *Scolopendra* sp. collected in the same locality (Gonzalez-Morales, 2009). Neofunctionalisation might also explain the low PLA_2 activity found in the venoms of *Otostigmus pradoi* and *Scolopendra viridicornis*, although the abundance of PLA_2 in these venoms were not determined (Malta *et al*., 2008). In some cases, PLA_2 appears to have been secondarily lost, such as in *Cormocephalus westwoodi* where no PLA_2 was detected in the venom proteome and only transcripts containing numerous stop codons were found in the venom-gland transcriptome (Undheim *et al*., 2014).

2.1.6.1.6 Other enzymes

In addition to the abundant and commonly recruited enzymes described above, a number of other less abundant or unusual enzymes have been found in centipede venoms. Among these is glucose-6-phosphate dehydrogenase (EC 1.1.1.49). The role of this enzyme in venoms remains to be determined, but proteomic data indicate that it is relatively abundant in *Scolopendrid* venoms and is potentially present in scutigerid venoms (Undheim *et al*., 2014). Glucose-6-phosphate dehydrogenase catalyses the first step of the pentose phosphate pathway (Bak, 2007) but this ancestral activity is unlikely to contribute to toxin processing

or venom toxicity. Thus, given its abundance, venom glucose-6-phosphate dehydrogenase likely represents a case of protein neofunctionalisation.

Perhaps the most novel enzyme found in centipede venom is centipede peptidyl arginine deiminase (centiPAD). This enzyme has not been reported from any other animal venom, but several isoforms were detected in venom from the scutigerid *T. longicornis* (Undheim *et al*., 2014). CentiPADs are distinct from mammalian PADs but similar to *Porphyromonas*-type peptidyl arginine deiminase, which catalyses deamination of the guanidino group on *C*-terminal arginine residues to yield ammonia and a citrullinated residue (Shirai *et al*., 2001). The function of CentiPADS in the venom or venom gland remains to be determined, but they might be involved in posttranslational modification of toxin arginine residues (Undheim *et al*., 2015).

Judging from proteomic data, esterases are among the least abundant enzymes in centipede venoms that are commonly found in other animal venoms. Esterases have been reported from the venoms of diverse taxa such as spiders (Rodrigues *et al*., 2006), snakes (Tu and Chua, 2006) and octopus (Undheim *et al*., 2010), and in fact the first enzymatic activity reported from centipede venom was esterase activity noted in venom-gland extracts of *Scolopendra morsitans* (Mohammed *et al*., 2003). This activity is likely due to type B carboxyl esterase, which was subsequently found in the venom of *Cormocephalus westwoodi* and identified in venom-gland transcriptomes from the scolopendrids *S. morsitans* and *S. alternans*, and the scutigerid *T. longicornis* (Undheim *et al*., 2014). Venom carboxyl esterases have been proposed to play a part in the release of endogenous purines during envenomation, which then act as "multitoxins" that cause a multitude of pharmacological effects including immobilization through hypotension (Dhananjaya and Souza, 2010). However, the function of centipede-venom esterases remains to be determined.

2.1.6.2 Non-enzymatic proteins

2.1.6.2.1 Centipede β-pore-forming toxins

Among the proteomically most abundant and most highly expressed proteins in centipede venoms are putative β-pore-forming toxins (β-PFTx) (Undheim *et al*., 2014). These toxins were probably recruited into an early common centipede ancestor more than 430 mya and have subsequently undergone extensive radiation (Fernandez *et al*., 2014). β-PFTx contain a pore-forming domain termed the β-complex domain. This structural domain, which is directly involved in pore formation, is characteristic of the aerolysin-like β-pore-forming toxin superfamily. Pore formation occurs via assembly of toxin monomers to form a β-barrel, which then undergoes a conformational change and inserts into the membrane to form a transmembrane pore (Knapp *et al*., 2010). Oligomerization of β-PFTx monomers is mediated by binding of the toxins to various cell-surface receptors via additional toxin domains; thus, the diversity of centipede β-PFTx might enable them to target a wide variety of cell types and tissues and assert multiple toxinological functions. Aerolysin requires proteolytic activation in order to oligomerize into a pore-forming heptamer, and this could be carried out by a number of proteases including S1 and S8 types (Knapp *et al*., 2010). Thus, one possible function of centipede-venom serine proteases might be activation of β-PFTx upon envenomation.

Although the pore-forming properties of centipede β-PFTx have yet to be directly demonstrated, they might be at least partly responsible for the cytolytic activity of centipede venoms (Malta *et al*., 2008). Pore-forming activity by β-PFTx might also explain the report that an 80-kDa centipede-venom protein induced an increased leak current in giant axons of the American cockroach *Periplaneta americana* (Stankiewicz *et al*., 2009). β-PFTx might also contribute to the myotoxic and oedematogenic activities of centipede venoms that are evident in the symptoms associated with human envenomations (Undheim and King, 2011).

2.1.6.2.2 CAP proteins

The CAP proteins have been widely recruited into animal venoms, where they can function as ion channel modulators, vasodilators, myotoxins, or even proteases (Moran *et al*., 2013). They constitute a major component of centipede venom, and phylogenetic analysis indicates that they have been recruited into centipede venom on three separate occasions: once in an early ancestor over 430 mya (Type 1; centiCAP1), once in a scolopendrid ancestor at least 200 mya (Type 2; centiCAP2), and once within the past 100 million years in the genus *Scolopendra* (Type 3; centiCAP3) (Joshi and Karanth, 2011). CentiCAP1 have only been found in the scutigerid *T. longicornis* and the scolopendrid *E. rubripes*, while centiCAP3 have been reported only in *S. morsitans* (Undheim *et al*., 2014). CentiCAP2 are the dominant form in *Scolopendrinae*, where they have diversified into multiple subtypes and undergone neofunctionalisation to include inhibitors of trypsin and voltage-gated calcium (CaV) channels (Gonzalez-Morales *et al*., 2014). The activities of centiCAP1 and centiCAP3, and most centiCAP2, remain to be determined but they might be of clinical relevance by virtue of their high abundance. CAP proteins are among the principal allergens in vespid and fire ant (*Solenopsis* spp.) venoms (Hoffman, 2006), and therefore the relatively frequent allergic reactions observed after centipede envenomation (Undheim and King, 2011) might be due at least in part to the abundant centiCAPs.

2.1.6.2.3 LDLA domain-containing proteins

In addition to β-PFTx and centiCAPs, proteomic analyses show that novel proteins containing a low-density lipoprotein receptor Class A repeat (LDLA) domain are a major constituent of centipede venoms (Undheim *et al*., 2014). The LDLA structural domain, which comprises a β-hairpin motif followed by a series of β turns, is present in a wide variety of proteins (Daly *et al*., 2005). The LDLA-proteins were recruited in an early

centipede ancestor at least 430 mya, and they have subsequently undergone substantial diversification (Undheim *et al*., 2014). The LDLA proteins have not been reported from any other venom, and the function of LDLA-containing centipede-venom proteins remains to be determined. Nevertheless, the abundance and diversification of centipede-venom LDLAs suggests that they are important components of the venom (Undheim *et al*., 2015).

2.1.6.2.4 Other non-enzymatic proteins

In addition to the abundant protein families described above, centipede venoms contain a number of other proteins that are probably non-enzymatic, including transferrin and cystatin. Transferrin has been identified in venom-gland transcriptomes from both scolopendrid subfamilies, the scutigerid *T. longicornis*, and the venom of *E. rubripes* and *S. morsitans* (Undheim *et al*., 2014). Centipede-venom transferrins may have an antibacterial function since invertebrate transferrins have been implicated in pathways involved in the reaction to secondary infections (Ramieez-Gomez, 2008).

Two isoforms of cystatin were identified in venom from the scolopendrid *E. rubripes* (Undheim *et al*., 2014). Cystatins are potent inhibitors of papain family cysteine proteases, although they have acquired new functions when recruited into reptile venom, *Lonomia* caterpillar bristles, and the saliva of ticks and mosquitoes (Fry *et al*., 2013). However, both of the centipede-venom isoforms contained the characteristic peptidase-interacting sequence Gln-Xaa-Val-Xaa-Gly as well as the cystatin type-1 like Pro-Gly pair, suggesting that they have retained their ancestral function as peptidase inhibitors (Abrahamson *et al*., 2003).

The majority of non-enzymatic protein families found in centipede venoms appear to be novel; they cannot be assigned a putative function or to a known protein family. Undheim and co-workers (2014) identified eleven protein families in venoms from three scolopendrids and one scutigerid, as well as two protein families containing only domains

of unknown function (DUF). One of these domains (DUF 1397) was identified only in scutigerid venom while the other (DUF 3472) was only found in the scolopendrid venoms and transcriptomes.

2.1.7 Physiological effects of centipede venom

Centipede venom comprises a complex mixture of toxins which show both structural and functional diversity. *Scolopendra mojiangica,* and S. *subspinipes mutilans* possess toxin-like molecules which display strong hemolytic and anti-insect activity (Liu *et al*., 2020). Centipede venom imposes severe pain and other multiple physiological effects such as anticoagulant, platelet aggregating, ion channel inhibition and nerve and muscle cell damage and necrosis (Ross *et al*., 2022). Centipede toxins showed myotoxic, cardiotoxic, and neurotoxic activities. Moreover, severity centipede envenomation increases with the time if treatment is not being made available to the victim, it becomes fatal. Therefore, centipede venom evaluation and management is quite important (Cooper *et al*., 2014). Centipede venoms showed the following effects:

2.1.7.1 Neurotoxic

Centipede venom possesses neurotoxins (*S. subspinipes dehaani*) which obstruct structure and function of ion channel. Centipede neurotoxins target brain or the thoracic ganglia affect limb movement (Dugon and Arthur, 2012). Toxin peptides isolated from *S. subspinipes dehaani* showed diverse physiological effects like platelet aggregating activity; anticoagulant, phospholipase A_2 activity; trypsin inhibiting activity; voltage-gated potassium channel activities; voltage-gated root ganglion neurons inhibition. SsmTx-I also act as a simple inhibitor or channel blocker rather than a gating modifier (Chen *et al*., 2014). It selectively blocks the Kv2.1 current with an IC_{50} value of 41.7 nm. These also showed voltage-gated calcium channel activities (Liu *et al*., 2012).

Neurotoxins bind directly to G-protein coupled receptors and these indirectly activate endogenous agonists as shown in figure 2.1. SsmTX-I possesses intra-molecular disulfide bridge motifs Cys1-Cys3 and Cys2-Cys4 and shows analgesic activity (Wang *et al*., 2017). SsmTx-I significantly blocked voltage-gated K^+ channels in dorsal (Chen *et al*., 2014).

2.1.7.2 Haemolytic activity

Centipede venom causes severe pain, paresthesia and oedema, which may develop into superficial necrosis. Centipede venom shows caseinolytic, fibrinogenolytic and gelatinolytic activities. *S. viridicornis* and *O. pradoi* venoms showed hyaluronidase activity. Most of the centipede venoms showed nociception, edema and myotoxicity in mice, but only *S. viridicornis* and *O. pradoi* venoms cause venoms intense direct hemolytic activity on human erythrocyte (Malta *et al*., 2008).

2.1.7.3 Cytotoxic

The purified proteins/peptides showed different pharmacological properties, like platelet aggregating activity; anticoagulant activity; phospholipase A activity; trypsin inhibiting activity; voltage-gated potassium channel activities; voltage-gated sodium channel activities; voltage-gated calcium channel activities (Liu *et al*., 2012). The centipede peptide toxins effect on ion channels, including Nav, Kv, Cav and the nonselective cation channel polymodal transient receptor potential vanilloid 1 (TRPV1) (Rong *et al*., 2015). *S. viridis* centipede venom possesses a variety of low-molecular-weight peptide toxins which after infliction shows immediate effects on their prey (Gonzalez-Morales *et al*., 2014). These target voltage-gated ion channels to interfere with the central system of prey and produce pain or paralysis for efficient hunting.

Centipede peptide toxins confer chemical, thermal and biological stability. These toxins possess the high potency and specificity and could be used as diagnostic tools and in the

treatment of human diseases. SsmTx displays a unique cysteine motif that is completely different from that of other venomous animal toxins (Undheim *et al*., 2015).

2.1.7.4 Muscle damage and necrosis

A different biochemical composition of *S. polymorpha* venom, based on the different effects of four venom fractions on the cells tested, according to statistical evidence. Fractions F6 and F7 caused the most important alterations. Venom-exposed EDL muscle showed signs of muscle damage including necrosis, loss of fascicular structure as well as mitochondrial accumulations and ragged red fibers (RRF), suggesting impairment in the normal mitochondrial arrangement. Nicotinamide adenine dinucleotide (NADH) and cytochrome c oxidase (CcO) tests also indicate that respiratory complexes might be affected (Robles *et al*., 2020).

2.1.7.5 Inflammatory

Centipede envenomation cause local inflammation, intense pain and edema. *Scolopendra viridicornis* centipede envenomation induce leukocyte infiltration mainly neutrophils and monocytes/ macrophages with production of pro-inflammatory mediators. Moreover, *S. viridicornis* venom stimulated the release of IL-6, MCP-1, KC, and IL-1β (Kimura *et al*., 2013). *S. viridicornis* venom shows cytotoxic effects and induces morphological changes in RBL-2H3 line. However, lower doses of the venom induced degranulation of both mast cell lines, as well as the secretion of MCP-1, IL-6 and IL-1β (Tavora *et al*., 2016). *S. viridicornis* venom exposure, mast cells and histamine are crucial for the establishment of the local inflammatory reaction.

2.1.7.6 Channel inhibitors

Centipede venom possesses low molecular weight toxins which after infliction from pod fangs reach into the blood stream. These selectively bind to the nociceptive transient receptor potential vanilloid 1 (TRPV1) ion channel. This is a polymodal receptor for

multiple painful stimuli, to which small peptide toxin RhTx2 from the Chinese red-headed centipede binds and strongly modulates TRPV1 activities (Zhu *et al*., 2020). The most abundant toxins expressed in the venom of *S. viridis* belonged to calcium and potassium ion-channel toxins, venom allergens, metalloproteases, and β-pore forming toxins (Ward and Rokyta, 2018).

A major toxin component [Ssm Spooky Toxin (SsTx)] in centipede venom inhibits the sales channel in conspecifics but not in heterospecifics to cause short-term, recoverable, and nonlethal envenomation. This same toxin causes fatal heterospecific envenomation, for example, by switching its target to the Shaker channels in heterospecifics without inhibiting the Shaker channel of conspecific *S. subspinipes* individuals. These findings suggest that the venom components exhibit intricate coevolution with their targets in both heterospecifics and conspecifics, which enables a single toxin to develop graded intraspecific and interspecific antagonistic interactions (Yang *et al*., 2020).

Ssm Spooky Toxin (SsTx) isolated from red-headed centipede is a potent blockage of the expressed potassium, calcium, and sodium gated (KCNQ) channels to simultaneously and efficiently disrupt cardiovascular, respiratory, muscular, and nervous systems. SsTx is a basic 53 residues compound that is main killer arsenal in centipedes' defense and predation. It inhibits KV1.3 channel, Both SsTx and its mutant SsTx-R12A inhibit cytokines production in T cells without affecting the level of KV1.3 expressions (Du *et al*., 2019). SsTx blocks KCNQ potassium channels to exert the lethal toxicity (Luo *et al*., 2011). Centipedes' venom has evolved to simultaneously disrupt cardiovascular, respiratory, muscular, and nervous systems by targeting the broadly distributed KCNQ channels, thus providing a therapeutic strategy for centipede envenomation (Luo *et al*., 2011). Venom toxinSsTx blocks KCNQ potassium channels and impose lethal toxic effects and kill in its prey (Hamanaka and Mori, 2020). Figure 2.1 below shows the summary of the physiological effects of centipede venom.

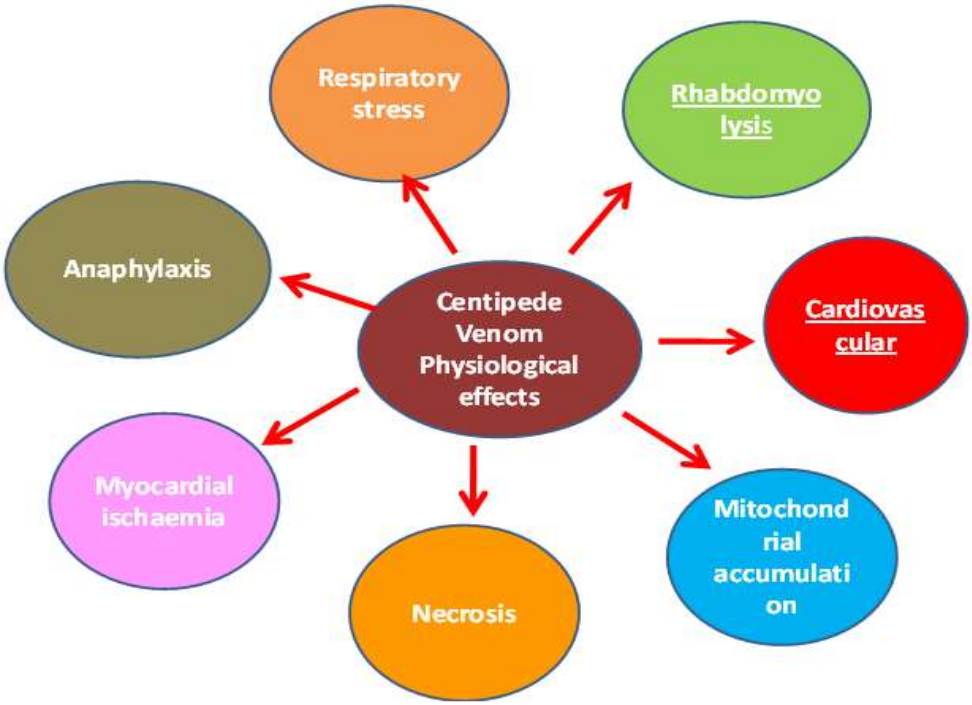


Figure 2.2: Physiological effects of centipede venom (Luo *et al*., 2011).

2.1.8 Therapeutic uses of centipede venom

Centipedes are venomous terrestrial predators; its venoms are complex cocktails of water, salts, small bioactive molecules, peptides, enzymes and larger proteins, with peptides usually comprising the majority of toxins (Herzig, 2018). Centipede venom is used in traditional medicine in China for the treatment of many disorders, such as stroke-induced hemiplegia, epilepsy, apoplexy, whooping cough, tetanus, burns, tuberculosis, as well as myocutaneous disease (Fratini *et al*., 2017). Centipede venom is also used for the treatment of cardiovascular diseases in Korea, China, and other Far Eastern Asian countries (Kong *et al*., 2013). Centipede venoms are highly complex chemical arsenals that are rich in dissolved-constrained peptides. Centipede venoms peptides target neuronal ion channels and receptors (Undheim *et al*., 2016).

The venom of centipedes could be an excellent source of peptides for developing drugs for treatments as well as bio-insecticides for agrochemical applications (Hakim *et al*., 2015). Its peptides, enzymes, exhibit a large array of anticancer and anti-pathogenic activities (Khusro *et al*., 2018). Voltage-gated sodium channel NaV 1.7 serves as an attractive target for chronic pain treatment. These could be used to make analgesic. The SP-TOX, selectivity and potency of Ssm6a upon NaV 1.7 decreases inflammatory pain in a rat model (Wang *et al*., 2017). Centipede venoms are complex mixtures of biochemically and pharmacologically active components such as peptides and proteins (Peng *et al*., 2010). These include ion channel modulators, antimicrobial peptides, different enzymes, enzyme inhibitors, anticancer peptides, antithrombotic peptides, as well as anticoagulants and centipede extracts (Kong *et al*., 2013). These possess novel pharmacology and three-dimensional structure and could be more useful for therapeutic purposes (Kong *et al*., 2013).

Centipede venom is a rich and complex natural source of bioactive proteins, peptides and other small molecules that aid in predation or defense. The venom can induce myotoxic, cardiotoxic, neurotoxic and other toxic effects. It also shows myocardial ischemia and infarction, hematuria, hemoglobinuria, rhabdomyolysis, hemorrhage, pruritus, eosinophilic cellulitis, as well as anaphylaxis. More prevalent are symptoms including pain, paresthesia, lethargy, localized necrosis, headache, dizziness and nausea. The constituents target different cellular processes and pathways which in turn trigger a cascade of physiological reactions in the victim (Ombati *et al*., 2018).

2.1.8.1 Allergic activity

Major centipede allergen Sco m 5 from *Scolopendra subspinipes mutilans.* Sera positive for Sco m 5 IgE-binding was cross-reactive against venom from the wasp Vespa mundane. The use of Sco m 5 to identify centipede-allergic individuals could be important, given the high potential allergenicity of Sco m 5 among the general Chinese population, along with the likely possibility of cross-reactivity against wasp venom among centipede-allergic patients (Lan *et al*., 2021).

2.1.8.2 Anticancer activity

Scolopendrasin VII isolated from the Centipede, *Scolopendra subspinipes mutilans* induce necrosis was mediated by specific interaction with phosphatidylserine. It makes pores in membrane of cancer cells (Lee *et al*., 2015). It stimulates macrophages and neutrophil activity. This is inhibited by the formyl peptide receptor 1 (FPR1) antagonist cyclosporine H (Park *et al*., 2015). A novel antimicrobial peptide acting via formyl peptide receptor 2 shows therapeutic effects against rheumatoid arthritis. In oriental medicine, centipede *Scolopendra subspinipe smutilans* has long been used as a remedy for rheumatoid arthritis (RA), a well-known chronic autoimmune disorder (Park *et al*., 2018). Scolopendrasin X strongly stimulates mouse neutrophils, results in intracellular increase of calcium ions, and

chemotactic migration through pertussis toxin-sensitive G-protein and phospholipase C pathway, and increased superoxide anion production in neutrophils. Another toxin peptide isolated from *Scolopendra subspinipe mutilans* peptide scolopin 1 (AMP-scolopin 1) showed broad-spectrum activities against bacteria, fungi, and tumor cells. Similar activity is reported in recombinant scolopin1 and synthetic scolopin 1 (Hou *et al*., 2013).

2.1.8.3 Analgesic activity

SsmTX-I specifically blocks Kv2.1 ion channel and showed analgesic potential. SsmTX-I contains intramolecular disulfide bridge that possess motifs specially having Cys1-Cys3 and Cys2-Cys4 (Wang *et al*., 2017). Animal venom peptides have proven to have potential as new types of analgesic medicine.

2.1.8.4 Arthritis

Scolopendra spp. venom is used to treat arthritis in Korean traditional medicine (Pemberton, 2009).

2.1.8.5 Antimicrobial activity

Centipedes possess novel peptides which show strong antimicrobial activity against various pathogens. These peptides (AMPs) also play important role in Ecdysis process of centipede *Scolopendra subspinipes subspinipes* (Chaperro *et al*., 2019). These also show broad-spectrum antibacterial activity (Ali *et al*., 2021). Centipede venoms contain toxins which serve as AMPs and were found active against several bacteria (Fratini *et al*., 2017). One of the most important cellular events in arthropods is the melting of the cuticle (Ecdysis). The centipede *Scolopendra subspinipe smutilans* is a medically important arthropod species. Although this species are increasingly applied as a reliable source of new antimicrobial peptides, the transcriptome of this species is a prerequisite for more rational selection of antimicrobial peptides (Yoo *et al*., 2014). Antifungal activity is reported in antimicrobial

peptide, scolopendin 1, derived from centipede *Scolopendra subspinipe mutilans* (Lee *et al*., 2017). Scolopendin 2 (AGLQFPVGRIGRLLRK) is also isolated from same species, it is a cationic antimicrobial peptide isolated from centipede. This, scolopendrasin II bound to the surface of bacteria through a specific interaction with lipoteichoic acid and a lipopolysaccharide, which was one of the bacterial cell-wall components. It shows broad-spectrum antimicrobial effects by forming pores in the cell membrane of pathogens (Lee *et al*., 2015) Scolopendrasin II may be useful for developing peptide antibiotics (Kwon *et al*., 2013).

It also Scolopendin 1 exerted an antimicrobial activity without inducing haemolysis of human erythrocytes (Choi *et al*., 2014). These antimicrobial peptidesalso evoke an innate immune response (Lee *et al*., 2017) the antimicrobial peptide scolopendrasin VII, derived from *Scolopendra subspinipe smutilans* stimulates macrophages, resulting in chemotactic migration via FPR1 signaling, and the peptide. *Scolopendra subspinipe smutilans* stimulates macrophage chemotaxis via formyl peptide receptor 1. It is also used to treat rheumatoid arthritis (RA), a well-known chronic autoimmune disorder (Park *et al*., 2018).

An anticancer activity of the antimicrobial peptide scolopendrasin VII derived from the Centipede, *Scolopendra subspinipe smutilans* induce necrosis that are mediated by specific interaction with Phosphatidylserine, which is enriched in the membrane of cancer cells. (Lee *et al*., 2015). Scolopendrasin X strongly stimulated mouse neutrophils, resulting in intracellular calcium increase, chemotactic migration through pertussis toxin-sensitive G-protein and phospholipase C pathway, and increased superoxide anion production in neutrophils (Park *et al*., 2017). AMPs from centipede venoms are promising biologically active molecules (candidates) which work against bacteria, protozoans, fungi and viruses (Primon and Jose, 2017).

Antimicrobial peptide scalloping 1 (AMP-scalloping 1) is a small cationic peptide identified from centipede venoms of *Scolopendra subspinipe smutilans*. It has broad-spectrum activities against bacteria, fungi, and tumor cells, which may possibly be used as an antimicrobial agent. The recombinant scolopin1 had similar antimicrobial properties to the synthetic scalloping 1 (Huo *et al*., 2013).

2.1.8.6 Insecticidal

Centipedes use the toxins as poisonous arsenals for prey capture and defense against predators. These possess insecticidal neuropeptides similar to polyamine-like compounds. These can be sued to make bioinsecticide to control insect pests. *Scolopendra subspinipe smutilans* crude venom showed strong insecticidal activity (Liu *et al*., 2018).

2.2 Wall gecko

The common house gecko (*Hemidactylus frenatus*) is a gecko native to South and Southeast Asia as well as Oceania. It is also known as the Asian house gecko, Pacific house gecko, wall gecko, house lizard, tiktiki, chipkali or moon lizard (Ota and Whitaker, 2010).

Most geckos are nocturnal, hiding during the day and foraging for insects at night. They can be seen climbing walls of houses and other buildings in search of insects attracted to porch lights, and are immediately recognisable by their characteristic chirping (Ota and Whitetaker, 2010).

Plate 2.1: Common house gecko (Carranza, 2006)

2.2.1 Taxanomy of wall gecko

Taxonomy work on the genus *Hemidactylus* is particularly difficult. Most taxonomic work of DNA sequencing is done on remote small species on remote islands. External features alone are not suitable for taxonomic identification since they are plastic in appearance and often their features appear differently within species and across geo-locations. This results in the difficulty of producing unambiguous identity keys based on morphology (Carranza, 2006).

Kingdom:	*Animalia*
Phylum:	*Chordata*
Subphylum:	*Vertebrata*
Class	*Reptilia*
Order:	*Squamata*
Suborder:	*Sauria*
Family:	*Gekkonidae*
Genus:	*Hemidactylus*
Species:	*frenatus*

2.2.2 Morphology and habitat of wall gecko

They grow to a length of between 7.5 – 15 cm (3–6 inches), and live for about 7 years. Most medium-sized to large geckos are docile, but may bite if distressed, which can pierce skin. The common house gecko is tropical, and thrives in warm, humid areas where it can crawl around on rotting wood in search of the insects it eats, as well as within urban landscapes in warm climates. The animal is very adaptable and may prey on insects and spiders, displacing other gecko species which are less robust or behaviourally aggressive. In parts of Australia and Papua New Guinea they are often confused with a similar native lizard, the dubious dtella (McKay *et al.*, 2009).

The common house gecko is by no means a misnomer, displaying a clear preference for urban environments. The synanthropic gecko displays a tendency to hunt for insects in close proximity to urban lights (Newbery and Junes, 2007). They have been found in bushland, but the current evidence seems to suggest they have a preference for urban environments, with their distribution being mostly defined by areas within or in close proximity to city bounds (Keim, 2002). https://en.wikipedia.org/wiki/Common_house_gecko - cite_note-7.

The selection of primarily urban habitats makes available the preferred foods of the common house gecko. The bulk of the diet of the gecko is made up of invertebrates, primarily hunted around urban structures (Newbery and Jones, 2007). Primary invertebrate food sources include cockroaches, termites, some bees and wasps, butterflies, moths, flies, spiders, and several beetle groupings (Newbery and Jones, 2007). There is limited evidence that cannibalism can occur in laboratory conditions, but this is yet to be observed in the wild (Gallina-Tessaro *et al.*, 2008).

2.2.3 Geographical distribution of wall gecko

The common house gecko appears to prefer areas in the light which are proximal to cracks, or places to escape. Geckos without an immediate opportunity to escape potential danger display behavioural modifications to compensate for this fact, emerging later in the night and retreating earlier in the morning (Rodder *et al.*, 2008). Without access to the urban landscape, they appear to prefer habitat which is composed of comparatively dense forest or eucalypt woodland which is proximal to closed forest (McKay *et al.*, 2008).

The selection of primarily urban habitats makes available the preferred foods of the common house gecko. The bulk of the diet of the gecko is made up of invertebrates, primarily hunted around urban structures (Brisbane *et al.*, 2021). Primary invertebrate food

sources include cockroaches, termites, some bees and wasps, butterflies, moths, flies, spiders, and several beetle groupings (Brisbane *et al.*, 2021).

In Mexico, *H. frenatus* was first collected in Acapulco, Guerrero, in March of 1895 and found to be well established there and in the surrounding regions by the early 1940s. It was likely introduced through shipping and cargo. *H. frenatus* now occurs throughout the lowlands of Mexico on both the Atlantic and Pacific versants including the Yucatan Peninsula, and Baja California, with records from 21 of the 32 Mexican states. Most records of *H. frenatus* in Mexico are from buildings such as homes, hotels, and other structure in cities and towns, with only a few reports of the species in natural habitat, and its impact, if any, on native fauna there is unknown (Farr, 2011).

2.2.4 Physiology of wall gecko

The common house gecko is ectothermic ("cold-blooded") and displays a variety of means of thermo-regulating through behaviour. Its physiology has ramifications for its distribution and nature of interaction with native species, as well as reproductive success as an introduced species (Griffing *et al.*, 2022).

Metabolically, the demand of the common house gecko is not significantly variable from other lizard species of a similar size, with oxygen consumption appearing congruent with trends observed in other tropical, subtropical and temperate species of gecko. Thermal independence exists between 26–35 degrees, with some capacity to self-regulate temperature. This means that where the environmental temperature is 26–35 degrees, the common house gecko can modify body temperature through behavioural adaptations. Breathing rates of geckos are temperature dependent above this maximal heat, but independent as it grows colder (Snyder and Weathers, 2006). There are behavioural

mechanisms of thermoregulation present, such as the selection of sunlight and the substrates on which they sit (Licht *et al.*, 2006).

The common house gecko can be best defined as quinodiurnal. This means they thermo-regulate during the daytime and forage at night (Dame and Petren, 2006). An active form of this thermoregulation includes the presence of the gecko in lighter environments, proximal to cracks in the substrate. As such, there is a close relationship between activity levels and correlated air temperature (Griffing *et al.,* 2022). Timing of the circadian rhythm of the common house gecko is further impacted by light levels. This rhythm tends to involve the highest population presence around midnight, with highest activity levels just after sunset (Dame and Petren, 2006), with a gradual reduction until dawn. Daily cycle differences from place to place can generally be explained by environmental factors such as human interaction, and structural features (Griffing *et al.*, 2022). A peak in hunting activity after dark places them in an ideal spot to take advantage of invertebrate congregation around artificial lighting in the urban environment.

2.3 Liver

Liver is an organ in the upper abdomen that aids in digestion and removes waste products and worn-out cells from the blood. It is a vital organ present in vertebrate and some other animals, which has a wide range of functions including detoxification and protein synthesis. The liver is our greatest chemical factory, it builds complex molecules from simple substances absorbed from the digestive tract, it neutralises toxins, it manufactures bile which aids fat digestion and removes toxins through the bowels (Maton *et al.,* 2003). But the ability of the liver to perform these functions is often compromised by numerous substances we are exposed to on a daily basis; these substances include certain medicinal agents which when taken in over doses and sometimes when introduced within therapeutic ranges injures the organ (Gagliano *et al.,* 2007).

Liver disease is a worldwide problem. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease in order to replace currently used drugs of doubtful efficacy and safety (Ozbek *et al.*, 2004). In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders.

However, we do not have satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver, so the search for effective hepatoprotective herbs continues.

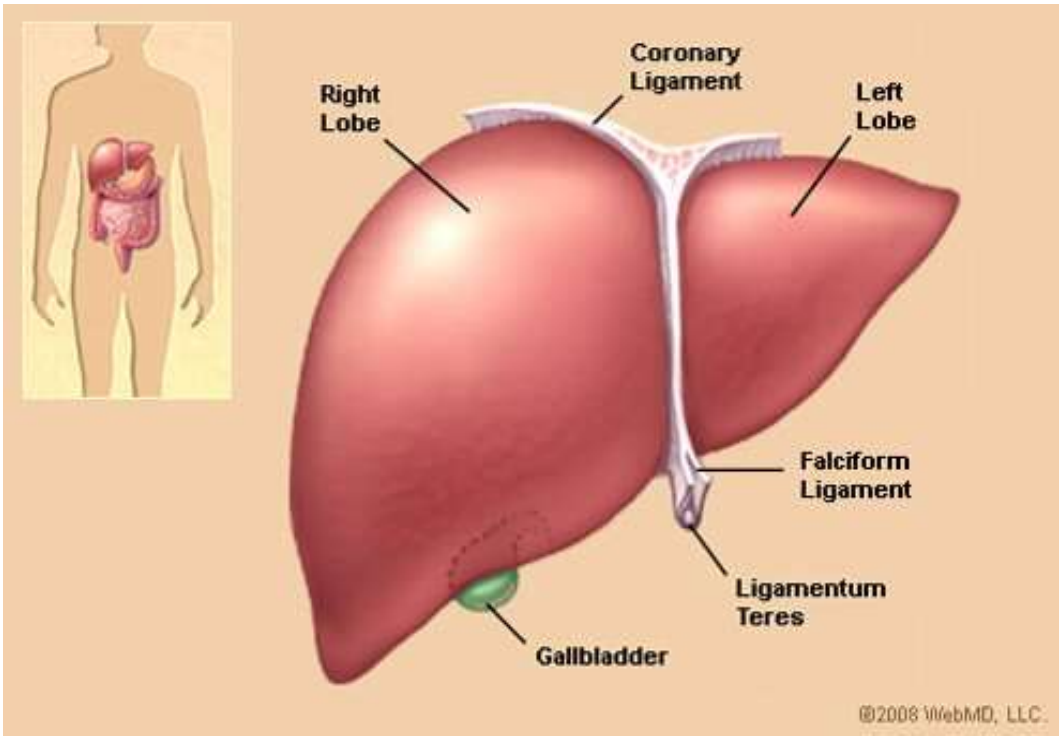


Figure 2.3: Intrahepatic vascular and biliary anatomy (Decker, 2007).

2.3.1 Livermarker enzymes

Liver function tests (LFTs or LFs), are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver (Lee, 2009). According to some, liver transaminases (AST/ALT (SGOT/SGPT) are *not* liver function tests, but are biomarkers of liver injury in a patient with some degree of intact liver function. Other sources include transaminase (McClatchey, 2002).

2.3.1.1 Aspartate aminotransferase (AST)

This is present in many tissues and is useful in evaluating muscle and liver damage in small and large animals. AST is not liver specific in any domestic animal species and the reference range in horses is rather broad. Skeletal muscle is the second largest source of AST in animals (Satué *et al.*, 2022).

Alanine aminotransferase is the most frequently relied biomarker of hepatotoxicity (Dufour *et al*., 2000). It is a liver enzyme that plays an important role in amino acid metabolism and gluconeogenesis. It catalyzes the reductive transfer of an amino group from alanine to α-ketoglutarate to yield glutamate and pyruvate. Normal levels are in the range of 5-50 U/L. Elevated level of this enzyme is released during liver damage. The estimation of this enzyme is a more specific test for detecting liver abnormalities since it is primarily found in the liver (Nathwani *et al*., 2005)

In combinations with the physical examination and history, the evaluation of other serum enzymes should aid in differentiating the source of increased AST levels. AST is present in both the cytoplasm and mitochondria of hepatocytes *(and many* other cells) and will elevate in states of altered membrane permeability. In such cases, levels are expected to be less than in states of frank necrosis, when both cytoplasmic and mitochondrial enzymes are released (Wacquier *et al.,* 2019).

2.3.1.2 Alanine aminotransferase (ALT)

ALT is considered to be liver specific in small animals. This enzyme is present in high concentrations in the cytoplasm of hepatocytes. Plasma concentrations increase with hepatocellular, damage/necrosis, hepatocyte proliferation, or hepatocellular degeneration. ALT is a cytoplasmic enzyme, and is considered to be liver specific in primates and some other small animal species. Aspartate aminotransferases is another liver enzyme that aids in producing proteins. It catalyzes the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. Besides liver, it is also found in other organs like heart, muscle, brain and kidney. Injury to any of these tissues can cause an elevated blood level (Nathwani *et al*., 2005). Normal levels are in the range of 7-40 U/L. It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury (Ozer *et al*., 2008) as it can also signify abnormalities in heart, muscle, brain or kidney (Dufour *et al*., 2000). The ratio of serum AST to ALT can be used to differentiate liver damage from other organ damage (Nathwani *et al*., 2005).

2.3.1.3 Alkaline phosphatase (ALP)

Alkaline phosphatase is a hydrolase enzyme that is eliminated in the bile. It hydrolyzes monophosphates at an alkaline pH. It is particularly present in the cells which line the biliary ducts of the liver. It is also found in other organs including bone, placenta, kidney and intestine. Several isozymes have been identified in humans and preclinical species. Normal levels are in the range of 20-120U/L. It may be elevated if bile excretion is inhibited by liver damage. Hepatotoxicity leads to elevation of the normal values due to the body's inability to excrete it through bile due to the congestion or obstruction of the biliary tract, which may occur within the liver, the ducts leading from the liver to the gallbladder, or the duct leading from the gallbladder through the pancreas that empty into the duodenum [small intestine]. Increase in alkaline phosphatase and/or bilirubin with little or no increase in ALT

is primarily a biomarker of hepatobiliary effects and cholestasis (Ramaiah *et al*., 2007). In humans, increased ALP levels have been associated with drug induced cholestasis (Wright *et al*., 2007).

2.3.2 Bilirubin

Bilirubin is the main bile pigment that is formed from the breakdown of heme in red blood cells. The broken down heme travels to the liver, where it is secreted into the bile by the liver. Normally, a small amount of bilirubin circulates in the blood. Serum bilirubin is considered a true test of liver function, as it reflects the liver's ability to take up, process, and secrete bilirubin into the bile.

Bilirubin production and excretion follows a specific pathway. When the reticuloendothelial system breaks down old red blood cells, bilirubin is one of the waste products. This "free bilirubin", is in a lipid-soluble form that must be made water-soluble to be excreted. The free, or unconjugated, bilirubin is carried by albumin to the liver, where it is converted or conjugated and made water soluble. Once it is conjugated into a water-soluble form, bilirubin can be excreted in the urine. An enzyme, glucuronyltransferase, is necessary for the conjugation of bilirubin. Either a lack of this enzyme, or the presence of drugs that interfere with glucuronyltransferase, impairs the liver's ability to conjugate bilirubin. Because the bilirubin is chemically different after it goes through the conjugation process in the liver, lab tests can differentiate between the unconjugated or indirect bilirubin and conjugated or direct bilirubin. The terms "direct" and "indirect" reflect the way the two types of bilirubin react to certain dyes. Conjugated bilirubin is water-soluble and reacts directly when dyes are added to the blood specimen. The non-water soluble, free bilirubin does not react to the reagents until alcohol is added to the solution. Therefore, the measurement of this type of bilirubin is indirect. Test results may be listed as "BU" for

unconjugated bilirubin and "BC" for conjugated bilirubin. Total bilirubin measures both BU and BC (Ye *et al.*, 2015).

2.3.2.1 Conjugated and unconjugated bilirubin

Bilirubin is a yellowish breakdown product of the heme. It is a part of the hemoglobin molecule that is in the red blood cells. It is thrown out of our body by means of bile or urine. Hence an increase in the level of bilirubin indicates the person could be suffering from certain diseases like jaundice. It is lipid soluble as it is a four ring structure known as tetrapyrrole. Bilirubin is split into two unconjugated versus conjugated bilirubin. Bilirubin when high is brown. However, when the level of bilirubin is slightly higher than normal it is yellowish. In some cases, depending on the level of bilirubin, when it is elevated it may show even on our skin and sclera (Harb and Thomas, 2007).

There are differences between unconjugated and conjugated bilirubin. Unconjugated bilirubin is not soluble with water and conjugated bilirubin is soluble with water. In order to explain this, tetrapyrrole is digested by reticuloendothelial cells that then result in unconjugated bilirubin. The Unconjugated bilirubin combines with albumin and is carried to the liver. From the liver it then joined or "conjugated" to glucuronide by an enzyme called UDP-glucuronyltransferase (Harb and Thomas, 2007).

Conjugated bilirubin reacts quickly as compared to unconjugated bilirubin. In order to produce Azobilirubin, which is a red-violet compound; dyes are added to blood sample. Conjugated bilirubin is known as direct bilirubin whereas unconjugated bilirubin is known as indirect bilirubin. Unconjugated bilirubin still produces Azobilirubin whereas conjugated bilirubin only produces it if dye is added (Harb and Thomas, 2007).

Unconjugated bilirubin is fat soluble however conjugated bilirubin is water-soluble and hence can be excreted through kidneys. An increase in the level of conjugated bilirubin means an indication towards hepatobiliary disease. Unconjugated bilirubin generally reacts slowly in the absence of an accelerator namely ethanol. In order to calculate indirect bilirubin the formula, Total bilirubin - Direct bilirubin, is used (Harb and Thomas, 2007).

Direct bilirubin is measured without an accelerator. Unlike unconjugated bilirubin which binds with neural tissue and also leads to kernicterus (a serious disorder damages the basal ganglia and other parts of the central nervous system) if left untreated or other forms of toxicity, conjugated bilirubin does not bind significantly to neural tissue neither does it lead to any form of toxicity or kernicterus. A very popular or well-known disease that is caused by elevated level of bilirubin in the body is Gilbert's syndrome. This is mainly hereditary and is caused by elevated level of unconjugated bilirubin but is not serious and may lead to mild jaundice if exerted (Harb and Thomas, 2007).

2.3.2.2 Cytotoxic effects of bilirubin

Neonatal Jaundice and Bilirubin Encephalopathy: Plasma unconjugated bilirubin (UCB) levels are usually elevated in normal infants during the first two weeks of postnatal life (< 200 μM) because of the marked and sudden breakdown of fetal erythrocytes at birth, coupled with a transient inability of the newborn to form bilirubin glucuronides in the liver and excrete them in the bile. Once the UGT*01 enzyme and the biliary excretory system reach maturity, at about 1 month of age, plasma UCB levels decrease and reach the adult levels of ~20 μM (Gourley, 2007). The “physiologic” neonatal hyperbilirubinemia (jaundice) may worsen because of: 1) increased bilirubin production resulting from increased hemolysis (e.g., in Rh or ABO incompatibility or in G6PD deficiency); 2) delayed maturation of the hepatic conjugation system (e.g., in prematurely born neonates); 3)

increased enterohepatic circulation of bilirubin; 4) genetic abnormalities (e.g., mutations in the UGT*01 gene, such as in patients with the Crigler-Najjar syndrome type 1) (Chuniaud *et al.*, 1996).

When the plasma levels of UCB are excessively elevated and surpass the capacity of albumin for high-affinity binding of UCB, the unbound (free) fraction of the pigment increases. This fraction may also be elevated in the plasma of newborns with "physiologic jaundice" in association with the following conditions: 1) low blood pH (acidosis); 2) reduced capacity of plasma albumin for high-affinity binding of UCB; and 3) use of drugs that compete with UCB for binding to plasma albumin (e.g., sulfonamides). Free UCB can easily enter the cells by passive diffusion and cause toxicity. The most vulnerable site is the central nervous system. UCB binds to discrete brain areas, such as the basal ganglia (kernicterus), and produces a wide array of neurological deficits collectively known as bilirubin encephalopathy. These include irreversible abnormalities in motor, sensory (auditory and ocular), and cognitive functions (Shapiro, 2003). Newborn infants display an increased susceptibility for brain damage because of the lower UCB-binding capacity of their plasma albumin and the temporal immaturity of their blood-brain barrier. Although bilirubin encephalopathy is a subject of great clinical importance, its pathogenesis and molecular basis are still not fully understood (Ostrow *et al.*, 2003a).

2.4 Kidney

The kidneys are organs that serve several essential regulatory roles in most animals, including vertebrates and some invertebrates. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid–base balance and regulation of blood pressure (via maintaining salt and water balance). They serve as a natural filter of the blood and remove wastes which are diverted to the

urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium, and they are also responsible for the reabsorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol, erythropoietin, and the enzyme renin. The kidneys excrete a variety of waste products produced by metabolism. These include the nitrogenous wastes called "urea", from protein catabolism, as well as uric acid, from nucleic acid metabolism. Formation of urine is also the function of the kidney. The concentration of nitrogenous wastes, in the urine of mammals and some birds, is dependent on an elaborate countercurrent multiplication system (Chuniaud *et al.*, 1996).

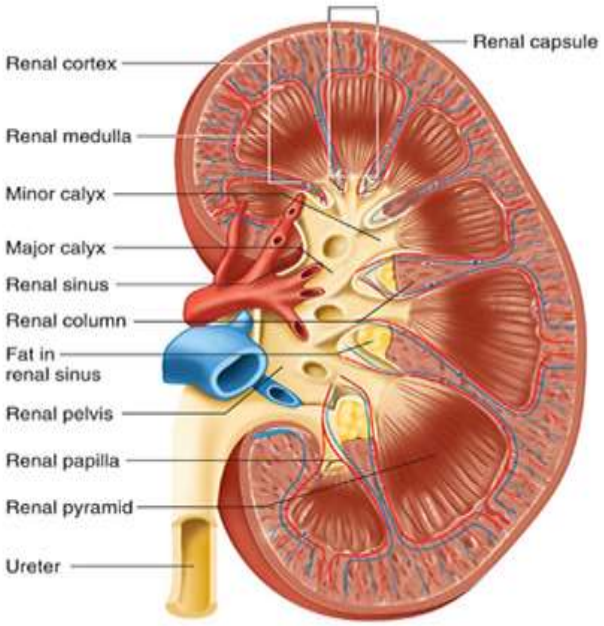


Figure 2.4: The Structure of Kidney (Shier *et al*, 2006).

2.4.1 Kidney function test

The kidneys, the body's natural filtration system perform many vital functions, including removing metabolic waste product from the bloodstream, regulating the body's fluid balance and maintaining the pH of the body system. The kidney function test helps to determine if the kidneys are performing their functions adequately.

2.4.1.1 Blood urea nitrogen test (BUN)

Urea is a small organic molecule (MW 60) comprising two amino (NH_2) groups and a linked carbamyl (C=O) group. It is the principal nitrogenous end product of protein and amino acid catabolism. Proteins are first degraded to constituent amino acids, which are in turn degraded (deaminated), with production of ammonia (NH_3), which is toxic. In a series of five enzymatically controlled reactions, known collectively as the "urea cycle", toxic ammonia resulting from protein breakdown is converted to non-toxic urea. In addition to ammonia and the five "urea cycle" enzymes, endogenous production of urea requires the presence of bicarbonate, aspartate and energy input in the form of adenosine triphosphate (ATP). Carbon dioxide (CO_2) is a secondary product of the urea cycle. Almost all of this urea production occurs in the cells of the liver (hepatocytes); the only other source is the cells of the kidneys. As might be expected, the rate of urea production is influenced by protein content of diet; low-protein diet is associated with reduced urea production and high-protein diet is associated with increased urea production. Starvation is, perhaps counter intuitively, associated with increased urea production but this is explained by the increased protein released from muscle tissue breakdown (autolysis) that occurs during starvation to provide an energy source. Any pathology associated with tissue breakdown is for the same reason associated with increased urea production. Detail of the urea cycle and its regulation is the subject of a recent review (Watford, 2003). A small amount (<10 %) of urea is eliminated via sweat and the gut, but most of the urea produced in the liver is transported in

blood to the kidneys where it is eliminated from the body in urine. This process of renal elimination, which is detailed in a recent review (Weiner *et al*., 2015), begins with filtration of blood at the glomeruli of the approximately 1 million nephrons contained within each kidney. During glomerular filtration, urea passes from blood to the glomerular filtrate, the fluid that is the precursor of urine. The concentration of urea in the filtrate as it is formed is similar to that in plasma so the amount of urea entering the proximal tube of the nephron from the glomerulus is determined by the glomerular filtration rate (GFR) (Weiner, 2015).

Urea is both reabsorbed and secreted (recycled back into the filtrate) during passage of the filtrate through the rest of the tubule of the nephron; the net effect of these two processes results in around 30-50 % of the filtered urea appearing in urine. The facility of the kidney to adjust urea reabsorption and secretion as the filtrate passes through the tubule determines an important role for urea in the production of a maximally concentrated urine, when this becomes necessary. The mechanism of this water-conserving action of urea within the nephron is well detailed by Weiner *et al.* (2015). Although often considered simply a metabolic waste product, urea has two important physiological functions outlined above: detoxification of ammonia and water conservation.

Measurement of Plasma/Serum Urea – a note on nomenclature and units around the world, essentially the same method of urea analyses is used, but the result is expressed in two quite different ways (Lamb *et al*., 2012). In the US and a few other countries, plasma or serum urea concentration is expressed as the amount of urea nitrogen. Although plasma or serum is used for the analysis, the test is still, somewhat confusingly, commonly referred to as blood urea nitrogen (BUN), and the unit of BUN concentration is mg/dL. In all other parts of the world, urea is expressed as the whole molecule (not just the nitrogen part of the molecule) in SI units (mmol/L). Since BUN reflects only the nitrogen content of urea (MW 28) and urea measurement reflects the whole of the molecule (MW 60), urea is

approximately twice (60/28 = 2.14) that of BUN. Thus BUN 10 mg/dL is equivalent to urea 21.4 mg/dL. Shils suggests that the rising blood urea is not due to the effect of xenobiotic directly on the kidney producing further impairment of renal function (Shils, 2002). Shils, 2002, 2003 postulates that the rise in the blood and urinary urea nitrogen is as a result of impaired protein synthesis due to the action of the xenobiotics on various enzyme mechanisms, (Weiner, 2015).

2.4.1.2 Blood creatinine level test

Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles (Wyss and Kaddurah-Daouk, 2000). Approximately 2% of the body's creatine is converted to creatinine every day (Taylor, 2009). Creatinine is transported through the bloodstream to the kidneys. The kidneys filter out most of the creatinine and dispose it off in the urine. The creatinine production normally remains essentially unchanged on a daily basis because the muscle mass in the body is relatively constant from day to day. Creatinine is a by-product of the filtration of the blood. This is a nitrous acid produced by the kidneys and is stored in muscle cells. The liver and the pancreas aid the kidneys in the synthesis of creatinine. If there is more creatinine than the normal range in a body, it is because the kidneys are malfunctioning and are unable to filter the creatinine out (Faull, 2007). Kidneys stones can often cause the creatinine levels to rise. These kidney stone in the kidney are extremely painful. They may even eventually cause injuries. A urinary tract infection may also cause higher levels of creatinine. Kidney diseases can also cause high levels of creatinine in the blood. When the urine protein creatinine ratio goes higher than the normal range, it can trigger the kidney being failing. It is normal for athletes and older people to have higher levels of creatinine in their bodies because their muscle mass continues to

fluctuate. However, this changing and often rather high amount of blood creatinine increases the risk of disease of the kidneys (Taylor, 2009).

Significance of serum creatinine levels

The kidneys maintain the blood creatinine in a normal range. Creatinine has been found to be a fairly reliable indicator of kidney function. Elevated creatinine level signifies impaired kidney function or kidney disease. As the kidneys become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys. It is for this reason that standard blood tests routinely check the amount of creatinine in the blood (Cockcroft and Gault, 2006).

A more precise measure of the kidney function can be estimated by calculating how much creatinine is cleared from the body by the kidneys. This is referred to as creatinine clearance and it estimates the rate of filtration by kidneys (glomerular filtration rate, or GFR) (Poggio and Hall, 2006). The creatinine clearance can be measured in two ways. It can be calculated by a formula using serum (blood) creatinine level, patient's weight, and age. Creatinine clearance can also be more directly measured by collecting a 24-hour urine sample (Gross, *et al*., 2005).

Blood urea nitrogen (BUN) level is another indicator of kidney function. Urea is also a metabolic byproduct which can build up if kidney function is impaired. The BUN-to-creatinine ratio generally provides more precise information about kidney function and its possible underlying cause compared with creatinine level alone. BUN also increases with dehydration (Melissa *et al.,* 2024).

Normal serum creatinine levels

Normal levels of creatinine in the blood are approximately 0.6 to 1.2 milligrams (mg) per deciliter (dl) in adult males and 0.5 to 1.1mg/dl in adult females (Poggio and Hall, 2006; Stevens *et al.,* 2006;).

Muscular young or middle-aged adults may have more creatinine in their blood than the norm for the general population. Elderly persons, on the other hand, may have less creatinine in their blood than the norm. Infants have normal levels of about 0.2mg/dl or more, depending on their muscle development. In people with malnutrition, severe weight loss, and long-standing illnesses, the muscle mass tends to diminish over time and, therefore, their creatinine level may be lower than expected for their age. A person with only one kidney may have a normal level of about 1.8 or 1.9mg/dl. Creatinine levels that reach 2.0mg/dl or more in babies and 10.0mg/dl or more in adults may indicate severe kidney impairment and the need for a dialysis machine to remove wastes from the blood (John, 2022).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and glass wares

Spectrophotometer (Spectrumlab, model 721),

Evaporation flask (Pyrex, England)

Oven (Gallenkamp, England)

Sohxlet extractor (Germany),

Rotary evaporator (Pyrex, England),

Centrifuge (Pacific, USA),

Conical flask and measuring cylinder (Pyrex, England)

Refrigerator (Haier Thermocool, England)

Water bath (Gallenkamp, London)

Weighing balance (Metler HAS, USA)

GCMS (Spectronic 20D, e2695 separations module)

Incubator (Gallenkamp, England).

Vacuum evaporator (Pyrex, England)

3.1.2 Chemicals and reagents

Formaldehyde (BDH)

Sodium chloride (BDH)

Distilled water

Liver function test kits (Randox)

Kidney function test kits (Randox)

3.1.3 Experimental animals

Thirty-five (35) male albino rats of body weights (*b.w.*) mean of 96g and 45 albino mice of body weight mean of 27g were purchased from the Animal Breeding Unit, University of Nigeria Nsukka, Nigeria.

3.2 Methods

3.2.1 Animal handling and grouping

The animals were kept in stainless–steel cages in a well–ventilated room of temperature 25 ± 2°C and relative humidity of 55–65% with a diurnal 12 h light cycle. The rats had access to water and pelletized standard finishers mesh (Vital finisher, United Africa Company Nigeria Plc., Jos, Nigeria) *ad libitum*. A period of 2 weeks was allowed for acclimatization of the rats to environmental conditions. The Thirty-five (35) albino rats were divided into seven (7) groups of five (5) rats each on weight basis. Ethical approval was obtained from the Department of Biochemistry Ethical Committee of Federal University of Technology, Owerri on use of experimental animals (Appendix I).

Table 3.1: Experimental design

Groups	Description	Treatments	No. of rats
One	Normal control	Feed and water only	5
Two	Degutted wall gecko (low dose)	10ml/kg body weight	5
Three	Degutted wall gecko (high dose)	20ml/kg body weight	5
Four	Whole wall gecko (low dose)	10ml/kg body weight	5
Five	Whole wall gecko (high dose)	20ml/kg body weight	5
Six	Centipede (low)	10ml/kg body weight	5
Seven	Centipede (high)	20ml/kg body weight	5

3.2.2 Preparation of wall gecko and centipede solution

Wall gecko and centipede used for this study were sourced around the school environment. The wall geckos were hunted at night at the School Cafeteria and hostel while the centipedes were sourced around Otamiri river in Federal University of Technology, Owerri. The wall gecko and centipede were killed by hitting the head gently with spatula.

Fifty grammes (4 wall gecko) of whole and degutted wall geckos (separately prepared) was weighed and boiled in 500ml distilled water for 5 minutes (after killing the animals) and allowed to cool to room temperature. The boiled solutions were filtered (Whatman no. 1) and stored for further use. The same procedure was used to prepare centipede solution. However, 5g of centipede (3 matured centipedes) was boiled in 100ml of distilled water (after killing the centipede).

3.2.3 GC-MS extraction and characterization of wall gecko and centipede solutions

Procedures:

The water sample from the animal extract was transferred into a clean beaker. Then, 10 ml of methanol was added to the sample. The sample was mixed thoroughly to ensure proper extraction of analytes into methanol. The sample and methanol mixture were allowed to stand for 24 hours. The mixture was filtered through a vacuum filtration setup into a clean beaker. The methanol extract was concentrated to 1ml (reducing the volume and increasing the analyte concentration) through a gentle stream of nitrogen gas. The concentrated extract was transferred into a Teflon-line crew cap vial ready for characterization using the GC-MS.

3.2.4 Acute toxicity test (LD_{50} determination)

The index of acute toxicity is the LD_{50}. The LD_{50} is the dose of a substance capable of producing death in 50% of the population of animal exposed to the substance. Locke's (1983) method was used with slight modification. This method has two different phases.

Phase I

Three groups containing three mice each were used. The prepared solution (either boiled degutted wall gecko, boiled whole wall gecko and boiled centipede water) was administered at concentrations of 5ml/kg body weight (b.wt) into group I mice, 10ml/kg b.wt into group II mice, and 20ml/kg b.wt into group III mice that formed the LD_{50} phase one groups. Administration of prepared solution was done orally. The animals were monitored for 24 hours for abnormal reaction or death.

Phase II

In this phase, three groups of mice were used with the prepared solution (either boiled degutted wall gecko, boiled whole wall gecko and boiled centipede water) in the concentration of 25ml/kg b.wt, 35ml/kg b.wt, and 50ml/kg, b.wt. to groups 1, group 2 and group 3 mice respectively that formed the phase two groups. They were monitored for 24 hours and the observations were recorded. Lethal dose LD_{50} of the extract was estimated by calculating the geometric mean of the maximum dose with 0% mortality and the minimum dose with mortality.

$$LD_{50} = \sqrt{maximium\ dose\ with\ 0\%\ mortality \times minimium\ dose\ with\ mortality}$$

3.2.5 Bioassay

Administration of wall gecko and centipede to the animals was done daily for 28 days through oral route, after which the rats were sacrificed, blood samples collected through ocular puncture and organs (liver and kidney) harvested for biochemical test and organ histology respectively.

3.2.6 Kidney function test

3.2.6.1 Determination of serum urea (Randox kit): This was done using the method of (Tariq *et al.,* 2019).

Principle: Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

$$NH_3 + hypochlorite + phenol \longrightarrow indophenol\ (blue\ compound)$$

Procedure: Three test tubes labelled T, S and B for test, standard and blank respectively were set up. And the following procedures listed below were taken.

10µl of serum was pipetted into test tube T, 10µl of creatinine standard was pipetted into test tube S, and 10µl of distilled water was pipetted into test tube B. 100 µl of R1 reagent was added into the all test tubes. Then all the test tubes were mixed well and incubated at 37^{o}C for 10 minutes. Then 2.5ml of R2 was added into all test tubes followed by the addition of 2.5ml of R3 to all test tubes. The content of the test tubes were mixed and incubated at 37^{o}C for 15 minutes. Before the absorbance reading of test and standard against the blank were taking at 492 λ nm.

$$Calculation: Urea\ conc.\ (mmol/l) = \frac{A_{test} \times conc.\ of\ standard\ (12.70 mmol/l)}{A_{standard}}$$

3.2.6.2 Determination of serum creatinine (Randox kit): This was done using the method of (Tariq *et al.,* 2019).

Principle: Creatinine in alkaline medium reacts with picrate to form a coloured complex whose colour intensity is directly proportional to the creatinine concentration in serum at 520nm

$$Creatine + picric\ acid \longrightarrow creatinine\text{-}picrate\ complex$$

Procedure: Two test tubes were labeled ‘sample’ and ‘standard’.

200µl of creatinine standard was pipetted into the test tube labelled standard and 200µl of serum sample into test tube labelled sample then 200µl of working reagent was added to the two test tubes.

The content of the individual tubes were mixed and a stopwatch started immediately. After 30 seconds, the absorbance A_1 was read at 492 nm. The absorbance A_2 was read again after exactly 2 mins. A_2-A_1 = A_{sample} or $A_{standard}$ as the case may be. The mixtures were allowed to stand for 20 minutes at 25 ^{0}C and the absorbance of the sample and standards read against the blank at 540 nm.

$$Calculation: Creatinine\ conc. = \frac{A_{sample} \times conc.\ of\ standard\ (170)}{A_{standard}}$$

3.2.6.3 Determination of serum electrolyte

(a) Serum sodium concentration

Serum sodium concentration was estimated using commercial kits and following standard protocols prescribed by the producer (CDC, 2016). Three test tubes properly labelled t est, standard and blank were set up and 1.0 ml of filtrate reagent was pipetted into each test tube. Exactly 50µl of the sample was added to the test tube labelled test and the same volume of the standard reagent to the standard and the same volume of distilled water to the blank. All test tubes were shaken vigorously to ensure adequate mixing of their contents. Each test tube was centrifuged at high speed for 10 minutes to obtain a supernatant above and precipitate below. Care was taken not to disturb the protein precipitate. The test proceeded to the stage with the supernatant only. Another set of three test tubes still labelled test, standard and blank were set up and 1 ml of the acid reagent was pipetted into each of the tubes. Then, 50µl of the supernatants was added to their corresponding test tubes and

mixed properly. Also, 50µl of the colour reagent was added to all the tubes and mixed. The spectrophotometer was zeroed with distilled water and then the absorbance of the content of each test tube was read at 492 nm wavelength and recorded. The concentration of sodium in the sample was calculated using the expression:

$$Sodium\ conc.\ in\ mEq/L = \frac{Abs.\ of\ blank - Abs.\ of\ test\ x\ Concentration\ of\ standard}{\text{Abs. of blank} - \text{Abs. of standard}}$$

Where concentration of standard = 150 mEq/L

(b) Serum chloride concentration

Serum chloride concentration was estimated using commercial kits and following standard protocols prescribed by the producer (CDC, 2016). Three test tubes properly labeled test, standard and blank were set up and 1.5ml of the chloride reagent was pipetted into each tube. Then 10µl of the sample was added to the test tube labeled test and the same volume of the standard reagent to the standard and the same volume of distilled water to the blank. All test tubes were shaken to mix and incubated at 25^{o}C for 5 minutes. Then, the absorbance of the content of each test tube was read at wavelength 500 nm after zeroing with the blank.

Chloride concentration in mEq/L was calculated with the expression:

$$Cl\ concentration\ in\ mEq/L = \frac{Absorbance\ of\ test\ \ x\ concentration\ of\ standard}{\text{Absorbance of standard}}$$

Where concentration of standard = 100 mEq/L

(c) Serum potassium concentration

Serum potassium concentration was estimated using commercial kits and following standard protocols prescribed by the producer (CDC, 2016). Three test tubes properly labeled test, standard and blank were set up and 1ml of the chloride reagent was pipette into each tube. Then 10µl of the sample was added to the test tube labelled test and the same

volume of the standard reagent to the standard and the same volume of distilled water to the blank. All test tubes shaken to mix and allow to stand at 25^{o}C for 3 minutes. Then the absorbance of the content of each test tube was read at wavelength 500 nm after zeroing with the blank.

Potassium concentration in mEq/L was calculated with the expression:

$$Potassium\ conc.\ in\ mEq/L\ =\ \frac{Absorbance\ of\ test\ \ x\ concentration\ of\ standard}{\text{Absorbance of standard}}$$

Where concentration of standard = 4 mEq/L

(d) Serum bicarbonate concentration

Serum bicarbonate concentration was estimated using commercial kits and following standard protocols prescribed by the producer (CDC, 2016). Carbon dioxide (CO_2) reagent was reconstituted with the volume of CO_2 free water indicated on the vial label, mixed by gentle inversion 5-6 times. Three cuvettes labelled test, standard and blank were set up and 1 ml of the reagent was pipetted into each tube and all tubes were incubated for 3 minutes at 37^{o}C. Exactly 10µl of the sample were added to the cuvette labelled test and the same volume of the standard reagent and distilled water to the standard and blank, respectively. The absorbance was read at 340 nm and the bicarbonate concentration calculated thus:

$$Bicarbonate\ conc.\ in\ mmol/L\ =\ \frac{Abs\ of\ blank\ -\ Abs\ of\ test\ \ x\ Conc.of\ standard}{\text{Abs of blank} -\ \text{Abs of standard}}$$

Where concentration of standard = 30 mmol/L

3.2.7 Liver function status

3.2.7.1 Determination of serum alkaline phosphatase

The serum activity of alkaline phosphatase (ALP) was determined using Randox test kits following the method of (Tariq *et al.,* 2019).

Principle: p-Nitrophenylphosphate is hydrolysed in the presence of magnesium ions by phosphatase to phosphate and p-nitrophenol. The p-nitrophenol liberation is proportional to the alkaline phosphatase activity and can be measured photometrically. The standared colorimetric method is according to the recommendation of the Deutsche Gesellschaft fur Klinische chemie.

$$\text{p-Nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{phosphate} + \text{p-nitrophenol}$$

Procedure: To 0.02ml of the serum sample in a cuvette was added 1.0ml of Randox ALP reagent. The content was mixed and the initial absorbance was taken, after which the readings were taken after 1, 2 and 3 min, at 405 nm in a spectrophotometer.

Calculations:

The ALP activity of the serum (U/L) = $2760 \times \Delta A405nm/min$

3.2.7.2 Determination of serum aspartate transaminase activity

The serum activity of aspartate transaminase (AST) or glutamate oxaloacetate transaminase (GOT) was determined using Randox test kits and following the method of (Tariq *et al.*, 2019).

Principle: The AST was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenylhydrazine.

$$\alpha\text{-Oxaloacetate} + \text{L-Aspartate} \xrightarrow{\text{GOT}} \text{L-Glutamate} + \text{Oxaloacetate}$$

Procedure: Two test tubes were set up labeled T_1 (reagent blank) T_2 (test sample). T_1 contained 0.10ml distilled water and 0.05ml Randox buffer solution, while T_2 contained 0.10ml serum sample and 0.50ml Randox buffer solution. The contents were mixed and

incubated for 30mins at 37°C. To each test tube was added 0.50ml of Randox 4-dinitrophenylhydrazine solution and the contents were mixed and allowed to stand for 20mins at 25°C. Then 5ml of the 0.4M NaOH solution was added to each of the tubes. The contents were mixed, and after 5mins, their absorbances were read at 546 nm against the reagent blank in a spectrophotometer.

Calculation

The AST activity was obtained by comparison to the table provided in the kits leaflet, as shown in Appendix.

3.2.7.3 Determination of serum alanine transaminase activity

The serum activity of alanine transaminase (ALT) or glutamate pyruvate transaminase (GPT) was determined using Randox test kits and following the method of (Tariq *et al.,* 2019).

Principle: The ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4 – dinitrophenylhydrazine (Tariq *et al.,* 2019).

A – Oxoglutarate + L-Alanine $\longrightarrow$ L-Glutamate + pyruvate

Procedures: Two test tubes were set up T_1 (reagent blank) and T_2 (test sample). T_1 contained 0.10mL distilled water and 0.50mL randox buffer solution, while T_2 contained 0.10mL serum sample and 0.50mL Randox buffer solution. The contents were mixed and incubated for 30min at 37°C. To each test tube was added 0.50mL of Randox 4 – dinitrophenylhydrazine solution and the contents were mixed and allowed to stand for 20min at 25^0C. Then 5mL of sodium hydroxide solution was added to each of the tubes. The contents were mixed and after 5min, their absorbance were read at 546nm against blank in a spectrophotometer.

Calculation

The ALT activity was obtained by comparison to the table provided in the kit's leaflet as shown in Appendix.

3.2.7.4 Serum total bilirubin content:

Serum bilirubin content was estimated using commercial kits (Randox, UK) and following standard protocols using the Tariq *et al.* (2019) method.

Principle: Colorimetric method based on that described by Jendrassik and Grof (1938). Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Procedure: Two test tubes were set up and labeled blank and sample. To the sample test tube, 200µl of Reagent 1 (0.17N hydrochloric acid), 50µl of Reagent 2 (38.5mmol/L of sodium Nitrite), 1000µl of Reagent 3 (0.52mmol/L sodium benzoate) and 200µl of serum were added and mixed properly while only 200µl of Reagent 1 and 1000µl of reagent R3 were added to the blank test tube. The 2 test tubes were then incubated at 20-25^{0}C for 10 minutes, after which 1000µl of reagent 4 (1.9N sodium Hydroxide) was added to both test tubes. The test tubes were incubated for a further 30 minutes at 25^{0}C before reading absorbance on a spectrophotometer at 546nm after zeroing with blank. To obtain total bilirubin in mg/dl the formular below was used.

Total Bilirubin = 10.8 x Absorbance of Sample

3.2.7.5 Serum direct bilirubin content:

Serum bilirubin content was estimated using commercial kits and following the method of (Tariq *et al.,* 2019).

Principle: Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue coloured complex.

Procedure: Two test tubes were set up and labeled blank and sample. To the sample test tube, 200µl of Reagent 1 (0.17N hydrochloric acid), 50µl of Reagent 2 (38.5mmol/L of

sodium Nitrite), 2000µl of 0.9% NaCl and 200µl of serum sample were added and mixed properly while only 200µl of Reagent 1 and 2000µl of 0.9% NaCl were added to the blank test tube. The two test tubes were then incubated at 20-25^{0}C for 10 minutes, before reading absorbance on a spectrophotometer at 546nm after zeroing with blank. Direct bilirubin in mg/dl was calculated using the formular below:

Direct Bilirubin (mg/dl) = 14.4 x Absorbance of Sample

3.2.7.6 Determination of total protein concentration: This was done using the method of (Kangle *et al.,* 2017).

Principle: Cupric ions interact with protein peptide bonds resulting in the formation of colored complex (Kangle *et al.,* 2017).

Procedure: Total protein concentration of the samples was determined using kit obtained from Randox Diagnostics Ltd, USA. An aliquot (0.01 ml) of distilled Water (reagent blank), standard, sample blank and serum samples were dispensed into their respective test tubes and 0.5 ml of Biuret reagent (containing 100, 16, 15 and 6 mmol/l of NaOH, Na-K-tartarate, potassiunm iodide and cupric sulphate, respectively) was added to all test tubes and mixed. The reaction mixture was incubated for 30 min at room temperature and read at 540 nm

$$\text{Total protein concentration (g/l)} = \frac{\text{Absorbance of sample X standard conc (g/l)}}{\text{Absorbance of Standard}}$$

3.2.7.7 Determination of albumin concentration

Principle: This assay is based on the quantitative binding of serum albumin to the indicator 3,3',5,5'- tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG) to form a complex which absorbs at 630 nm (Grant, 1987).

Procedure: Aliquots (0.01 ml) of distilled water (reagent blank), standard and serum samples were dispensed into their appropriately labeled test tubes and mixed with 3.0 ml of

BCG reagent. The reaction mixture was incubated at room temperature for 5 min and read at 630 nm. Absorbance of the sample and standard were measured against the reagent blank.

$$\text{Albumin concentration (g/l)} = \frac{\text{Absorbance of sample X standard conc. (g/l)}}{\text{Absorbance of Standard}}$$

3.2.7.8 Estimation of serum globulin

Since, bromocresol green (BCG) – albumin complex absorbs light at a different wavelength from the unbound dye, the method may overestimate albumin by binding to other proteins (George, 2009). Hence, the total globulin fraction is generally determined by subtracting the albumin fraction from the total protein fraction.

3.2.8 Histological study

This was carried out as described by Bancroft and Stevens (2002).

Preservation/Fixation: The aim was to make the tissue appear as life-like manner as possible. The volume of the fixative was 3 times the volume of the tissue. Cotton was used to press down the tissue, so that uniform fixation will be achieved. This was done because fatty tissues and lung may float on the fixative.

Preparation of 10% formalsaline: A 10% formal-saline is commonly used as routine fixative and is composed of formaline 10ml, sodium chloride 0.85gm and distilled water 90ml.

Tissue processing

(a) Dehydration: This was done in a gradual manner so that the architecture of the tissue will not be altered as a result of rupture difference. This was achieved by bathing the tissue with graded ethanol ranging from 40%, 50%, 60%, 70%, 80%, 90% to absolute.

(b) **Clearing:** This step involves immersing the tissue in a wax soluble solvent (xylene). The essence of this step is to replace ethanol with a paraffin soluble solvent (xylene).

(c) **Parafin wax infiltration:** The tissues were then transferred in a parafin wax at the melting point of the paraffin which is 54 to 60C, the volume of the parafin wax was 20 times that of the tissue. The time for wax impregnation was 2 hours.

(d) **Embedding:**

Impregnated tissues were placed in a mould with their labels and then fresh melted wax poured in it and allowed to settle and solidify. Once the block has cooled sufficiently to form a surface skin it was immersed in cold water rapidly to cool it. After the block has completely cooled it was cut into individual blocks and each was trimmed (tissue sectioning).

(e) Staining: The staining method used is the haematoxylin and eosin staining procedure. Which is as follows:

i. The section fixed on slide was put in xylene for 10 minutes.

ii. Then it was transferred to absolute alcohol for 3 minutes.

iii. Then transferred to 95% alcohol for 3 minutes. The slide was agitated gently in an up-down like motion

iv. The slide was finally place in 80% alcohol for 3 minutes.

v. The slide was then washed in running tape water for 3 minute and put in Harris's Haematoxylin for 3- 5 minutes.

vi. The slide was rinsed in running tape water for 30 seconds

vii. Excess dye was removed by washing in acid-alcohol (35% ethanol with 0.1M HCl).

viii. Then neutralised in alkaline alcohol (35% ethanol with pinch sodium carbonate) until tissue attain blue colour.

ix. The slide was then placed in 70% alcohol for 1 minute

x. Then counter stained with eosin for 3-5 minutes.

xi. Placed briefly in 70% ethanol to rinse off excess eosin.

xii. The slide was then placed in 95% ethanol for 1 minute after which it was removed and placed in second change of 95% ethanol for another 1 minute

xiii. The slide was then placed in an absolute (100%) ethanol for 1 minute and then removed and placed in a second change of 100% ethanol for another 1 minute

xiv. The slide was then cleared in xylene and mounted with Canada balsam (mounting medium) and covered with a slip and allowed to dry.

xv. Microscopy

3.2.9 Statistical analysis

The data generated in this study were analyzed using one-way analysis of variance (ANOVA). Values were considered significant at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 GC-MS characterization of wall gecko and centipede boiled solutions

The GC-MS characterization of sample solution showed the presence of twenty-nine (29) active components in degutted wall gecko boiled solution, thirty (30) chemical components in whole wall gecko boiled solution and thirty (30) chemical compounds in centipede boiled

solution (tables 4.2, 4.3 and 4.4). Notable among the compounds present in whole wall gecko boiled solution are hexadecanoic acids, 11-octadecanoic acid, methyl stearate with peak area of 33.42, 27.16 and 12.86 respectively. Hexadecanoic acid, 9,10-epoxy-18-(trimethylsiloxy)-, methyl stearate, 6-Octadecenoic acid and n-hexadecanoic were relatively present in high level in degutted wall gecko boiled solution with peak area of 29.44, 23.25, 10.96, 7.22 and 6.46, respectively. 9-octadecanoic acid, hexadecanoic acid and methyl stearate were also present in centipede boiled solution with peak values of 24.84, 24.64 and 9.63, respectively (tables 4.2, 4.3 and 4.4).

Table 4.1: GC-MS characterization of boiled whole wall gecko solution

s/no	RT(mins)	Compound name	Molecular formula	Molecular weight	Peak area
1	2.930	cis-2-Ethyl-2-hexen-1-ol	$C_8H_{16}O$	128.12	0.17
2	3.170	L-Methionine	$C_6H_{13}NO_2S$	163.07	0.19
3	3.517	Methenamine	$C_6H_{12}N_4$	140.11	0.87

4	3.913	1-Deoxy-d-mannitol	$C6H_{14}O_5$	166.08	0.24
5	4.491	Docosanoic acid	$C_{29}H_{58}O_2$	438.44	0.16
6	5.245	Heptadecanoic acid	$C_{18}H_{36}O_2$	284.27	0.64
7	6.457	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.22	2.85
8	6.874	2,3-Dihydroxybenzoic acid	$C_{25}H_{48}O_4Si_3$	496.29	0.20
9	7.028	Pentadecanoic acid	$C_{16}H_{32}O_2$	256.24	0.81
10	7.365	Tungsten	$C_{16}H_{29}NW$	419.18	0.18
11	7.468	9-Hexadecenoic acid	$C_{17}H_{32}O_2$	268.24	4.33
12	7.571	Hexadecanoic acid	$C_{17}H_{34}O_2$	270.26	33.42
13	7.828	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24	1.16
14	7.897	Acetamide	$C_{21}H_{20}F_3NO_6$	439.12	0.17
15	7.971	Octadecanoic acid	$C_{18}H_{36}O_2$	284.27	0.38
16	8.091	methyl ester	$C_{18}H_{36}O_2$	284.27	0.95
17	8.343	Cyclohexane	$C_9H_{13}NO_4$	199.08	0.22
18	8.446	9,12-Octadecadienoic acid	$C_{19}H_{34}O_2$	294.26	2.33
19	8.468	11-Octadecenoic acid	$C_{19}H_{36}O_2$	296.27	27.16
20	8.588	Methyl stearate	$C_{19}H_{38}O_2$	298.29	12.86
21	8.720	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356.29	2.32
22	8.829	Colchiceine	$C_{21}H_{20}F_3NO_6$	439.12	0.23
23	8.903	Protocatechoic acid	$C_{25}H_{48}O_4Si_3$	496.29	0.67
24	9.069	Methyl hexadecyl ether	$C_{17}H_{36}O$	256.28	0.32
25	9.200	13-Methyl-Z-14-nonacosene	$C_{30}H_{60}$	420.47	0.18
26	9.411	cis-11-Eicosenoic acid	$C_{21}H_{40}O_2$	324.30	2.67
27	9.520	1-Triacontanol	$C_{30}H_{62}O$	438.48	1.35
28	10.286	Androsta[17-16-b]furan-5'-imine	$C_{29}H_{45}NO_2$	439.35	1.62
29	10.412	Methanesulfonamide	$C_{25}H_{27}N_3O_7S$	513.16	1.04
30	13.304	5-(4-Chlorophenyl)-6-ethylpyrimidine-2,4-diamine	$C_{16}H_{11}ClF_6N_4O_2$	440.05	0.31

Table 4.2: GC-MS characterization of boiled degutted wall gecko solution

s/no	RT(mins)	Compound name	Molecular formula	Molecular weight	Peak area
1	3.542	Methenamine	$C_6H_{12}N_4$	140.11	1.21
2	4.491	2,2-Dichloroethyl isobutyl carbonate	$C_7H_{12}Cl_2O_3$	214.02	0.20

3	5.239	Cyclopentanetridecanoic acid	$C_{19}H_{36}O_2$	296.27	0.79
4	5.702	Benzenamine	$C_{16}H_{14}BrN_5$	355.04	0.19
5	6.547	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.22	2.61
6	6.748	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	259.24	0.25
7	7.023	Pentadecanoic acid	$C_{16}H_{32}O_2$	256.24	0.75
8	7.360	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324.30	0.23
9	7.463	9-Hexadecenoic acid	$C_{17}H_{32}O_2$	268.24	3.16
10	7.566	Hexadecanoic acid	$C_{17}H_{34}O_2$	270.26	29.44
11	7.743	N-[4-Aminobutyl]aziridine	$C_6H_{14}N_2$	114.12	0.43
12	7.817	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24	6.46
13	7.891	Ethyl oxamate	$C_4H_7NO_3$	117.04	0.22
14	7.971	2-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324.23	0.33
15	8.091	Heptadecanoic acid	$C_{18}H_{36}O_2$	284.27	0.84
16	8.234	[5-(5-Bromopyridin-3-yl)-2H-1,2,4- triazol-3-yl]acetic acid	$C_9H_7BrN_4O_2$	281.97	0.22
17	8.406	1,3-Bis(hydroxymethyl)urea	$C_3H_8N_2O_3$	120.05	0.19
18	8.440	Oleic Acid	$C_{18}H_{34}O_2$	282.26	1.91
29	8.463	9,10-epoxy-18-(trimethylsiloxy)-	$C_{22}H_{44}O_4Si$	400.30	23.25
20	8.583	Methyl stearate	$C_{19}H_{38}O_2$	298.29	10.96
21	8.714	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.26	7.22
22	8.817	Cyclopentanetridecanoic acid	$C_{19}H_{36}O_2$	296.27	1.97
23	8.897	5Alpha-cyano-3-methoxymethylenecholestane	$C_{30}H_{49}NO$	439.38	0.64
24	9.057	1H-Imidazole	$C_5H_{10}N_2$	98.08	0.29
25	9.412	Methyl 9-eicosenoate	$C_{21}H_{40}O_2$	324.30	2.47
26	9.520	Methyl 18-fluoro-octadecanoate	$C_{19}H_{37}FO_2$	316.28	0.76
27	10.280	Androsta[17-16-b]furan-5'-imine	$C_{29}H_{45}NO_2$	439.35	1.58
28	10.406	Docosanoic acid	$C_{23}H_{46}O_2$	354.35	1.07
29	16.864	5-(4-Chlorophenyl)-6-ethylpyrimidine-2,4-diamine	$C_{16}H_{11}ClF_6N_4O_2$	440.05	0.19

Table 4.3: GC-MS characterization of boiled centipede solution.

s/no	RT(mins)	Compound name	Molecular formula	Molecular weight	Peak area
1	3.548	Methenamine	$C_6H_{12}N_4$	140.11	0.57

2	4.131	Octadecanoic acid	$C_{21}H_{42}O_2$	326.32	0.27
3	5.234	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.22	0.69
4	6.457	Tridecanoic acid	$C15H_{30}O_2$	242.22	2.14
5	6.760	Cyclopropanecarboxylic acid	$C_{12}H_{20}N_2O$	208.16	0.56
6	6.822	γ-Sitosterol	$C_{29}H_{50}O$	414.39	0.33
7	6.862	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.26	0.22
8	7.023	Pentadecanoic acid	$C_{17}H_{34}O_2$	270.26	0.61
9	7.251	Ethanone	$C_7H_8N_6O_2$	208.07	0.23
10	7.365	Halcinonide	$C_{24}H_{32}ClFO_5$	454.19	0.25
11	7.463	9-Hexadecenoic acid	$C_{17}H_{32}O_2$	268.24	3.58
12	7.565	Hexadecanoic acid	$C_{18}H_{36}O_2$	284.27	24.64
13	7.743	Flurandrenolide	$C_{24}H_{33}FO_6$	436.23	0.36
14	7.817	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24	4.99
15	8.086	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.26	0.75
16	8.440	9,12-Octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O_2$	294.26	3.43
17	8.463	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	282.26	24.84
18	8.491	11-Octadecenoic acid	$C_{19}H_{36}O_2$	296.27	5.83
19	8.583	Methyl stearate	$C_{19}H_{38}O_2$	296.29	9.63
20	8.714	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.26	7.67
21	8.817	Octadecanoic acid	$C_{18}H_{34}O_2$	282.26	1.10
22	8.903	3,5-Dihydroxybenzoic acid	$C_{25}H_{48}O_4Si_3$	496.29	0.52
23	9.057	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.26	0.41
24	9.149	Oleic Acid	$C_{18}H_{34}O_2$	282.25	0.24
25	9.406	Hexacosanol	$C_{29}H_{62}OSi$	454.46	2.37
26	9.520	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326.31	1.02
27	10.206	Protocatechoic acid	$C_{25}H_{48}O_4Si_3$	496.29	0.31
28	10.275	Androsta[17-16-b]furan-5'-imine	$C_{29}H_{45}NO_2$	439.35	1.18
29	10.400	Protocatechoic acid	$C_{25}H_{48}O_4Si_3$	496.29	0.91
30	10.550	5-(4-Chlorophenyl)-6-ethylpyrimidine-2,4-diamine	$C_{16}H_{11}ClF_6N_4O_2$	440.05	0.36

4.1.2 The results of the acute toxicity (LD_{50}) of boiled centipede and wall gecko solutions

The results of the acute toxicity (LD_{50}) of the boiled solutions are presented in the table 4.1 below:

Table 4.4: LD_{50} of boiled solutions

Phase I

Groups	Whole wall gecko		Degutted wall gecko		Centipede	
Groups	No. of mice	Mortality	No. of mice	Mortality	No. of mice	Mortality
I	3	-	3	-	3	-
II	3	-	3	-	3	-
III	3	-	3	-	3	-
Phase II						
IV	3	-	3	-	3	-
V	3	-	3	-	3	-
VI	3	-	3	-	3	-

No mortality was observed at the study level.

4.1.3 Effect of boiled wall gecko and centipede solutions on some kidney function parameters of rats

The result presented below showed a significant ($p<0.05$) increase in urea (Fig. 4.1), creatinine (Fig. 4.2), sodium ion (Fig. 4.3) and chloride ion (Fig. 4.5) levels in groups 4 and 5 (boiled whole wall gecko solution), and groups 6 and 7 (boiled centipede solution) when compared to group 1 (normal control group). However, there was no significant ($p>0.05$) difference in urea, creatinine, sodium ion and chloride ion concentrations in group 2 and 3 (boiled degutted wall gecko solution) when compared to the normal control group. The result also showed a significant ($p<0.05$) decrease in bicarbonate ion concentration (Fig.4.4) following administration of boiled whole wall gecko solution (group 4 and 5) and high dose of boiled centipede solution (group 7) when compared to the normal control group (group1). The result further showed no significant difference in bicarbonate ion level following oral administration of boiled degutted wall gecko solution (group 2 and 3) and low dose of boiled centipede solution (group 6) when compared to the normal control group.

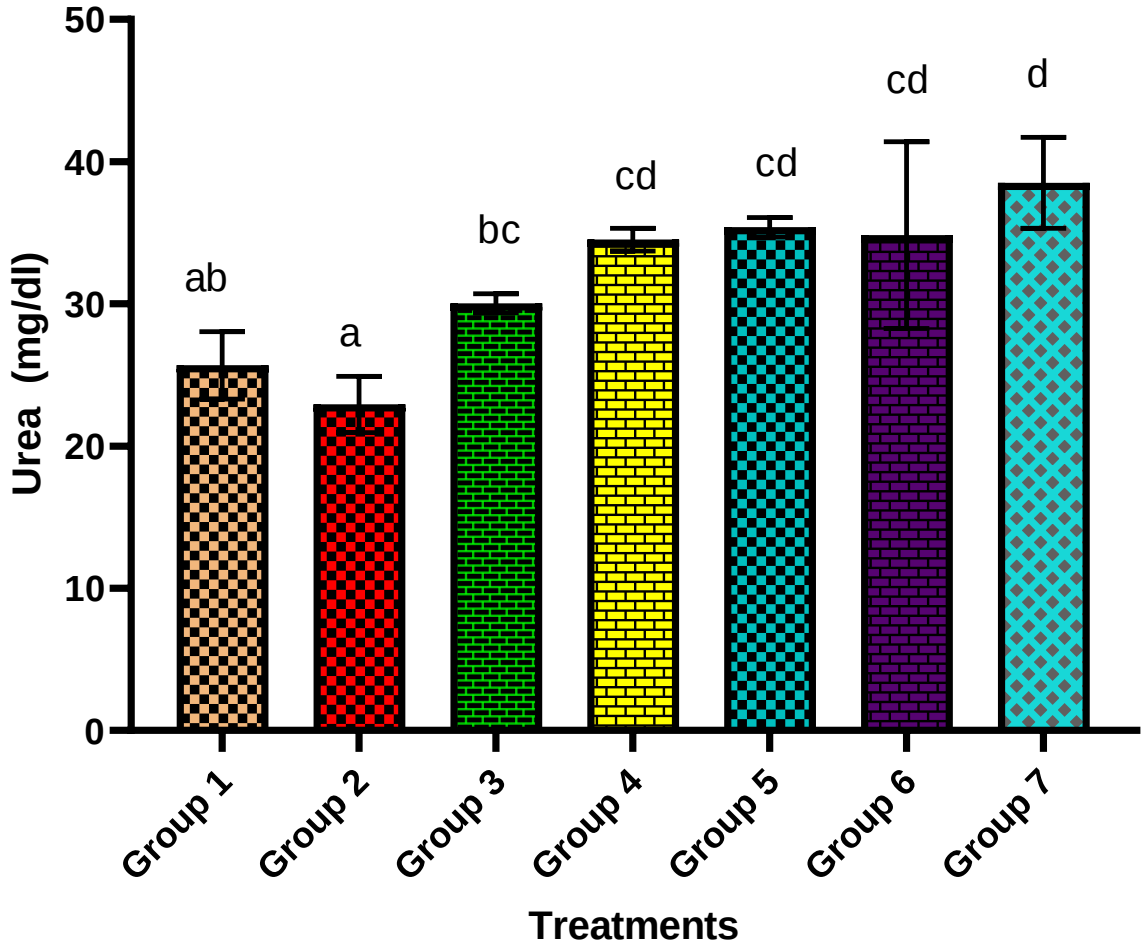


Figure 4.1: Effect of boiled wall gecko and centipede solution on urea level of rats. Bars with different alphabets are statistically ($p<0.05$) significant. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

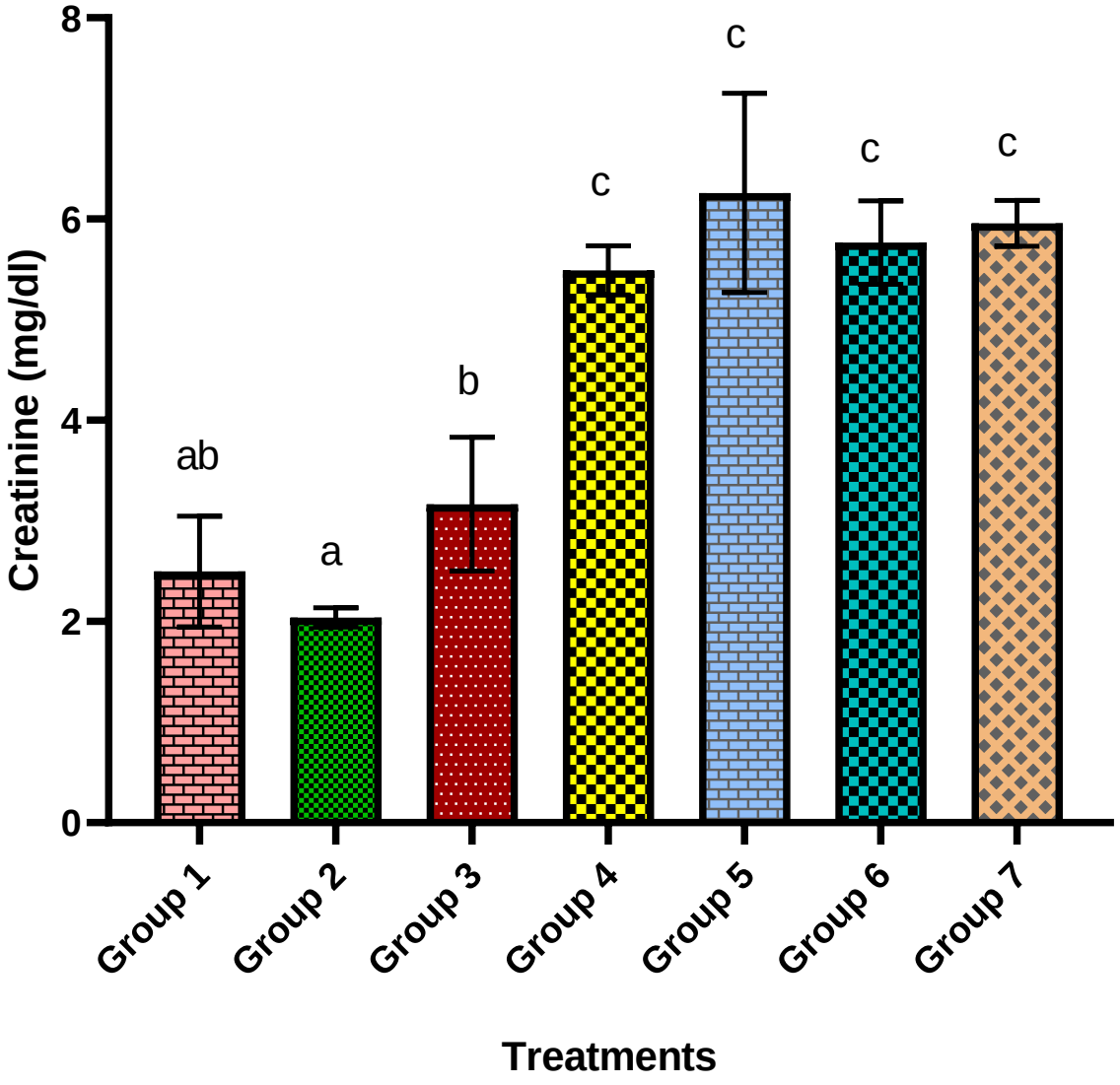


Figure 4.2: Effect of boiled wall gecko and centipede solution on creatinine level of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

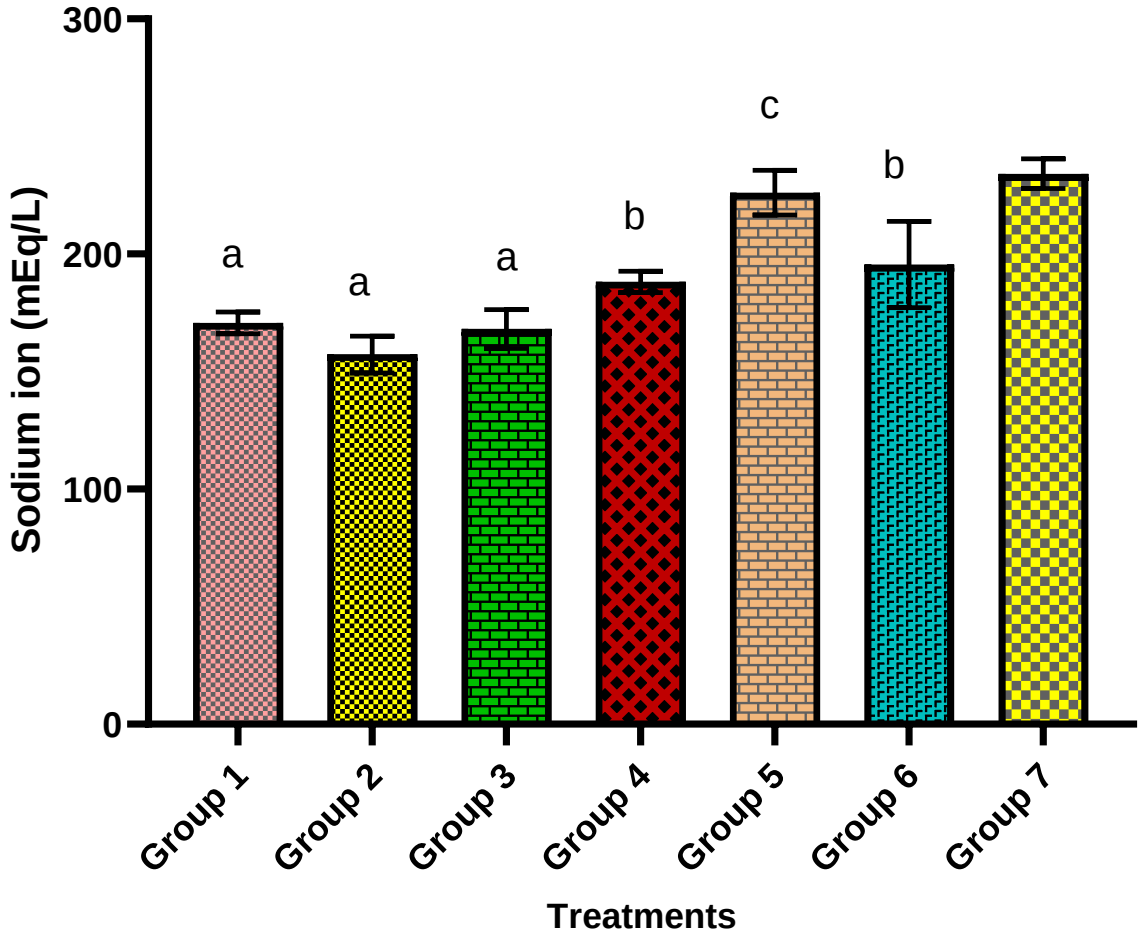


Figure 4.3: Effect of boiled wall gecko and centipede solution on sodium ion level of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

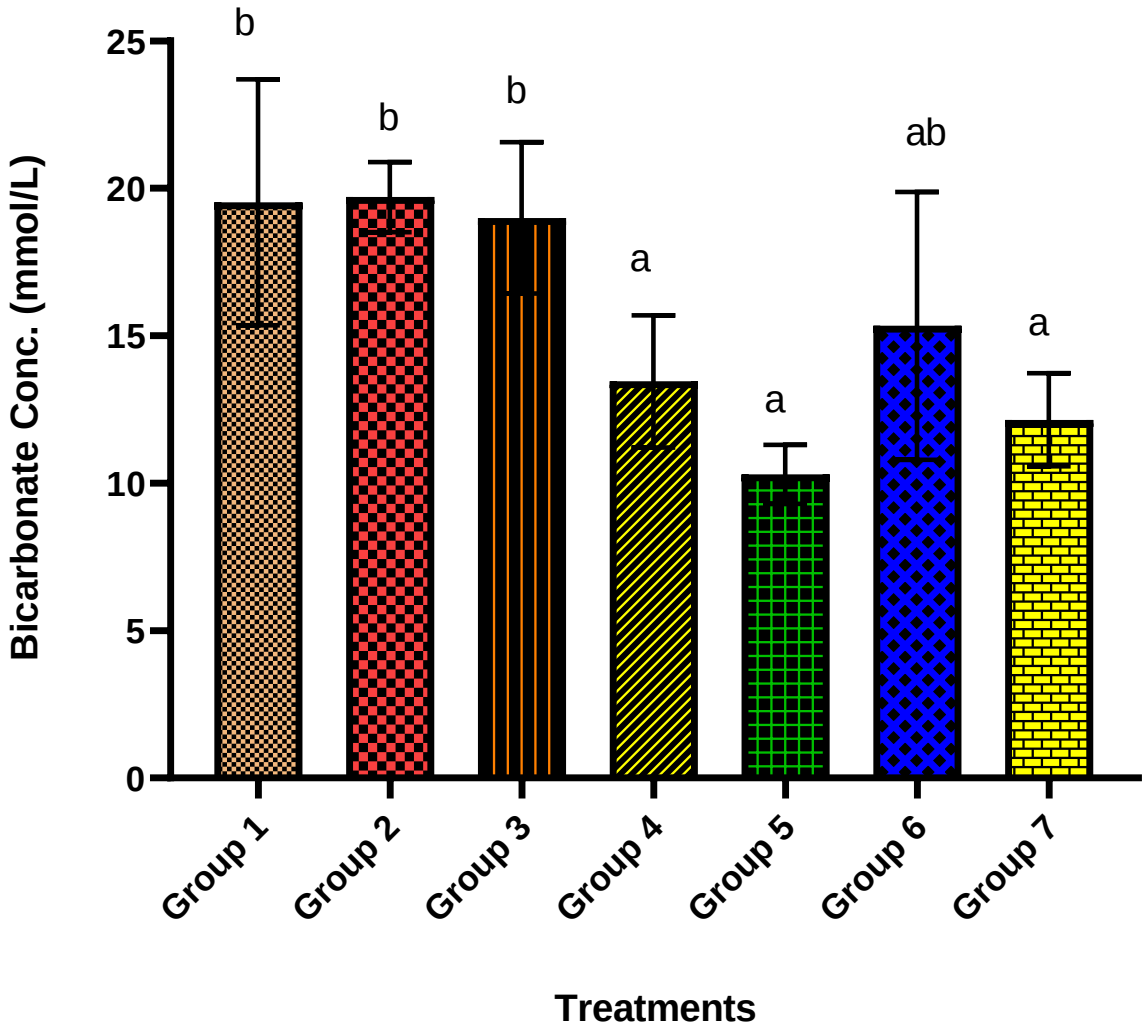


Figure 4.4: Effect of boiled wall gecko and centipede solution on bicarbonate ion level of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

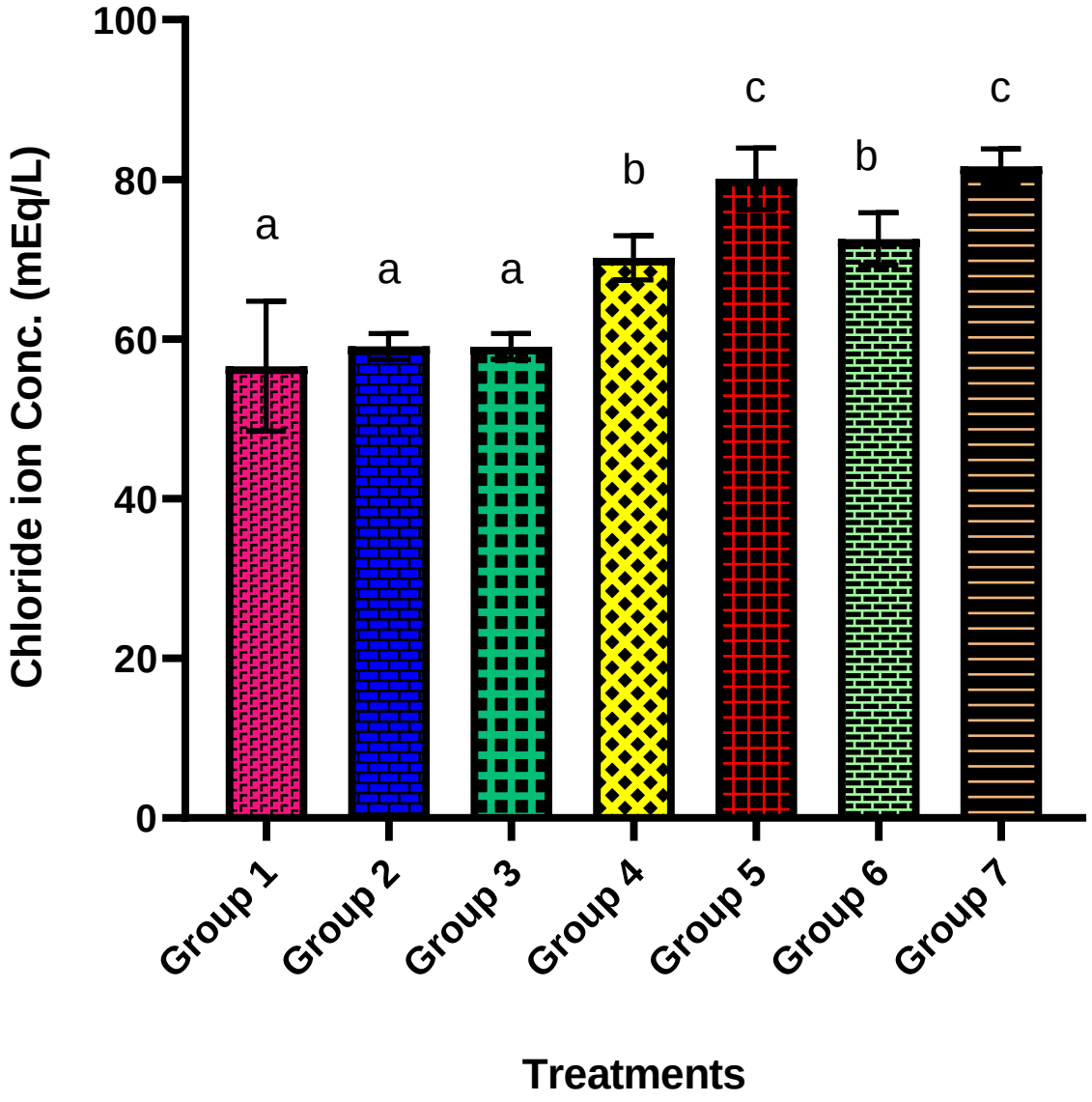


Figure 4.5: Effect of boiled wall gecko and centipede solution on chloride ion level of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

4.1.4 Effect of boiled wall gecko and centipede solutions on liver function parameters of rats

The result presented below showed a significant ($p<0.05$) increase in ALP activity (Fig. 4.6), ALT activity (Fig. 4.7), total bilirubin level (Fig. 4.9) and direct bilirubin concentration (Fig. 3.10) following administration of boiled degutted wall gecko solution (group 2 and 3), boiled whole wall gecko solution (group 4 and 5) and boiled centipede solutions (group 6 and 7) when compared to the normal control group (group 1). The result also showed a significant ($p<0.05$) increase in AST activity (Fig. 4.8) following oral administration of high dose of boiled degutted (group 3) and whole (group 5) wall gecko when compared to the control group (group 1). The result also showed a significant ($p<0.05$) increase in AST activity in group 6 and 7 (boiled centipede solution) when compared to the normal control group. However, there was no ($p>0.05$) significant difference in AST activity when group 2 and 4 are compared to the normal control group. The result further showed a significant decrease ($p<0.05$) in total protein level (Fig. 4.11) and globulin level (Fig. 4.13) in group 4 and 5 (boiled whole wall gecko) and group 6 and 7 (boiled centipede solution) when compared to the normal control group. However, there was no significant ($p>0.05$) difference in total protein and globulin level in group 2 and 3 (boiled degutted wall gecko) when compared to normal control group. There was a significant ($p<0.05$) decrease in albumin level (Fig. 4.12) in group 5, 6 and 7 when compared to the normal control group, however, the result also showed no significant ($p>0.05$) difference in albumin level in group 2, 3 and 4 when compared to the control group.

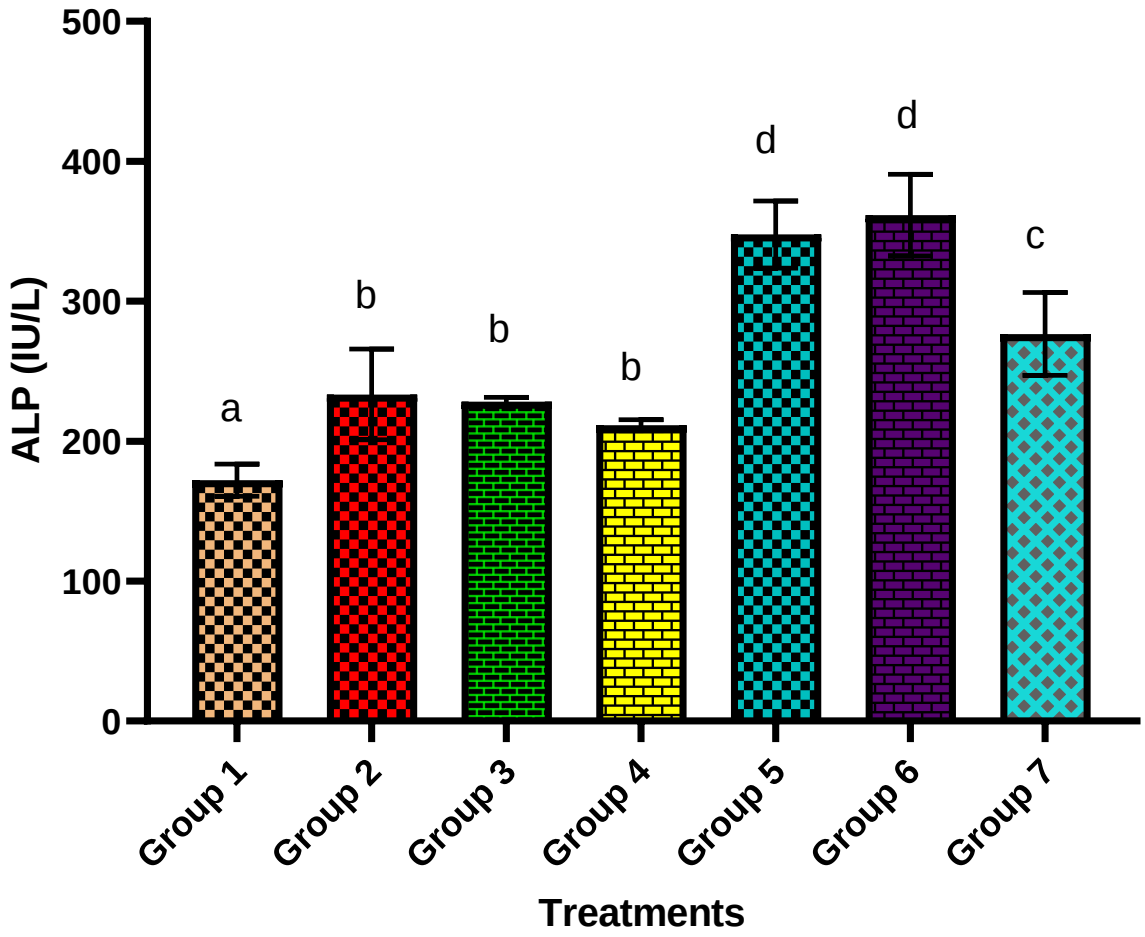


Figure 4.6: Effect of boiled wall gecko and centipede solution on ALP activity of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

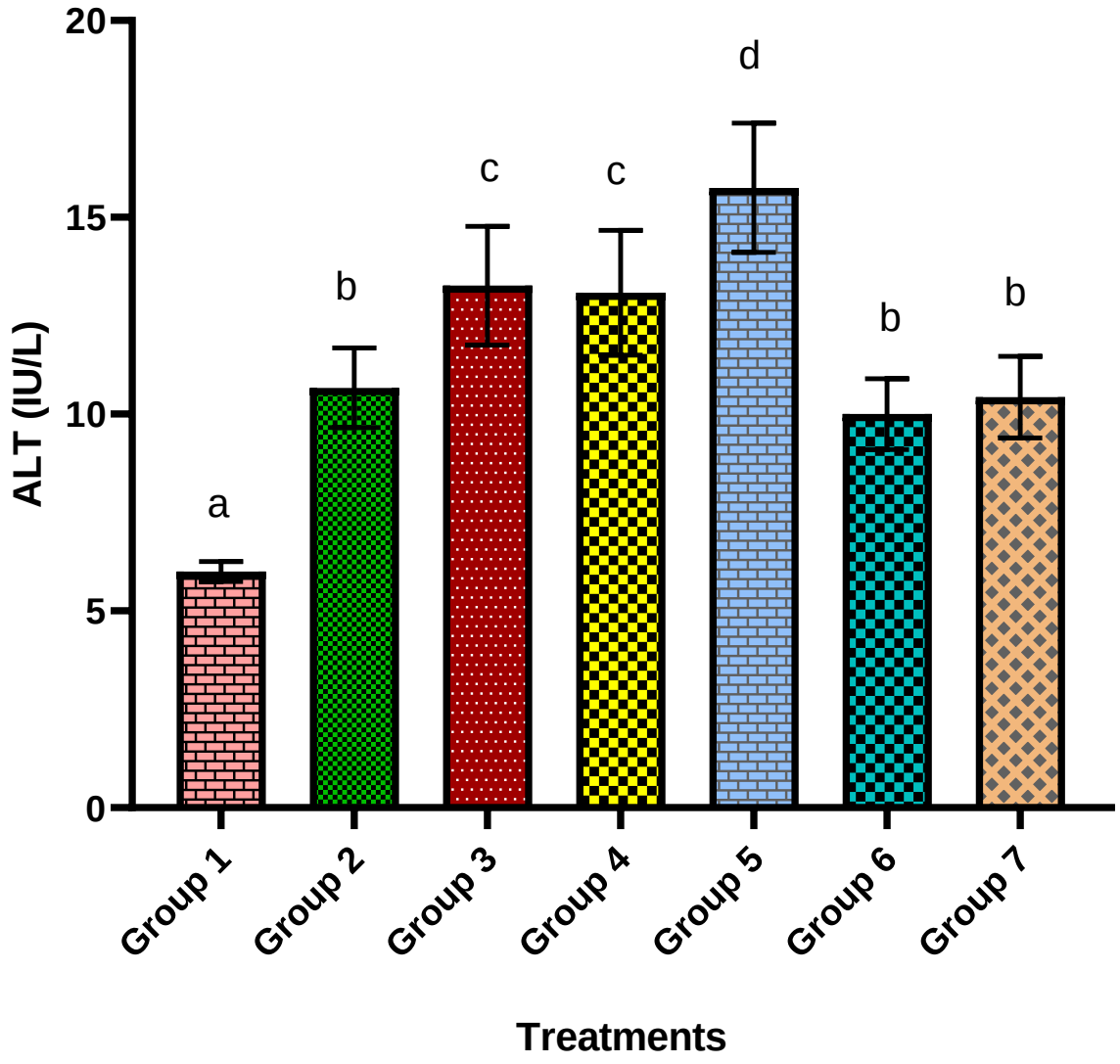


Figure 4.7: Effect of boiled wall gecko and centipede solution on ALT activity of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

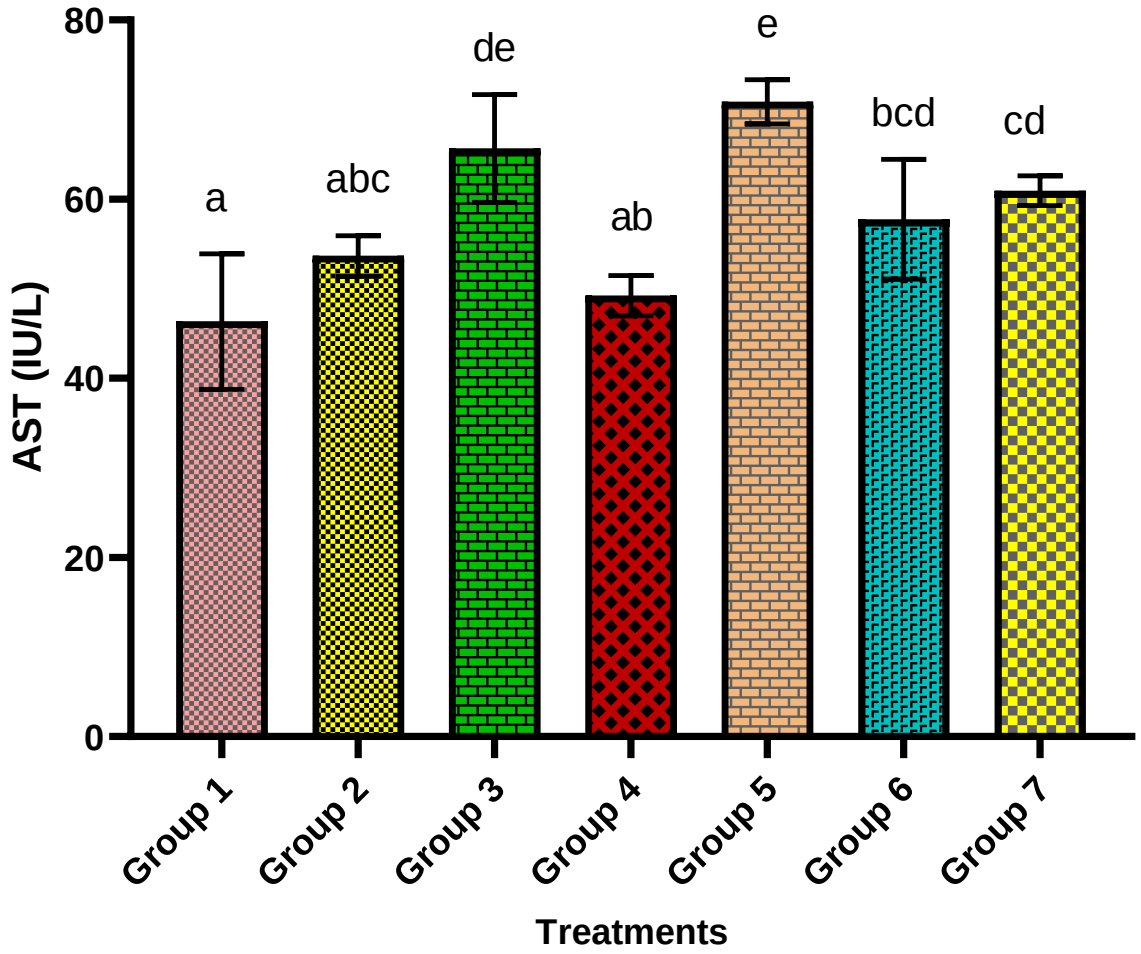


Figure 4.8: Effect of boiled wall gecko and centipede solution on AST activity of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

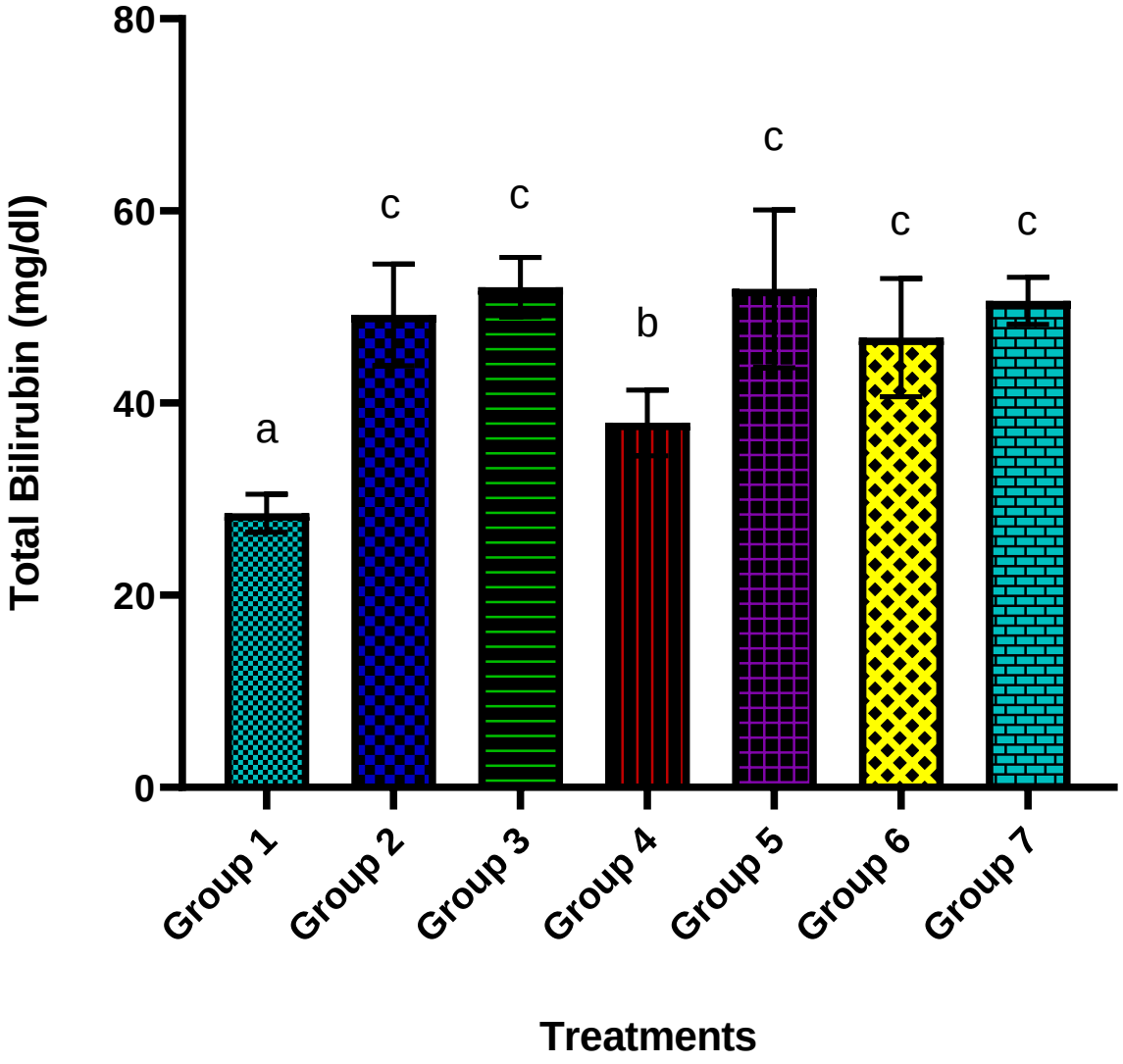


Figure 4.9: Effect of boiled wall gecko and centipede solution on total bilirubin level of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

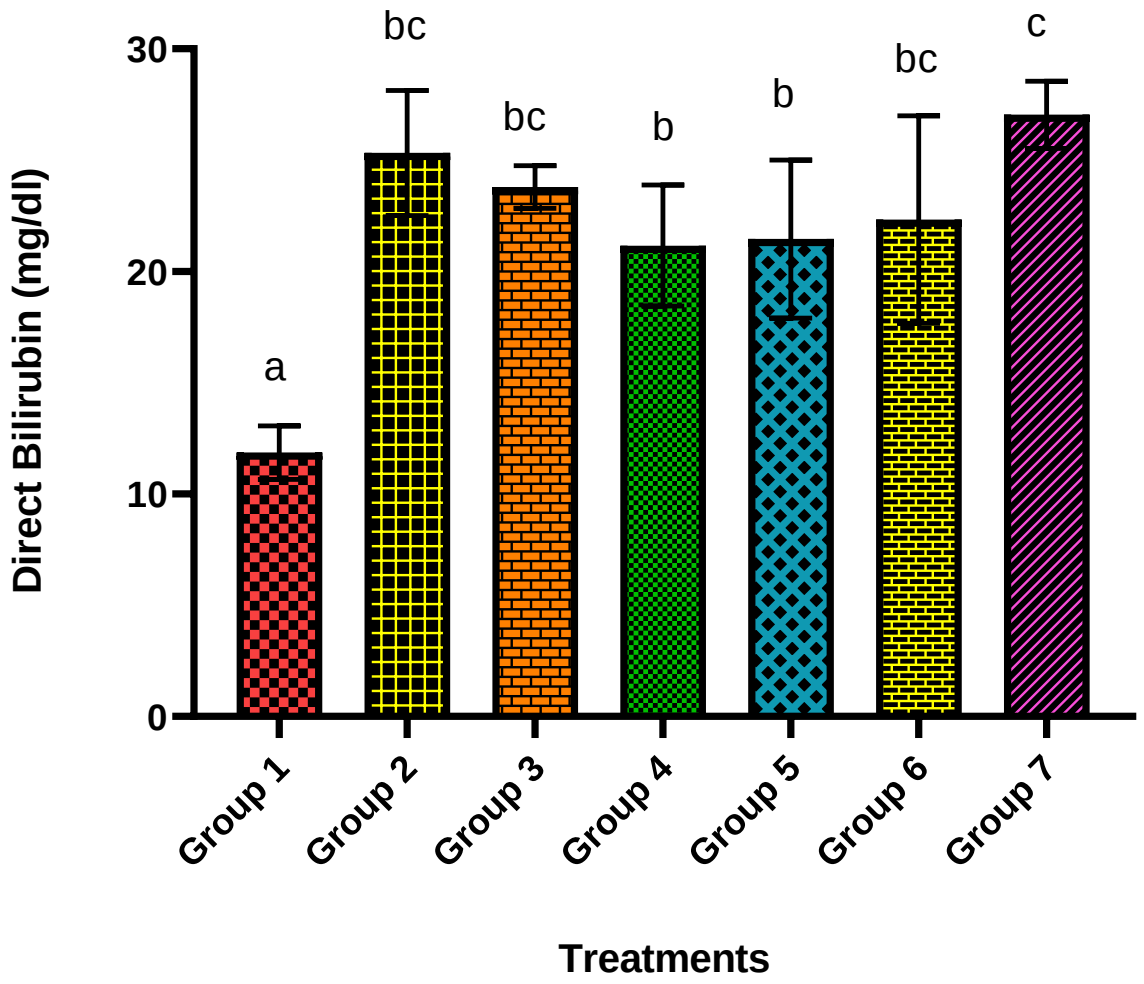


Figure 4.10: Effect of boiled wall gecko and centipede solution on direct bilirubin level of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

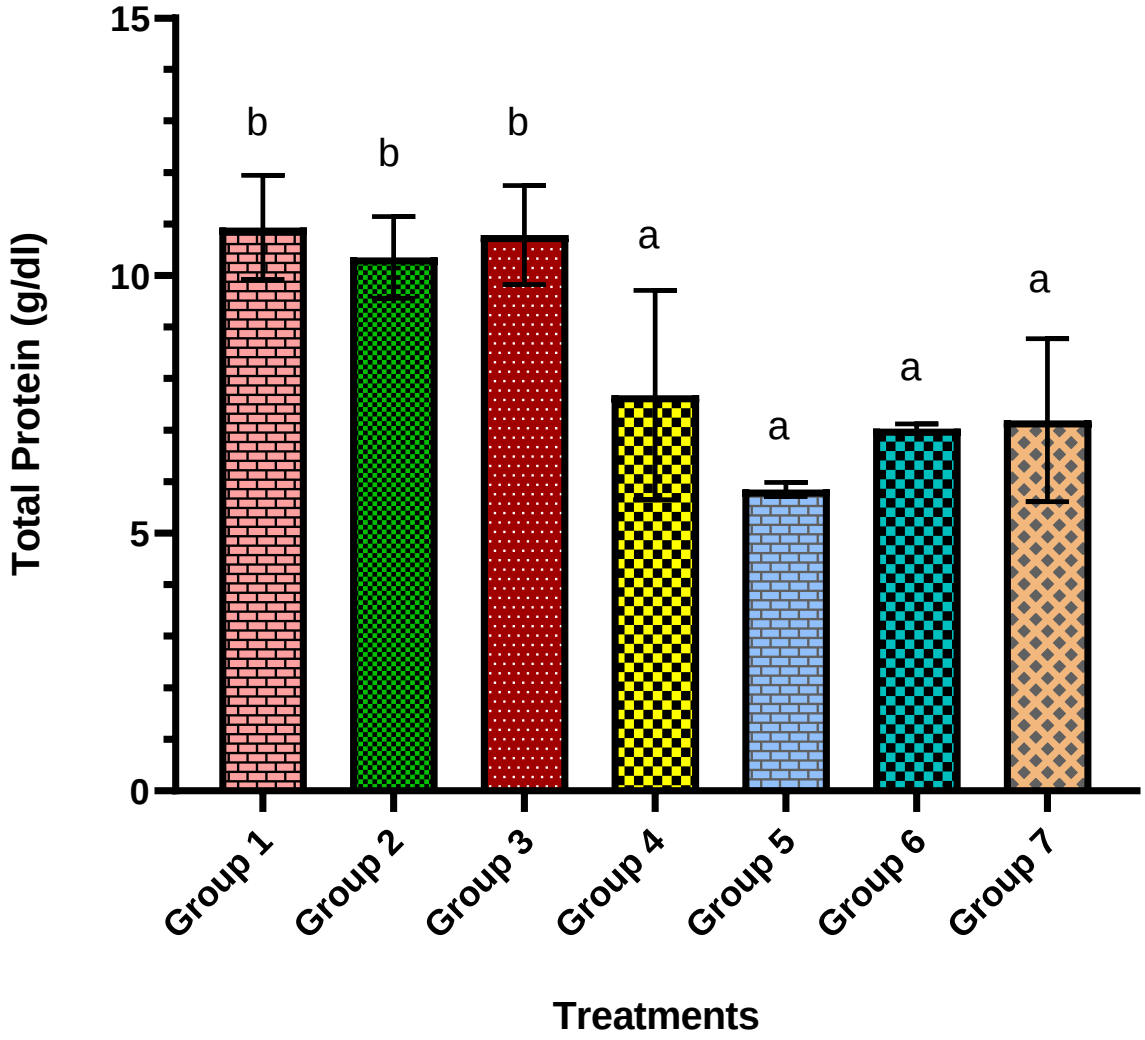


Figure 4.11: Effect of boiled wall gecko and centipede solution on total protein level of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

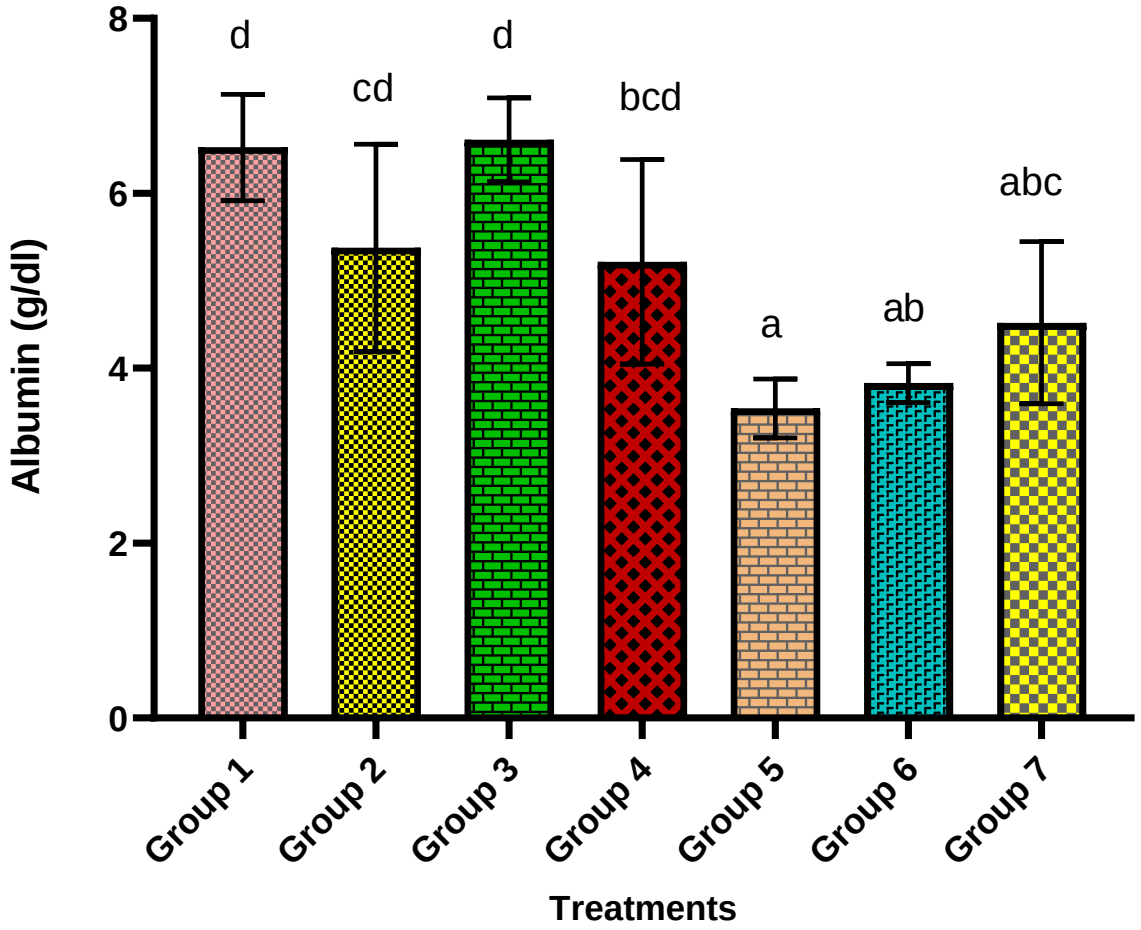


Figure 4.12: Effect of boiled wall gecko and centipede solution on albumin level of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

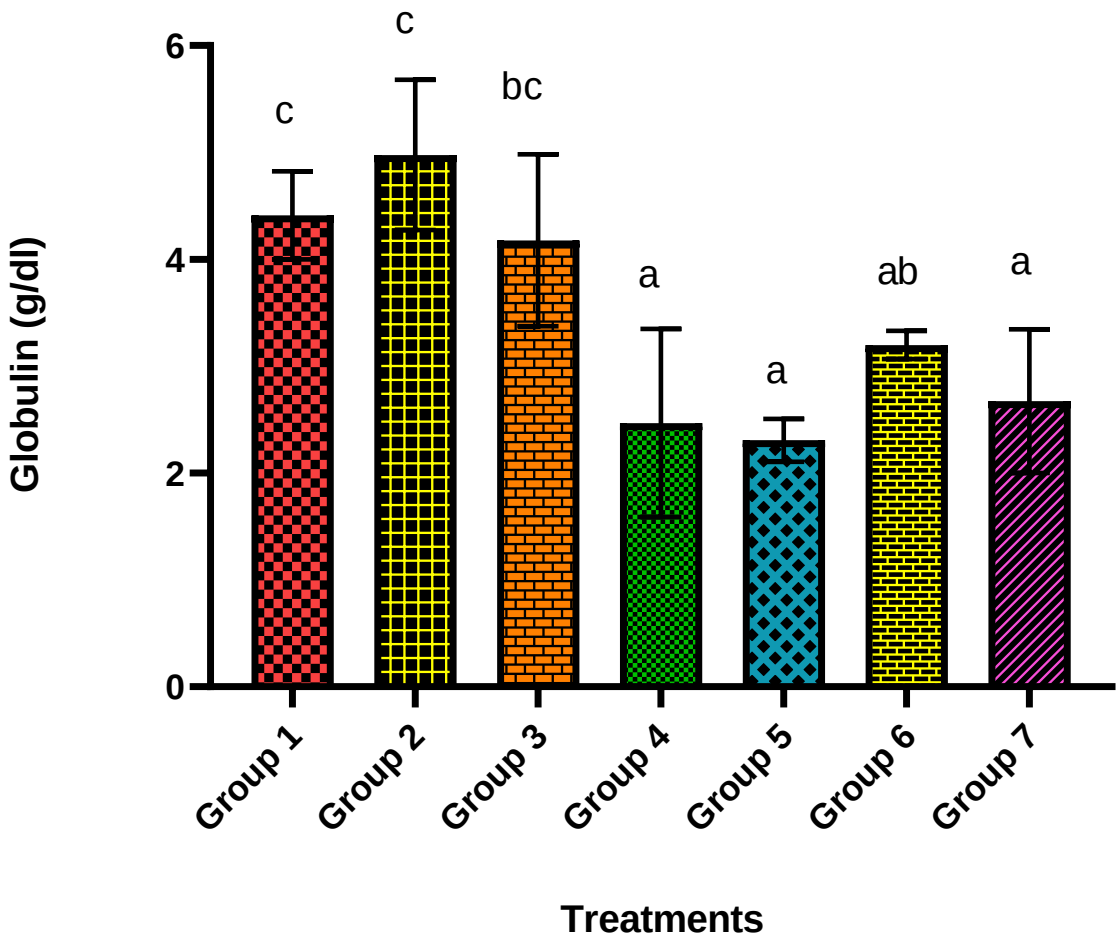


Figure 4.13: Effect of boiled wall gecko and centipede solution on globulin level of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

4.1.5 Effect of boiled wall gecko and centipede solutions on relative organ/body weight changes of rats

The result presented below showed no significant ($p>0.05$) difference in organ/body weight ratio in the experimental groups when compared to the normal control group (figures 4.14, 4.15 and 4.16).

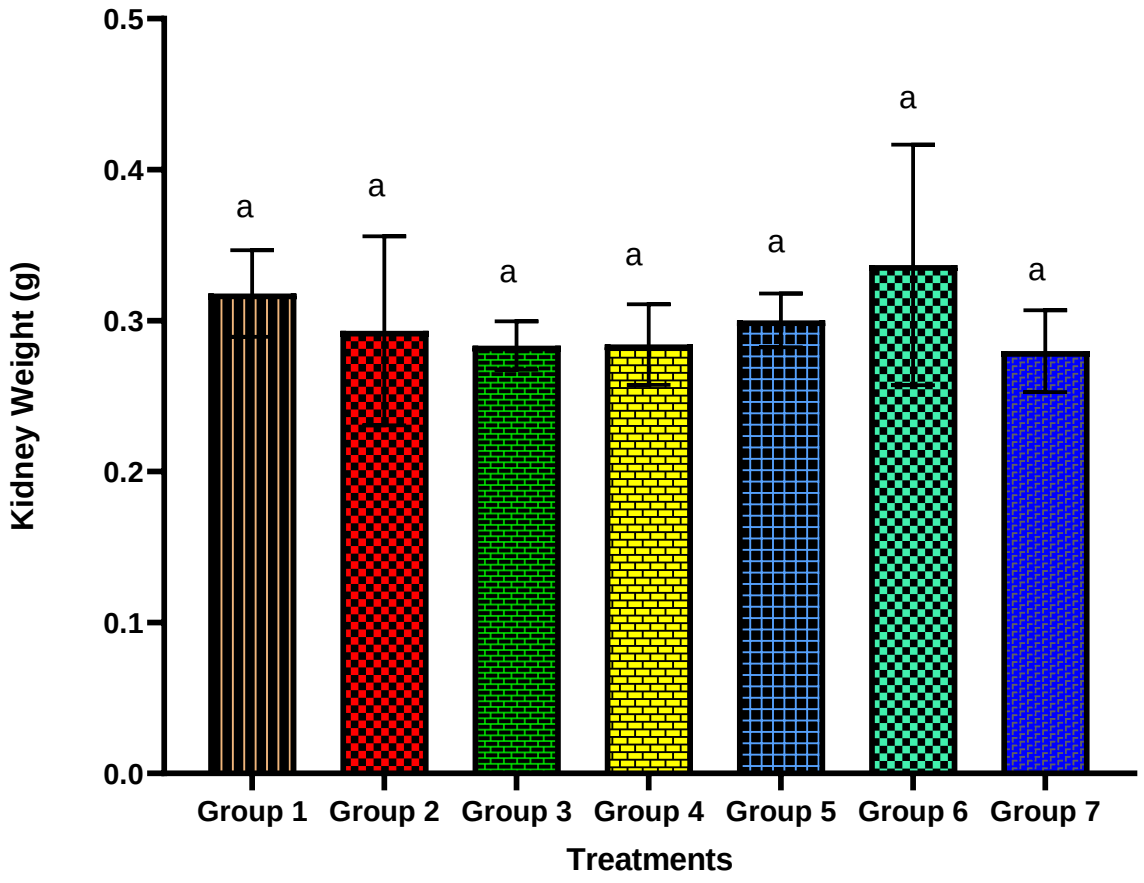


Figure 4.14: Effect of boiled wall gecko and centipede solution on kidney/body weight ratio of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

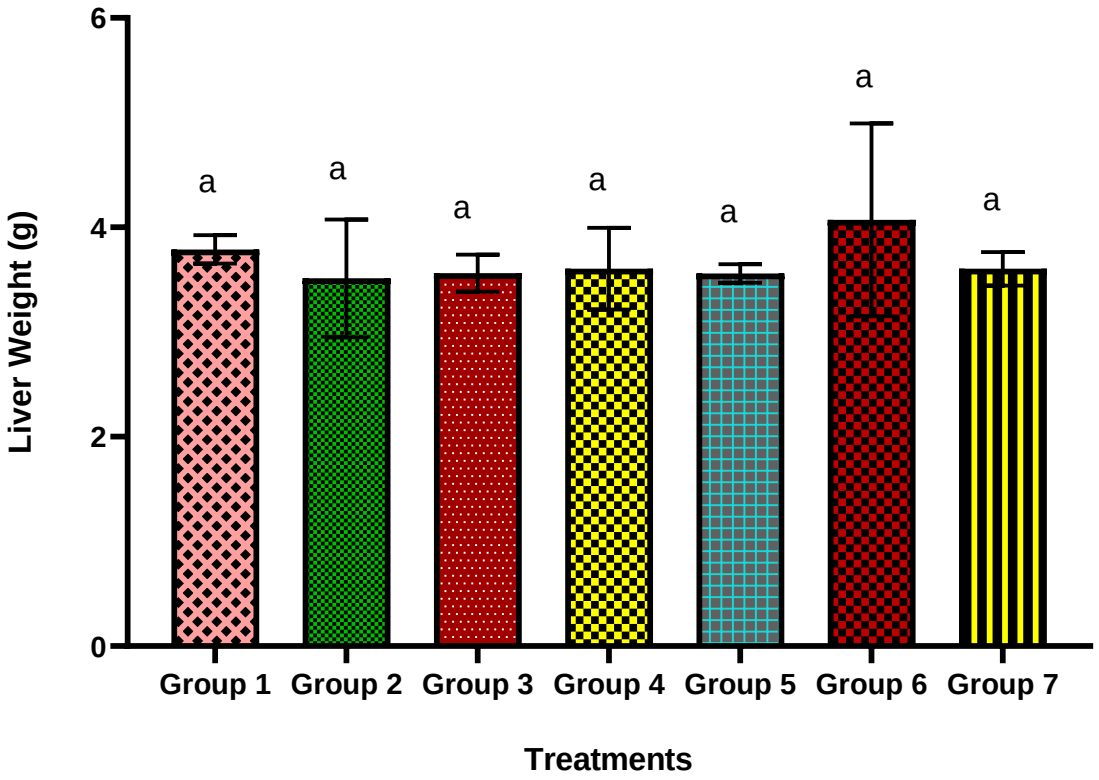


Figure 4.15: Effect of boiled wall gecko and centipede solution on liver/body weight ratio of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

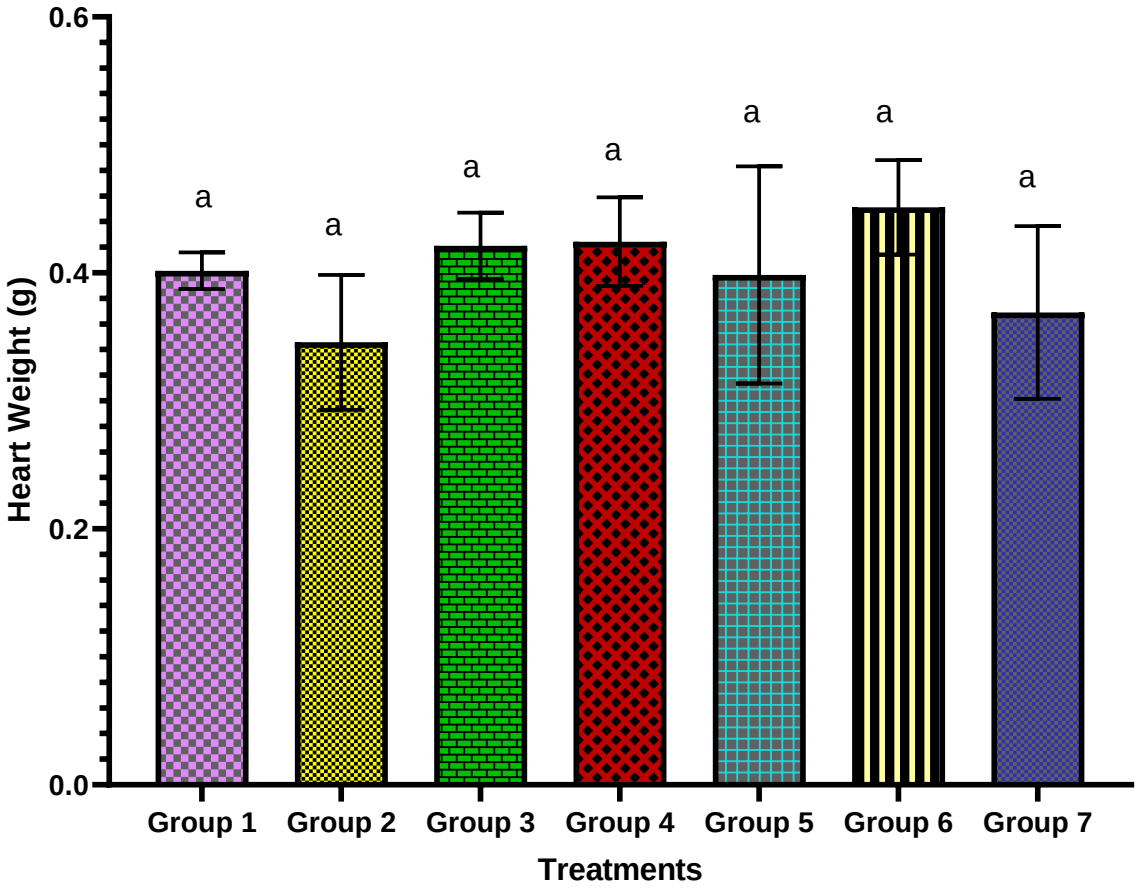
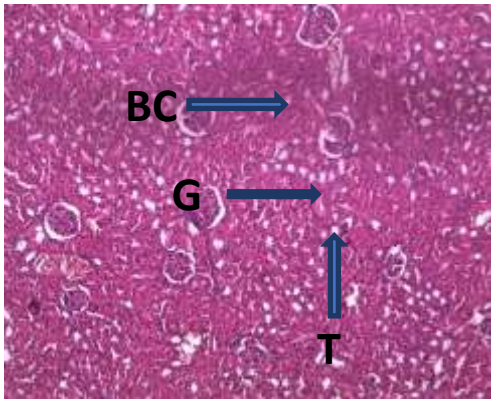


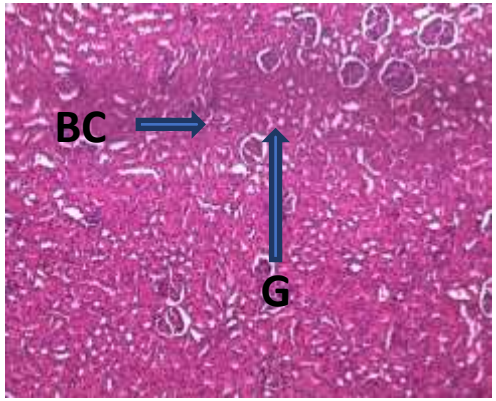
Figure 4.16: Effect of boiled wall gecko and centipede solution on heart/body weight ratio of rats. Bars with different alphabets are statistically ($p<0.05$) significant different. Group 1 = normal control, group 2 = degutted wall gecko low dose, group 3 = degutted wall gecko high dose, group 4 = whole wall gecko low dose, group 5 = whole wall gecko high dose, group 6 = centipede low dose, group 7 = centipede high dose.

4.1.6 Effect of boiled wall gecko and centipede solutions on kidney histopathology

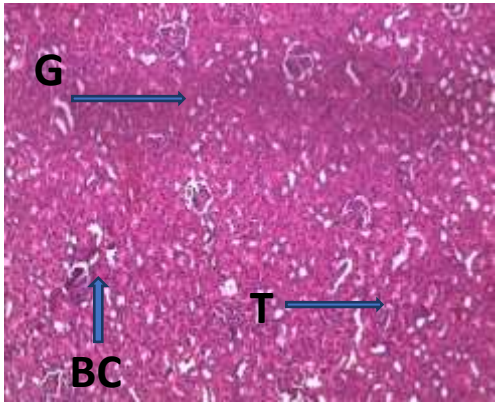
Histology sections of the kidney of rat in group 1 (normal control) and 2 (low dose of boiled degutted wall gecko solution) showed normal tissue architecture. The glomeruli, Bowman's capsule and tubules appear normal. Histology sections of the kidney of rat in group 3 (high dose of boiled degutted wall gecko solution) and 4 (low dose of boiled whole wall gecko solution) showed glumeruli closely adherent to the Bowman's capsule with congested stroma while that of the kidney of rat in group 5 (high dose of boiled whole wall gecko solution) showed slightly shrunken glomeruli with increase bowman's capsular space. Few of the tubules appear diluted and the stroma is congested (plate 4.1). There are few cystically diluted spaces. Histology section of the kidney of rat in group 6 (low dose of boiled centipede solution) showed marked shrunken glomeruli with markedly increase Bowman's capsule majority of the tubules are markedly dilated. Also seen is a focus of haemorrhage while that of group 7 showed glomeruli closely adherent to the Bowman's capsule with congested stroma showing haemorrhagic areas. Some of the tubules are compressed into slit-like channels (plate 4.1).



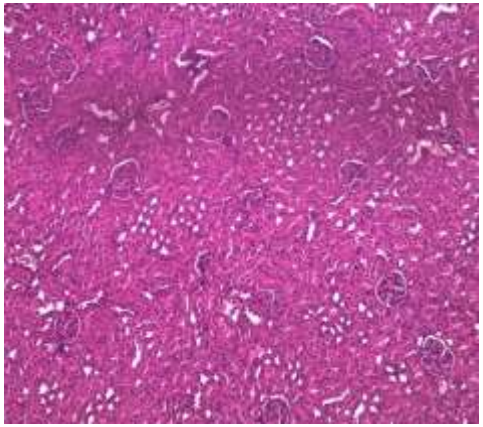
Group 1: Normal control rat's kidney (x400), stain: H and E.



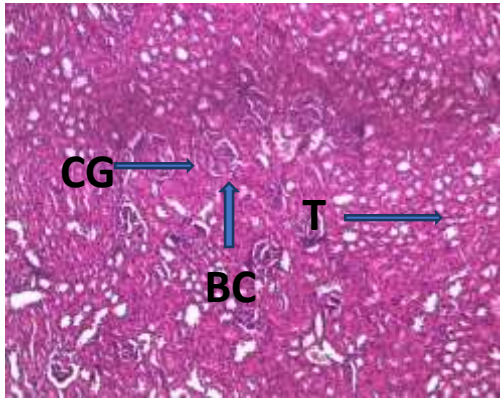
Group 2: Histology of rat's kidney administered 10 ml/kg of boiled degutted wall gecko solution (x400), stain: H and E.



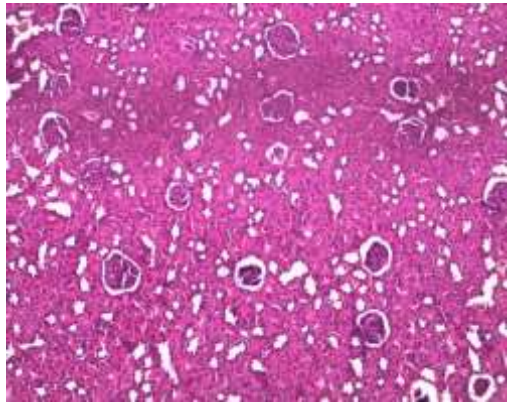
Group 3: Histology of kidney of rats administered 20 ml/kg of boiled degutted wall gecko solution (x400), stain: H and E.



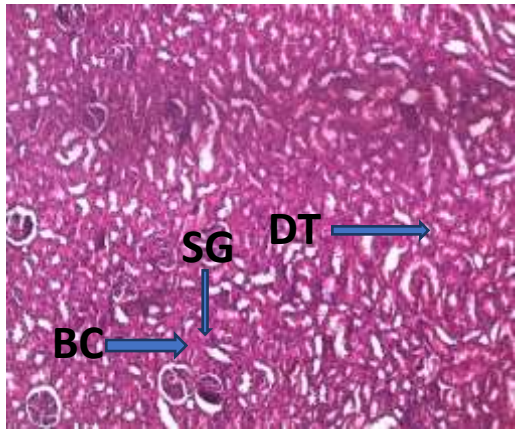
Group 7: Histology of kidney rats administered 20 mg/kg of boiled centipede solution (x400), stain: H and E.



Group 4: Histology of kidney of rats administered 10 ml/kg of boiled whole wall gecko solution (x 400), stain: H and E).



Group 5: Histology of kidney of rats administered 20 ml/kg of boiled whole wall gecko solution, (x400), stain: H and E.



Group 6: Histology of kidney of rats administered 10 mg/kg of boiled centipede solution (x400), stain: H and E.

(**CT, CG** and **BC** indicate congested tubule, glomeruli closely adherent & Bowman's capsule respectively).

H and E: Hematolxycilin & Eosin

Plate 4.1: Histology of kidney of the experimental animals.

Plate 4.1: Histopathological sections of kidneys of rats

BC = Bowman's capsule

T = tubule

G = glomerulus

CG = glomeruli closely adherent

T = tubule, G = glomeruli

CG = glomeruli closely adherent

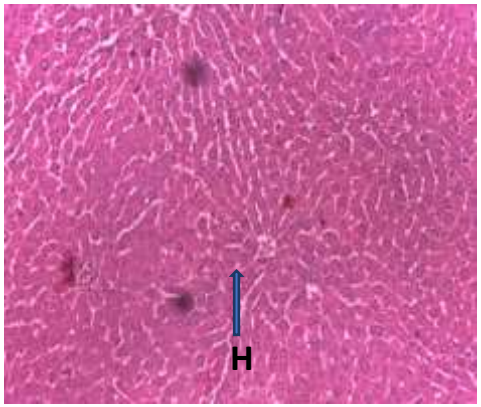
DT = dilated tubule

CT = congested tubule

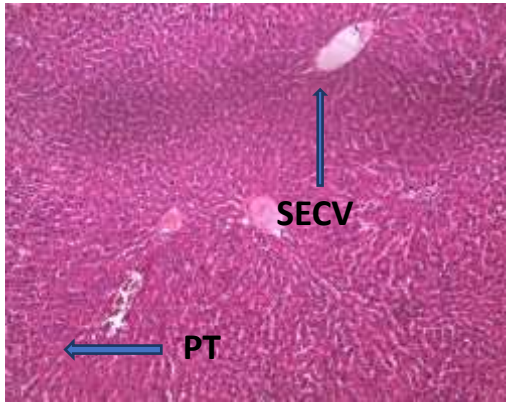
SG = shrunken glomeruli

4.1.7 Effect of boiled wall gecko and centipede solutions on liver histopathology

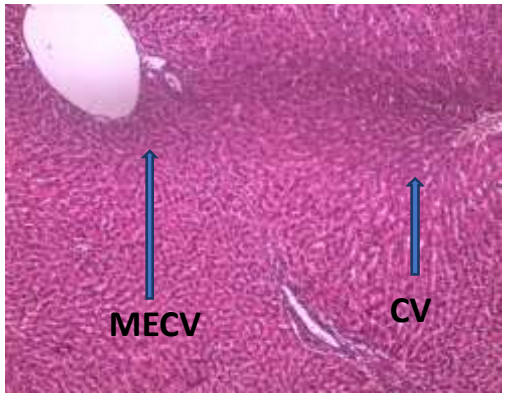
Histology section of the liver of group 1 (control group) showed normal liver architecture, all tissue element appeared normal that of group 2 and 3 (boiled degutted wall gecko solution) and 5 (high dose of boiled whole wall gecko solution) showed normal architecture. However, the central vein appears slightly enlarged. Other tissue elements appear unremarkable (plate 4.2). Histology section of the liver of rats in group 4 (low dose of boiled whole wall gecko solution) and 6 (low dose of boiled centipede solution) showed slightly enlarged central vein with majority of the hepatocytes showing vacuolation. Other tissue elements appear unremarkable however, in some areas the sinusoids appear congested while that of group 7 (high dose of boiled centipede solution) showed congested stroma with unremarkable central vein containing blood clot. Some of the hepatocytes appeared to have clear zone around the nuclei. The sinusoids appeared markedly compressed and the lamellar of the hepatocytes arrangement appeared distorted (plate 4.2).



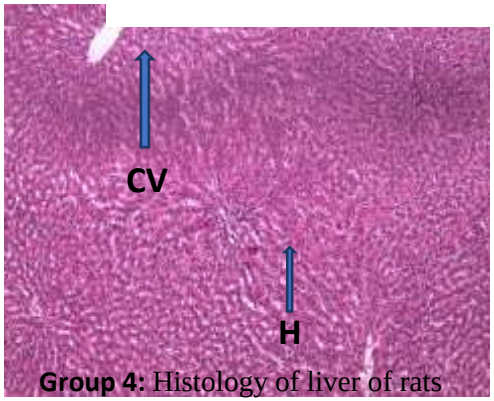
Group 1: Histology of normal rat's liver (x400), stain: H and E.



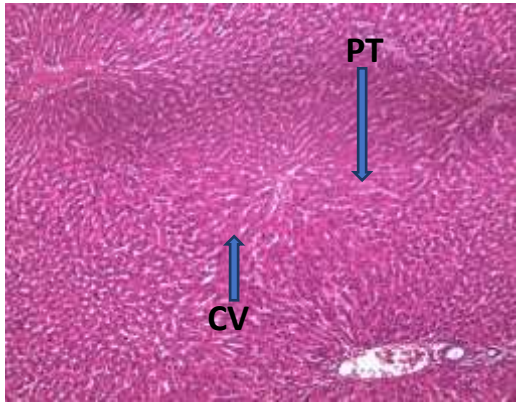
Group 2: Histology of liver of rats administered 10 ml/kg of boiled degutted wall gecko solution (x400), stain: H and E.



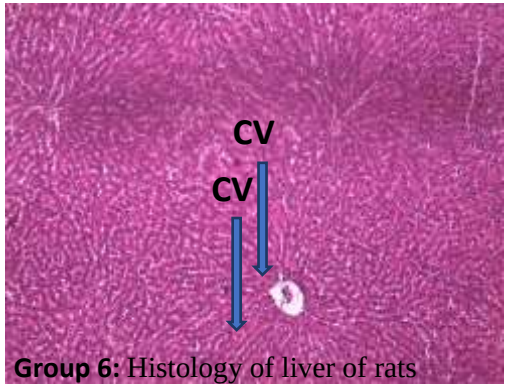
Group 3: Histology of liver of rats administered 20 ml/kg of boiled degutted wall gecko solution (x400), stain H and E (MECV indicates markedly enlarged central vein).



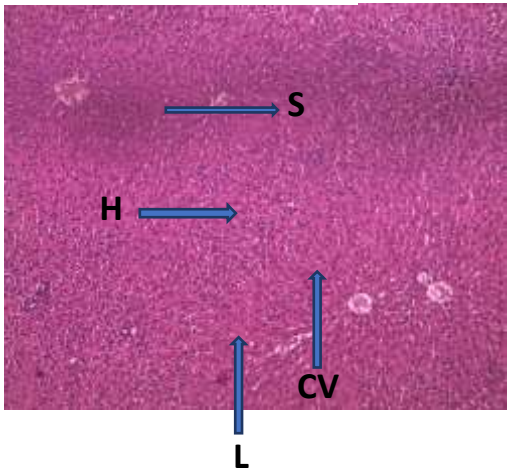
Group 4: Histology of liver of rats administered 10 ml/kg of boiled whole wall gecko solution (x400), stain: H and E.



Group 5: Histology of liver of rats administered 20 ml/kg of boiled whole wall gecko solution (x400) stain: H and E



Group 6: Histology of liver of rats administered 10 ml/kg of boiled centipede solution (x400), stain: H and E.



Group 7: Histology of liver of rats administered 20 mg/kg of boiled centipede solution (x400), stain: H and E.

(**H, S** and **CV:** indicate Hepatocyte, sinusoids and central vein respectively)

Plate 4.2: Histology of the liver of the experimental animals.

Plate 4.2: Histopathological sections of liver of rats

H = hepatocyte

CV = central vein

MECV = markedly enlarged central vein

PT = portal triad

SECV = slightly enlarged central vein

L = lamella

S = sinusoid

4.2 Discussion

The cellular toxicity effects induced by snakes, scorpions and arachnid venoms have been widely reported in the scientific literature, mostly by their direct implications in acute envenomation in humans or domestic animals (Undheim *et al.,* 2015). In contrast, centipede venoms and wall gecko toxicity have received less attention since there is a comparatively lower incidence of clinical cases (Undheim *et al.,* 2015). When evaluating the effect of boiled solutions of centipede and wall gecko on rats, we found them to induce alterations and changes in kidney and liver of rats.

The result obtained from GCMS characterization of sample solution showed the presence of twenty-nine (29) active components in degutted wall gecko boiled solution, thirty (30) chemical components in whole wall gecko boiled solution and thirty (30) chemical compounds in centipede boiled solution. Notable among the compounds present in boiled whole wall gecko solution were hexadecanoic acids, 11-octadecanoic acid, methyl stearate. Hexadecanoic acid, 9,10-epoxy-18-(trimethylsiloxy)-, methyl stearate, 6-Octadecenoic acid and n-hexadecanoic acids were relatively present in high level in degutted wall gecko boiled solution. The 9-octadecanoic acid, hexadecanoic acid and methyl stearate were also present in centipede boiled solution.

The enzyme kinetics study proved that n-hexadecanoic acid inhibits phospholipase A_2 in a competitive manner. Phospholipase A2 (PLA_2, E.C.3.1.1.4) catalyses the hydrolysis of ester bonds at the sn-2 position of membrane phospholipids which releases fatty acids, such as arachidonic acid (AA) and lysophospholipids. It is the initiating step in the formation of potent inflammatory mediators (Ueno and Rosenberg, 2000). Fatty acid derivatives of bee venom and sea weeds such as n-hexadecanoic in micro molar concentrations caused >90% inhibition of PLA_2 (Mayer *et al.,* 2003).

Methyl ester or methyl stearate is a saturated 19 carbon-chained compound and it is also known as octadecanoic acid methyl ester (OA). This fatty acid has various antiviral activities against viruses. Methyl stearate was able to inhibit the replication of HCV and synergistic effect with IFN-α was observed by Leu *et al*. (2004).

The 9,10-epoxy-18-(trimethylsiloxy)- is an omega-hydroxy fatty acid anion that is the conjugate base of 18-hydroxy-9,10-epoxyoctadecanoic acid arising from deprotonation of the carboxylic acid function; major species at pH 7.3. It is an omega-hydroxy-long-chain fatty acid anion and an epoxy fatty acid anion (Guzman *et al*., 2014). It is functionally related to an octadecanoate. Its major biological function is in the biosynthesis of cutin (framework of plant curticle) (Guzman *et al.*, 2014).

Some of these chemicals detected through GC-MS analysis are toxic to human consumption which have been implicated in liver injury. Other toxic chemicals detected were acetamide, cyclohexane, colchiceine, ethyl oxamate and N-(4-Aminobutyl) aziridine. Benzenamine also known as aniline is irritating to the skin, eyes, and respiratory tract. Its effect can result from all routes of exposure. Benzenamine induces methemoglobinemia, which impairs the delivery of oxygen to tissues. Benzenamine may also cause the destruction of red blood cells, which manifests as acute or delayed hemolytic anemia (Muir, 2001). Methenamine is an antibiotic that stops the growth of bacteria in urine. When the urine is acidic, methenamine turns into formaldehyde to kill the bacteria. Methenamine also possesses some toxic side effects like stomach upset, vomiting, diarrhea, stomach cramps and loss of appetite (Altinoz *et al*., 2019).

The 1H-imidazole that was detected is an imidazole tautomer which has the migrating hydrogen at position one. 1H-imidazole-based histidine compounds play very important roles in intracellular buffering. Histidine can be decarboxylated to histamine. Histamine can cause urticaria (hives) when it is produced during allergic reaction. This compound is a

strong antifungal agent. The imidazole derivatives inhibit the transformation of blastospores of *Candida albicans* into the invasive mycelial form. This inhibition probably facilitates the task of host defense cells and may be the principal factor leading to clearance of infection (Hochachka and Somero, 2002). The 1H-imidazole has been found to possess toxic effect in humans. The substance is corrosive to the skin and is severely irritating to the eyes. The substance is irritating to the respiratory tract. Imidazole has low oral acute toxicity in rats as indicated by its LD_{50} of 970 mg/kg (Ebel *et al.,* 2002). Halcinonide is a corticosteroid indicated for the relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses (Fredriksson *et al.,* 2000). The precise mechanism of action of topical corticosteroids is unclear. However, they possess anti-inflammatory, antipruritic, and vasoconstrictive actions. New research indicates that halcinonide activates MBP (myelin basic protein) expression via smoothened receptor activation. Halcinonide has been reported to cause side effects such as hives, difficulty in breathing, swelling of the face, lips, tongue, or throat, worsening of the skin condition, redness, warmth, swelling, oozing, or severe irritation of any treated skin, increased thirst, increased urination, dry mouth (Fujino *et al.,* 2005). Acetamide (60-35-5) is a dipolar solvent finding many uses in chemical processing and in the preparation of many chemical compounds. The chemical is easily absorbed and, to a large extent, excreted unchanged in the urine (U.S. Department of Health and Human Services, 1993). The acute toxicity is very low with lethal dose 50 percent (LD_{50}) values in the g kg^{-1} range. Acetamide was found to block the action of both endogenous and exogenous testosterone and, in addition, to being a potent inhibitor of testosterone-stimulated prostatic DNA synthesis. Moreover, it is capable of inhibiting prostatic nuclear uptake of androgen (IARC, 2007). Cyclohexane adversely affects the human nervous system. Effects range from headaches to anaesthesia, tremors, and convulsions. Contact with cyclohexane liquid or vapour can damage the eyes (Campbell, 2011). Colchicine is an

alkaloid derived from Colchicum autumnale, commonly known as autumn crocus. It prevents cells from forming spindles during mitosis, preventing chromosomal segregation during anaphase. As a result, colchicine induces multiple sets of chromosomes (Manzoor *et al.*, 2019). Colchicine is an important mutagen that works by preventing the microtubules formation and doubles the number of chromosomes (Gracheva *et al.*, 2020). Oxamate analogues have potential for use in contraception because N-isopropyl and propyl oxamates were selective inhibitors of LDH-C4 from testes (Rodrıguez-Pa´ez *et al*., 2011). Furthermore, N-propyl oxamate (NPOX) significantly reduced ATP levels and capacitation of mouse sperm (Wong *et al.,* 2007).

The result of this study also showed increases in some kidney function indices such as urea, creatinine, sodium and chloride ion levels following administration of boiled solutions of whole wall gecko and centipede. However, there was no alteration in these parameters following administration of boiled solution of degutted wall gecko in groups 2 and 3 when compared to the control group.

The kidneys perform lots of functions in animals such as homeostasis and acid-base balance, regulation of the balance of electrolytes in the blood, removal of waste products of metabolism, secretion of some enzymes and hormones, metabolism and osmoregulation. Any alteration or abnormality associated with the kidneys could lead to non-performance or inefficiency in carrying out these functions by the kidney. The abnormalities associated with kidney function could be ascertained by evaluating the levels of some kidney function parameters such as blood urea, creatinine, serum electrolytes and also histological examination of the organ, among others (Kamianowska *et al*., 2019).

The increase in urea level observed in this study was a sign of alteration and could possibly result from the effects of the boiled solution of whole wall gecko and centipede administered, thereby altering the rate at which the kidney excretes urea. Urea is the final degradation

product of protein and amino acid metabolism. It is synthesized in the liver from ammonia produced as a result of deamination of proteins. Filtration of urea from the blood into the urine by the renal glomeruli is the major means by which excess nitrogen is eliminated from the body.

Among the renal causes of increased urea levels are acute glomerulonephritis, chronic nephritis, polycystic kidney, nephrosclerosis, and tubular necrosis. Any type of obstruction of the urinary tract is a post-renal cause for elevated blood urea nitrogen levels (Tietz, 2007). Other causes of increased level of blood urea is cardiac decompensation, water depletion due to decreased intake and excessive loss, increased protein catabolism and high protein diet. Elevation of blood urea level was mostly recorded in groups 4 and 5 animals administered low and high concentration of boiled solution of whole wall gecko and in groups 6 and 7 animals administered low and high concentration of boiled solution of centipede. Both urea and creatinine are cleared by the renal glomeruli. However, urea is subsequently partially reabsorbed by the renal tubules, while creatinine is not.

Blood creatinine levels in all the groups administered the boiled solutions of whole wall gecko and centipede obviously increased when compared with the normal control.

Measuring serum creatinine is a simple test, and it is the most commonly used indicator of renal function. There were alterations in creatinine levels in the groups administered low and high dose of boiled solution of degutted wall gecko. The effect of the boiled solution was dose-dependent. Serum creatinine (a blood measurement) is an important indicator of renal health because it is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys (Imo and Uhegbu, 2015b). The elevation of creatinine levels (figure 4.1) showed that more creatinine was retained in the blood of all the groups administered boiled solutions of whole wall gecko and centipede. Nephrotoxicity is

by significant elevation in serum level of creatinine and urea. Creatinine retention in the blood is evidence of kidney impairment (Imo and Uhegbu, 2015b).

Creatinine is removed from the blood chiefly by the kidneys, primarily by glomerular filtration, but also by proximal tubular secretion. Little or no tubular reabsorption of creatinine occurs. A rise in blood creatinine level is observed majorly with marked damage to functioning nephrons (Imo and Uhegbu, 2015b). The elevation of the blood creatinine levels in this study was believed to be as a result of the boiled solutions that were administered. It is possible that the chemical constituents of the boiled solutions were responsible for these negative effects.

Electrolytes (sodium, potassium, chloride and bicarbonate) balance in the blood is a good indicator of how well the kidneys and heart function. Knowing which electrolytes are out of balance can help in the determination of a course of treatment. Sodium is regulated by the kidneys and adrenal glands. Sodium is the major cation of the extracellular fluid. It plays a central role in the maintenance of the normal distribution of water and the osmotic pressure in the various fluid compartments. Too much sodium (hypernatremia) or too little sodium (hyponatremia) can cause cells to malfunction, and extremes in the blood sodium levels (too much or too little) can be fatal (Imo and Unegbu, 2015).

Chloride is important in the maintenance of the cation/anion balance between intra and extracellular fluids. This electrolyte is essential to the control of proper hydration, osmotic pressure, and acid/base equilibrium. Low serum chloride values are found with extensive burns, excessive vomiting, intestinal obstruction, nephritis, metabolic acidosis, and in Addisonian crisis. Elevated serum chloride values may be seen in dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary obstruction (Imo *et al*., 2018).

The serum or plasma bicarbonate content is an indicator of electrolyte dispersion and anion deficit. Low alteration of bicarbonate and CO_2 dissolved in plasma are characteristics of acid-base imbalance, which may be due to renal tubular acidosis, hyperkalemic acidosis, renal failure or keto acidosis (Imo *et al*., 2019). Sodium, bicarbonate, chloride and potassium stability in the blood is known to be a good indicator of effective functioning of the kidneys and heart (Imo and Uhegbu, 2015b). Imo and Uhegbu (2015b) reported that alterations in the concentrations of these body electrolytes is indicative of poor renal functions or renal impairment.

The result of this study showed increases in ALP and ALT activities and in total bilirubin and direct bilirubin levels following administration of boiled degutted wall gecko solution (groups 2 and 3), boiled whole wall gecko solution (groups 4 and 5) and boiled centipede solutions (groups 6 and 7) when compared to the normal control group (group 1).

There was also an increase in AST activity (Fig. 4.8) following oral administration of high doses of boiled degutted (group 3) and whole wall gecko (group 5) solutions when compared to the control group (group 1).

The liver is one of the most vital organs that functions as a centre for metabolism of nutrients and excretion of waste metabolites. The liver handles the metabolism and excretion of xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them (Ozougwu and Eyo, 2014). Given the liver's strategic function in the body, it is continuously exposed to xenobiotics and chemotherapeutic agents (Gupta and Misra, 2006).

The AST is primarily found in the liver mitochondria and cytoplasm. It is also found in the heart, muscle, kidney and brain. Its serum level increases in hepatic necrosis, myocardial infarction and muscle injury (Srinivasan *et al*., 2007). In this present research, the rise in the activity of serum AST observed may be attributed to some damage to the liver or other

organs where AST is produced. The increase in the activity of AST in this research was in agreement with the report of other investigators who reported increases in the activity of AST subsequent to toxicant administration (Onyema *et al*., 2006; Thomas *et al*., 2009). Rene *et al.* (2014) observed a gradual increase in AST activity following administration of monosodium glutamate as a toxicant.

The ALT is a sensitive biomarker of hepatic status (Al-mamary *et al*., 2002). Although ALT and AST are synthesized in the liver, they are also present in the serum and in various tissues. The ALT serum activity becomes elevated during liver disease, and therefore, it is considered a more specific marker of liver injury than AST (Kunutsor *et al*., 2013). In this present study, the increase in serum ALT activities when comparism is made to the control group was in agreement with the report of Rene *et al.* (2014*)* where an increase in ALT activity was observed.

Phosphatase (ALP) is a biomarker enzyme for assessing the integrity of plasma membrane (Akanji *et al*., 2003). Alteration in the activities of alkaline phosphatase is an indication that there could be damage due to cytotoxic effect of toxicant (Ortiz *et al*., 2006) thereby resulting to leakage of this enzyme from the liver into serum. Such increase in alkaline phosphatase activities can constitute threat to the lives of cells that are dependent on a variety of phosphate esters for their vital processes since there may be indiscriminate hydrolysis of phosphate esters in the tissue (Yakubu *et al*., 2006). Altered activity of ALP can occur due to *de novo* synthesis by liver cells. The ALP is a reliable marker of hepatobiliary dysfunction due to damage (Muriel and Escobar, 2003). The increase in ALP activity may have suggested that boiled solution of centipede and wall gecko at this study level could pose serious harm to the liver (Moss and butterworth, 2004). The result was in line with the report of Rocek *et al*. (2001) who demonstrated that MSG administration could alter the intestinal function thereby releasing intestinal ALP.

The increase of bilirubin concentrations in rats administered boiled solution of wall gecko or centipede in this study may be due to excessive haem destruction and blockage of biliary tract resulting in inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes (Ali *et al*., 2010). Bilirubin has a protective role against oxidative damage of cell membrane induced by toxicant (Noriega *et al.*, 2003).

The decrease in serum total protein (figure 4.11) observed in this study following 28 days administration of boiled solutions of whole wall gecko and centipede may be due to both hepatic and renal damage-induced by the toxicant present in these solutions (Ahmed and Shalaby, 1999), or may be due to binding of these toxicant to plasma proteins, where it causes alteration in a high number of enzymes and can also disturb protein synthesis in hepatocytes (Goering, 1993). The decreased serum total protein concentrations may be attributed to a decreased utilization of free amino acids for protein synthesis (Moussa and Bashandy, 2008).

Albumin, which is the most abundant blood plasma protein, is produced by the liver and study has shown that its production can be decreased by toxicant (Ogutcu *et al*., 2008). Since reductions in albumin levels are generally suggestive of liver disease, it was possible that high doses of boiled solutions of whole wall gecko and centipede may have altered protein and free amino acid metabolism and their synthesis in the liver (Ncibi *et al.*, 2008). Qualitative and quantitative disturbance of protein synthesis is a consequence of impaired hepatic function (Celia and Wilkinson, 2003). Hypoalbuminemia is a liver disorder thought to be a consequence of decreased hepatic synthesis of albumin (Burtis and Edward, 2004).

Histopathological lesions have been widely used as biomarkers for the health evaluation of organisms exposed to toxicant (Rekha and Hamid, 2013). Histopathological biomarker in xenobiotic monitoring is of significant advantage as it permits examining specific target

organs which are responsible for vital functions. Moreover, the changes noticed in these vital organs are usually easier to identify as compared to functional ones and can be used as warning symptoms for organism health. Histological examinations of the hepatic tissues of rats exposed to centipede and wall gecko boiled solutions revealed degenerative changes in the liver in the form of parenchymatous degeneration and hepatocyte degeneration (Rezg *et al*., 2008). Toxicants are known to induce various histopathological changes in liver tissues (Gokcime and Artiran, 2007), such as induce hemorrhage, inflammatory cell infiltration, tissue damage, and necrosis (Kalender *et al.,* 2006). Such liver damage may arise from the toxic effects of the boiled centipede and wall gecko solutions, which may have disturbed the detoxification mechanisms of the liver. Similar findings to the current observations were also reported in kidney tissue of rats administered with boiled solutions of wall gecko and centipede. The most notable effect of these solutions after subchronic exposure in rats was vacuolar degeneration (Grothe *et al*., 2002). Stebbins *et al*. (2002) reported that mice given toxicants had vacuolation in liver and kidney tissues. In addition, it is possible that these toxicants adversely affected the cytochrome P450 system or the mitochondrial membrane transport system of hepatocytes (Gokcime *et al.,* 2007). In the present study, hepatic and renal morphological changes observed in boiled wall gecko and centipede solution-treated rats correlated with altered levels of evaluated biochemical parameters.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In the light of the aforementioned results, it can be seen that changes in the biochemical parameters are in agreement with histopathological observations which showed degenerative changes in liver and kidneys of rats treated with boiled solutions of wall gecko and centipede in a dose-related manner. The result also revealed that the toxicity effect was more pronounced on boiled whole wall gecko solution when compared to the degutted wall gecko solution. This may suggest that the gut of wall gecko might be responsible or contained agents responsible for the toxicity of wall gecko.

5.2 Recommendations for further study

The following recommendations are made for further study:

(a) A study of these boiled solutions on other organs should be undertaken.

(b) More study is needed in this field to elucidate the chemical or pathological profile of wall gecko's gut to be able to understand the mechanism of the toxicity of the reptile.

(c) It is suggested that the study should be continued at doses above 50 ml/kg body weight.

(d) It is suggested that the effect of boiled solutions on haematological parameters be carried out.

5.3 Contribution to knowledge

i. The boiled centipede and wall gecko solutions were toxic

ii. The extra toxicity of whole wall gecko solutions stemmed from the contents of the wall gecko's gut.

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APPENDIX I

ETHICAL LETTER

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI
SCHOOL OF BIOLOGICAL SCIENCES
DEPARTMENT OF BIOCHEMISTRY

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Date: 06/06/2023

DEPARTMENTAL ETHICAL COMMITTEE

Mrs Jane Esseh
Department of Biochemistry
Postgraduate School
Federal University of Technology
Owerri.

Dear Mrs Esseh,

RE: REQUEST FOR ETHICAL APPROVAL

We hereby respond to your application seeking approval for the use of rats for your research on the effects of the boiled solutions of wall gecko and centipede on some biochemical, organ weights and physiological parameters in rats.

Your application has been considered by members and approved. You are to ensure that the animals/rats are handled humanely; in such ways that meet the requirements of the National Institutes of Health, NIH (2011).

Congratulation!

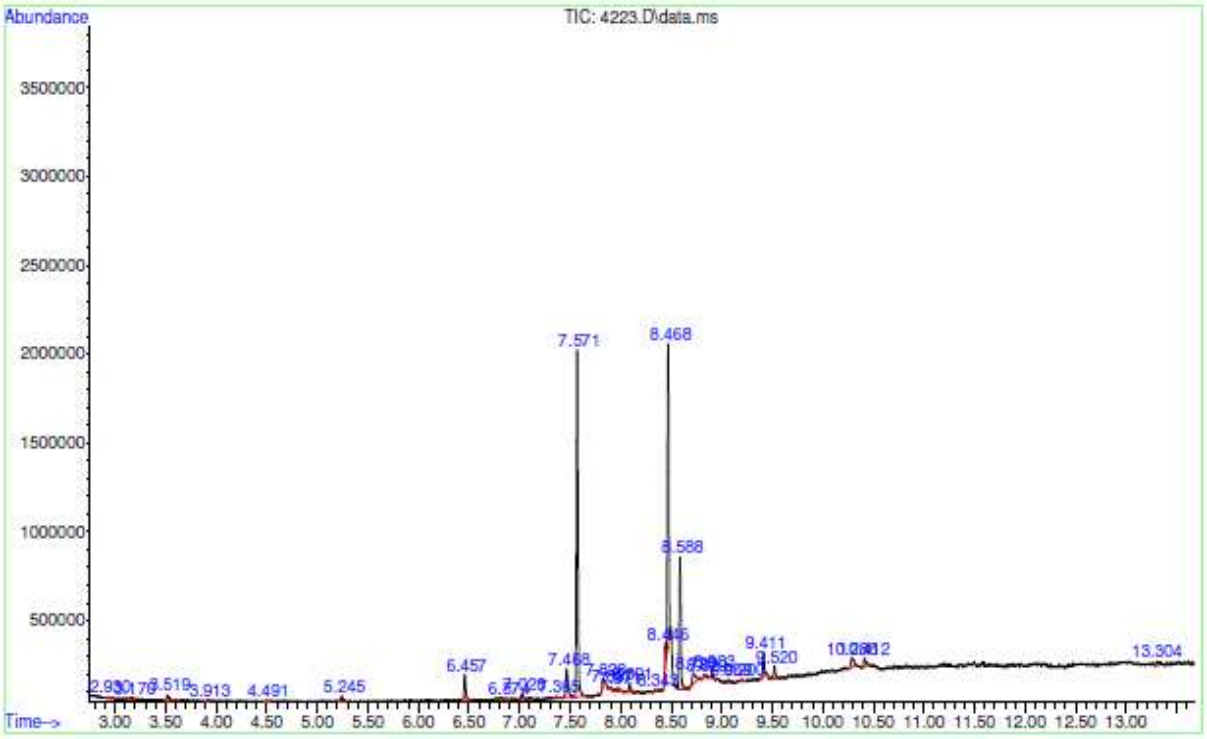
16/01/2024

Prof. R.N. Nwaoguikpe
(Chairman)

APPENDIX II

GCMS analysis of whole wall gecko

```
File       :C:\gcms\1\data\ESSEH\4223.D
Operator   : EISL LAB
Acquired   : 12 Oct 2023  10:35     using AcqMethod PAHS_LC.M
Instrument :    GCMSD
Sample Name: SAMPLE A
Misc Info  :
Vial Number: 2
```



Area Percent Report

Data Path : C:\gcms\1\data\ESSEH\
Data File : 4223.D
Acq On : 12 Oct 2023 10:35
Operator : EISL LAB
Sample : SAMPLE A
Misc :
ALS Vial : 2 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : OFF Filtering: 9
Sampling : 1 Min Area: 1 Area counts
Start Thrs: 0.001 Max Peaks: 30
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Tangent else baseline drop >
Peak separation: 1

Method : C:\gcms\1\methods\PAHs_lc.M
Title : PAH_LC

Signal : TIC: 4223.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.930	58	61	67	rBB8	7916	10196	0.50%	0.168%
2	3.170	98	103	107	rBB8	7924	11502	0.57%	0.189%
3	3.519	161	164	175	rBB	31840	52647	2.59%	0.865%
4	3.913	228	233	237	rBB7	8764	14471	0.71%	0.238%
5	4.491	331	334	341	rBB9	6418	9979	0.49%	0.164%
6	5.245	461	466	470	rBB3	30251	38992	1.92%	0.641%
7	6.457	674	678	687	rBB	140858	173304	8.52%	2.849%
8	6.874	748	751	755	rBB6	8449	12072	0.59%	0.198%
9	7.028	772	778	783	rBB5	33649	49332	2.43%	0.811%
10	7.365	835	837	841	rBB5	10658	11015	0.54%	0.181%
11	7.468	847	855	868	rBB2	162568	263232	12.95%	4.327%
12	7.571	868	873	886	rBB	1951130	2033094	100.00%	33.418%
13	7.828	914	918	921	rBB3	46043	70500	3.47%	1.159%
14	7.897	928	930	934	rBB4	10848	10167	0.50%	0.167%
15	7.971	941	943	951	rBB9	16459	23341	1.15%	0.384%
16	8.091	960	964	969	rBB	51006	57940	2.85%	0.952%
17	8.343	1004	1008	1011	rBB6	11444	13620	0.67%	0.224%
18	8.446	1023	1026	1027	rBB2	121538	141709	6.97%	2.329%
19	8.468	1027	1030	1033	rBB	1713535	1652524	81.28%	27.163%
20	8.588	1046	1051	1059	rBB	743667	782515	38.49%	12.862%

21	8.720	1067	1074	1082	rBB	59537	140918	6.93%	2.316%
22	8.829	1089	1093	1094	rBB4	10340	14261	0.70%	0.234%
23	8.903	1103	1106	1112	rBB7	37533	40735	2.00%	0.670%
24	9.069	1131	1135	1138	rBB6	15359	19286	0.95%	0.317%
25	9.200	1155	1158	1162	rBB6	14290	10657	0.52%	0.175%
26	9.411	1191	1195	1199	rBB2	133961	162497	7.99%	2.671%
27	9.520	1210	1214	1220	rBB3	70798	82427	4.05%	1.355%
28	10.286	1344	1348	1355	rBB10	51348	98535	4.85%	1.620%
29	10.412	1366	1370	1375	rBB6	46156	63428	3.12%	1.043%
30	13.304	1873	1876	1878	rBB4	22252	18869	0.93%	0.310%

Sum of corrected areas: 6083765

PAHs_lc.M Thu Oct 12 11:18:44 2023

Library Search Report

Data Path : C:\gcms\1\data\ESSEH\
Data File : 4223.D
Acq On : 12 Oct 2023 10:35
Operator : EISL LAB
Sample : SAMPLE A
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: C:\Database\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - rteint.p

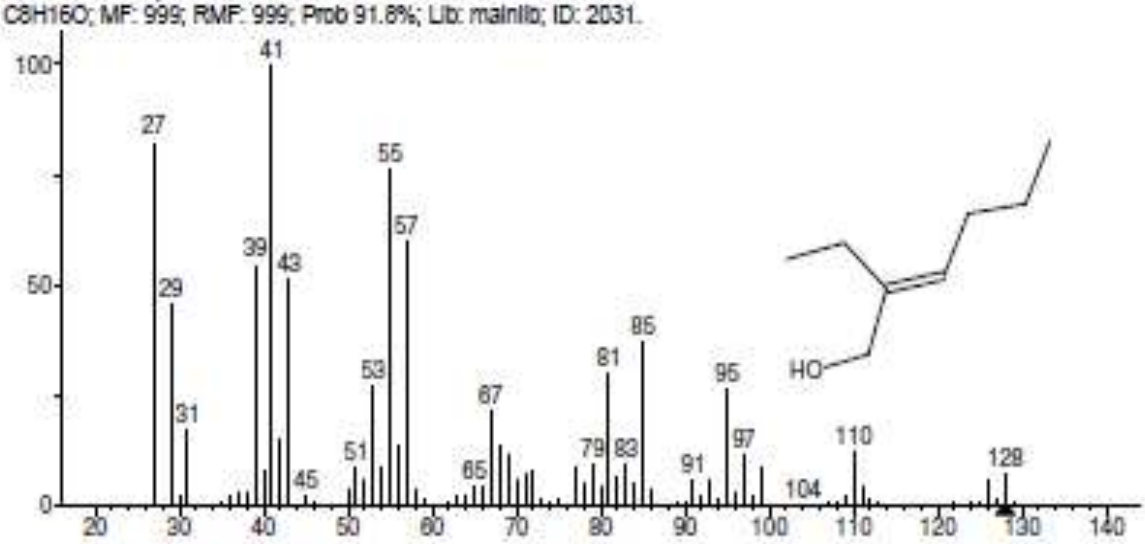
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	2.930	0.17	C:\Database\NIST14.L			
			cis-2-Ethyl-2-hexen-1-ol	12828	1000139-52-4	46
			2H-Pyran, 2-(3-butynyloxy)tetrahydro-	28583	040365-61-5	43
			2-Furanmethanol	3125	000098-00-0	43
2	3.170	0.19	C:\Database\NIST14.L			
			L-Methionine, methyl ester	34259	010332-17-9	27
			N-Methyl-N-methoxy-bistrifluoromethylacetamide	101459	078191-01-2	27
			Metobromuron	119630	003060-89-7	27
3	3.519	0.87	C:\Database\NIST14.L			
			Methenamine	18654	000100-97-0	83
			Methenamine	18655	000100-97-0	72
			Methenamine	18653	000100-97-0	72

1	2.930	0.17	C:\Database\NIST14.L			
			cis-2-Ethyl-2-hexen-1-ol	12828	1000139-52-4	46
			2H-Pyran, 2-(3-butynyloxy)tetrahydro-	28583	040365-61-5	43
			2-Furanmethanol	3125	000098-00-0	43
2	3.170	0.19	C:\Database\NIST14.L			
			L-Methionine, methyl ester	34259	010332-17-9	27
			N-Methyl-N-methoxy-bistrifluoromethylacetamide	101459	078191-01-2	27
			Metobromuron	119630	003060-89-7	27
3	3.519	0.87	C:\Database\NIST14.L			
			Methenamine	18654	000100-97-0	83
			Methenamine	18655	000100-97-0	72
			Methenamine	18653	000100-97-0	72
4	3.913	0.24	C:\Database\NIST14.L			
			1-Deoxy-d-mannitol	36777	060965-81-3	50
			6-Desoxy-l-altritol	36781	1000130-02-3	40
			1,2-Dichloroethylene	2723	000540-59-0	30
5	4.491	0.16	C:\Database\NIST14.L			
			Docosanoic acid, heptyl ester	253357	1000405-21-8	9
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	272253	019095-24-0	9
			4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid methyl ester	197842	1000301-45-7	9
6	5.245	0.64	C:\Database\NIST14.L			
			Undecanoic acid, 10-methyl-, methyl ester	78103	005129-56-6	81
			Heptadecanoic acid, 16-methyl-, methyl ester	157954	005129-61-3	72
			Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	117491	062691-05-8	64
7	6.457	2.85	C:\Database\NIST14.L			
			Methyl tetradecanoate	104286	000124-10-7	98
			Tridecanoic acid, 12-methyl-, methyl ester	104333	005129-58-8	95
			Methyl tetradecanoate	104288	000124-10-7	94
8	6.874	0.20	C:\Database\NIST14.L			
			12,17-Diacetoxy-5,6,11,18-trinaphthylenetetrone	271139	096722-26-8	4
			11,18-Diacetoxy-5,6,12,17-trinaphthylenetetrone	271137	096722-33-7	2
			butanamide, N-[3-(acetylamino)-5-chloro-2-hydroxyphenyl]-2-[2,4-bis(1,1-dimethylpropyl)phenoxy]-	266659	1000399-63-5	1

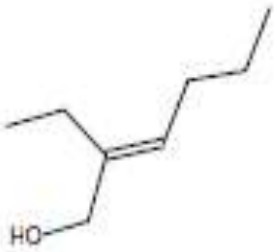
9	7.028	0.81	C:\Database\NIST14.L			
			Pentadecanoic acid, methyl ester	117470	007132-64-1	94
			Methyl 13-methyltetradecanoate	117456	1000336-31-4	94
			Tetradecanoic acid, 12-methyl-, me thyl ester	117485	005129-66-8	94
10	7.365	0.18	C:\Database\NIST14.L			
			Tungsten, tris(.eta.-3-allyl)-1-(b utylimido)propyl)	246689	1000156-85-9	4
11	7.468	4.33	C:\Database\NIST14.L			
			9-Hexadecenoic acid, methyl ester, (Z)-	128700	001120-25-8	99
			9-Hexadecenoic acid, methyl ester, (Z)-	128693	001120-25-8	99
			Methyl hexadec-9-enoate	128645	010030-74-7	99
12	7.571	33.42	C:\Database\NIST14.L			
			Hexadecanoic acid, methyl ester	130813	000112-39-0	99
			Hexadecanoic acid, methyl ester	130820	000112-39-0	98
			Hexadecanoic acid, methyl ester	130822	000112-39-0	98
13	7.828	1.16	C:\Database\NIST14.L			
			n-Hexadecanoic acid	117419	000057-10-3	96
			n-Hexadecanoic acid	117418	000057-10-3	96
			n-Hexadecanoic acid	117417	000057-10-3	95
14	7.897	0.17	C:\Database\NIST14.L			
			n-Hexadecanoic acid	117419	000057-10-3	95
			Pentadecanoic acid, methyl ester	117469	007132-64-1	42
			Dodecanoic acid, 10-methyl-, methy l ester	91484	005129-65-7	35
15	7.971	0.38	C:\Database\NIST14.L			
			Octadecanoic acid	144271	000057-11-4	90
			n-Hexadecanoic acid	117419	000057-10-3	55
			Undecanoic acid, 10-bromo-	124097	018294-93-4	42
16	8.091	0.95	C:\Database\NIST14.L			
			Hexadecanoic acid, 14-methyl-, met hyl ester	144337	002490-49-5	97
			Heptadecanoic acid, methyl ester	144319	001731-92-6	95
			Hexadecanoic acid, 15-methyl-, met hyl ester	144340	006929-04-0	93
17	8.343	0.22	C:\Database\NIST14.L			
			Cyclohexane, 1R-acetamido-2,3-cis- 4-epoxy-4-cis-formyloxy-	64579	1000153-72-6	10
			S-(Sec-buthoxythiocarbonyl)thiohyd roxylamine	35783	035659-83-7	9
			Heptane, 1-(ethenylthio)-	31420	021961-05-7	9
18	8.446	2.33	C:\Database\NIST14.L			
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	153889	000112-63-0	99
			9,12-Octadecadienoic acid, methyl ester	153873	002462-85-3	99
			10,13-Octadecadienoic acid, methyl ester	153881	056554-62-2	98
19	8.468	27.16	C:\Database\NIST14.L			
			11-Octadecenoic acid, methyl ester	155736	052380-33-3	99
			9-Octadecenoic acid, methyl ester	155719	002345-29-1	99
			11-Octadecenoic acid, methyl ester	155737	052380-33-3	99
20	8.588	12.86	C:\Database\NIST14.L			
			Methyl stearate	157884	000112-61-8	99
			Methyl stearate	157883	000112-61-8	99

21	8.720	2.32	C:\Database\NIST14.L			
			2,3-Dihydroxypropyl elaidate	210328	002716-53-2	86
			15-Octadecenoic acid, methyl ester	155729	004764-72-1	70
			6-Octadecenoic acid, (Z)-	142084	000593-39-5	62
22	8.829	0.23	C:\Database\NIST14.L			
			Colchiceine, N-desacetyl-N-TFA-	253459	071295-34-6	35
			Cyclodisilazane-2,2,4,4-tetramine, N,N,N',N'-tetramethyl-1,3-bis[tris(methylamino)silyl]-	253605	034665-55-9	22
			2,5-Dihydroxybenzoic acid, 3TBDMS derivative	265792	1000332-90-2	22
23	8.903	0.67	C:\Database\NIST14.L			
			Protocatechoic acid, 3TBDMS derivative	265789	1000352-46-7	47
			4-Benzothiazol-2-yl-2,5-diphenyl-3-trifluoromethyl-3,4-dihydro-2H-pyrazol-3-ol	253485	1000318-41-0	46
			2,5-Dihydroxybenzoic acid, 3TBDMS derivative	265792	1000332-90-2	40
24	9.069	0.32	C:\Database\NIST14.L			
			Methyl hexadecyl ether	117557	007307-53-1	10
			2-Dodecanol	52995	010203-28-8	10
			2-Trifluoroacetoxytetradecane	168810	1000245-47-4	10
25	9.200	0.18	C:\Database\NIST14.L			
			13-Methyl-Z-14-nonacosene	247276	1000131-19-0	11
			2-Propenoic acid, 2-methyl-, 2-propenyl ester	11447	000096-05-9	9
			Acetamide, 2-chloro-N-(2-cyanoethyl)-	22366	017756-81-9	9
26	9.411	2.67	C:\Database\NIST14.L			
			cis-11-Eicosenoic acid, methyl ester	182571	1000333-63-8	60
			1,30-Triacontanediol	257954	036645-68-8	49
			cis-13-Eicosenoic acid, methyl ester	182570	1000333-52-1	41
27	9.520	1.35	C:\Database\NIST14.L			
			Eicosanoic acid, methyl ester	184596	001120-28-1	98
			Nonadecanoic acid, 11-methyl-, methyl ester	184631	055334-33-3	93
			eicosanoic acid, 2-methyl-	184586	1000402-37-5	58
28	10.286	1.62	C:\Database\NIST14.L			
			Androsta[17-16-b]furan-5'-imine, 4'-methylene-3-methoxy-N-cyclohexyl	253599	1000196-04-9	46
			Pentacosanoic acid, tert-butyldimethylsilyl ester	265942	1000333-15-1	35
			N,N'-Diacetylbenzidine, 2TBDMS derivative	265821	1000314-43-7	32
29	10.412	1.04	C:\Database\NIST14.L			
			methanesulfonamide, N-[4-[3-[4-[bis[2-(acetyloxy)ethyl]amino]phenyl]-2-cyano-1-oxo-2-propen-1-yl]phenyl]-	267923	1000399-67-5	64
			Methyl 20-methyl-heneicosanoate	209123	1000336-47-4	55
			Docosanoic acid, methyl ester	209113	000929-77-1	42
30	13.304	0.31	C:\Database\NIST14.L			
			5-(4-Chlorophenyl)-6-ethylpyrimidine-2,4-diamine, N,N'-bis(trifluoroacetyl)-	253638	1000373-24-2	41

Hit 1 : cis-2-Ethyl-2-hexen-1-ol
C8H16O; MF: 999; RMF: 999; Prob 91.8%; Lib: mainlib; ID: 2031.

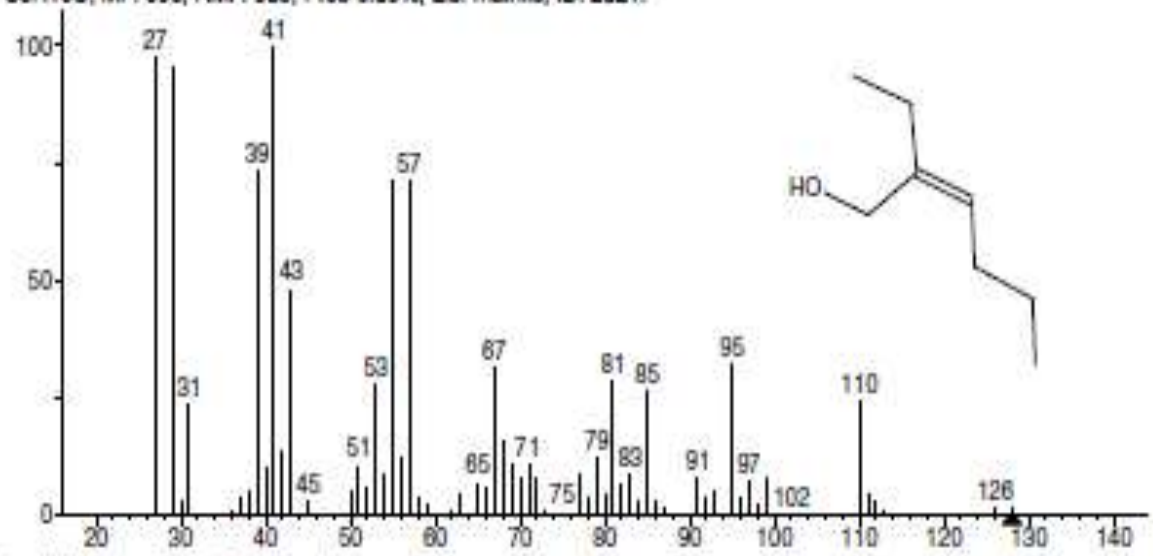


(mainlib) cis-2-Ethyl-2-hexen-1-ol

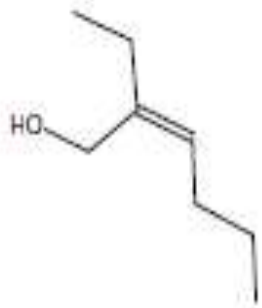


Name: cis-2-Ethyl-2-hexen-1-ol
Formula: $C_8H_{16}O$
MW: 128 Exact Mass: 128.120115 NIST#: 139524 ID#: 2031 DB: mainlib
Contributor: B. Derendyaev, Novosibirsk Institute of Organic Chemistry
InChIKey: JSRFYJBJQPGAAA-SOFGYWHQSA-N Non-stereo
10 largest peaks:
41 999 | 27 926 | 55 784 | 57 600 | 39 549 | 43 517 | 29 461 | 85 372 | 81 301 | 53 276 |

Hit 2 : trans-2-Ethyl-2-hexen-1-ol
C8H16O; MF: 893; RMF: 905; Prob 6.09%; Lib: mainlib; ID: 2021.

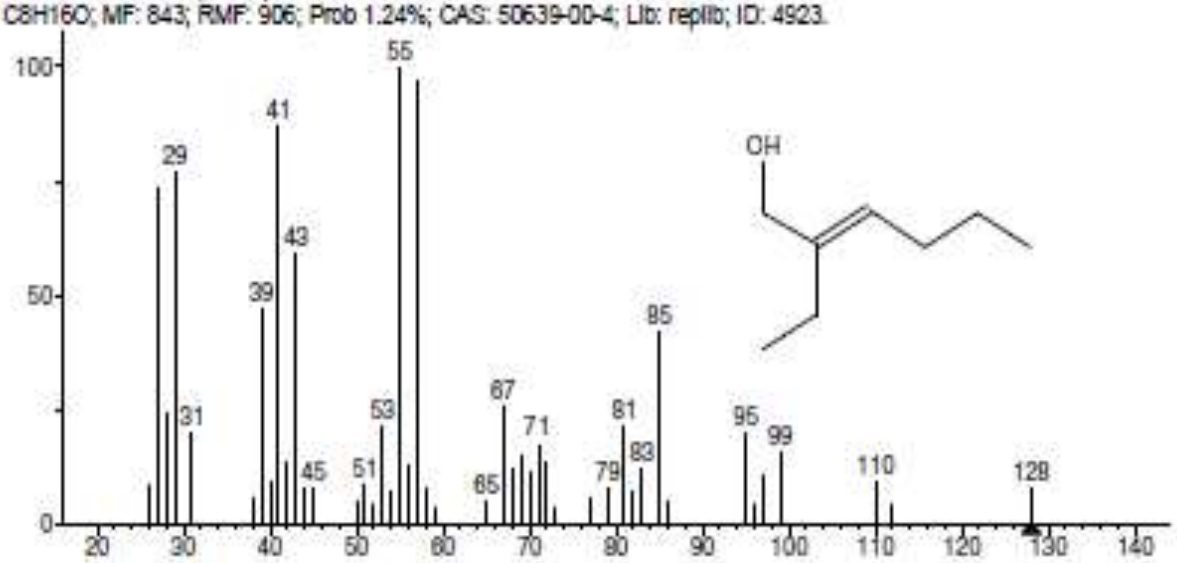


(mainlib) trans-2-Ethyl-2-hexen-1-ol

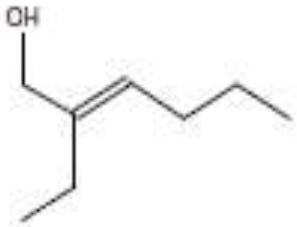


Name: trans-2-Ethyl-2-hexen-1-ol
Formula: $C_8H_{16}O$
MW: 128 Exact Mass: 128.120115 NIST#: 139523 ID#: 2021 DB: mainlib
Contributor: B. Derendyaev, Novosibirsk Institute of Organic Chemistry
InChIKey: JSRFYJBJQPGAAA-VURMDHGXSA-N Non-stereo
10 largest peaks:
41 999 | 27 979 | 29 949 | 39 740 | 57 713 | 55 709 | 43 484 | 95 322 | 67 318 | 81 285 |

Hit 3 : 2-Hexen-1-ol, 2-ethyl-
C8H16O; MF: 843; RMF: 906; Prob 1.24%; CAS: 50639-00-4; Lib: replib; ID: 4923.



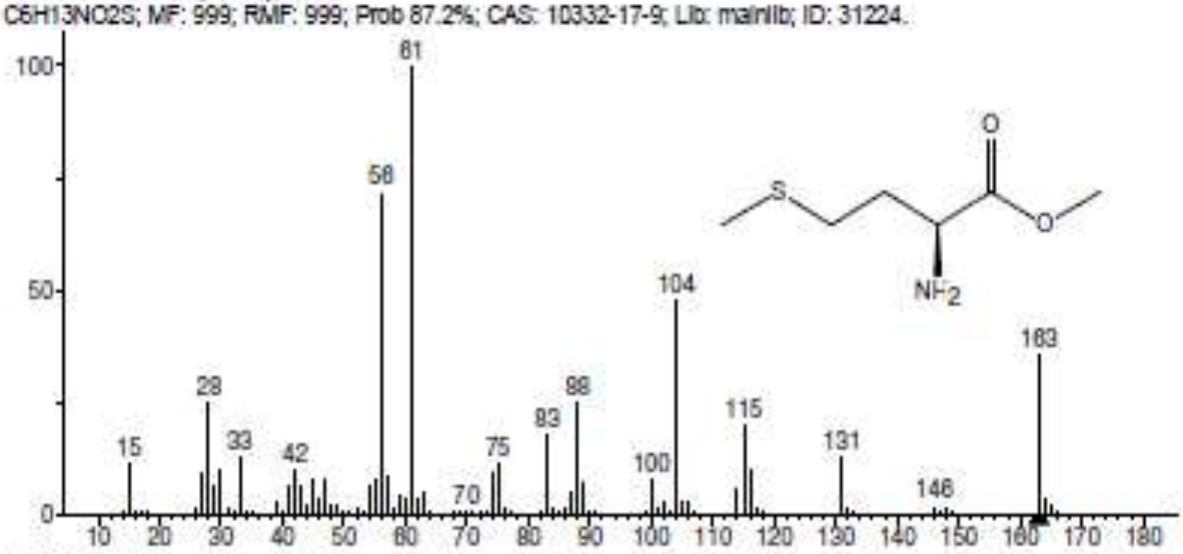
(replib) 2-Hexen-1-ol, 2-ethyl-



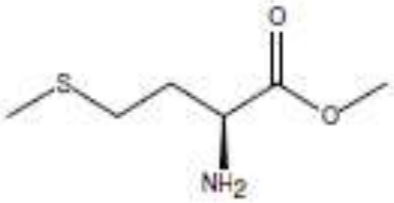
Name: 2-Hexen-1-ol, 2-ethyl-
Formula: $C_8H_{16}O$
MW: 128 Exact Mass: 128.120115 CAS#: 50639-00-4 NIST#: 2688 ID#: 4923 DB: replib
Other DBs: Fine, EINECS
InChIKey: JSRFYJBJQPGAAA-SOFGYWHQSA-N Non-stereo
10 largest peaks:
55 999 | 57 965 | 41 874 | 29 772 | 27 731 | 43 597 | 39 471 | 85 422 | 67 260 | 28 236 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:996±7 (2)
Standard non-polar: 1051±N/A (1)
Polar: 1518±N/A (1)

Hit 1 : L-Methionine, methyl ester
C6H13NO2S; MF: 999; RMF: 999; Prob 87.2%; CAS: 10332-17-9; Lib: mainlib; ID: 31224.



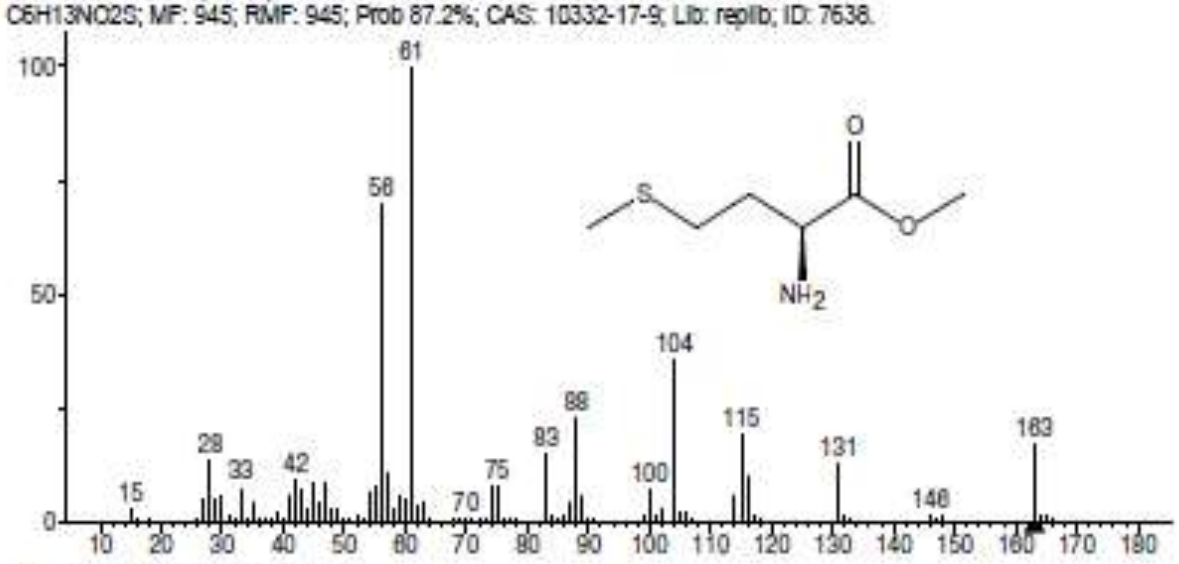
(mainlib) L-Methionine, methyl ester



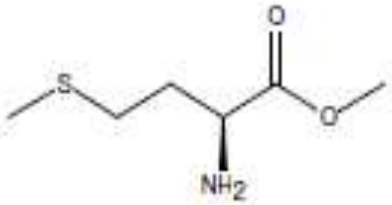
Name: L-Methionine, methyl ester
Formula: $C_6H_{13}NO_2S$
MW: 163 Exact Mass: 163.0667 CAS#: 10332-17-9 NIST#: 236637 ID#: 31224 DB: mainlib
Other DBs: EINECS
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-7009
InChIKey: UIHPNZDZCOEZEN-YFKPBYRVSA-N Non-stereo
10 largest peaks:
61 999 | 58 719 | 104 478 | 163 359 | 28 252 | 88 252 | 115 208 | 83 182 | 131 133 | 33 132 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1290±N/A (1)

Hit 2 : L-Methionine, methyl ester
C6H13NO2S; MF: 945; RMF: 945; Prob 87.2%; CAS: 10332-17-9; Lib: replib; ID: 7638.



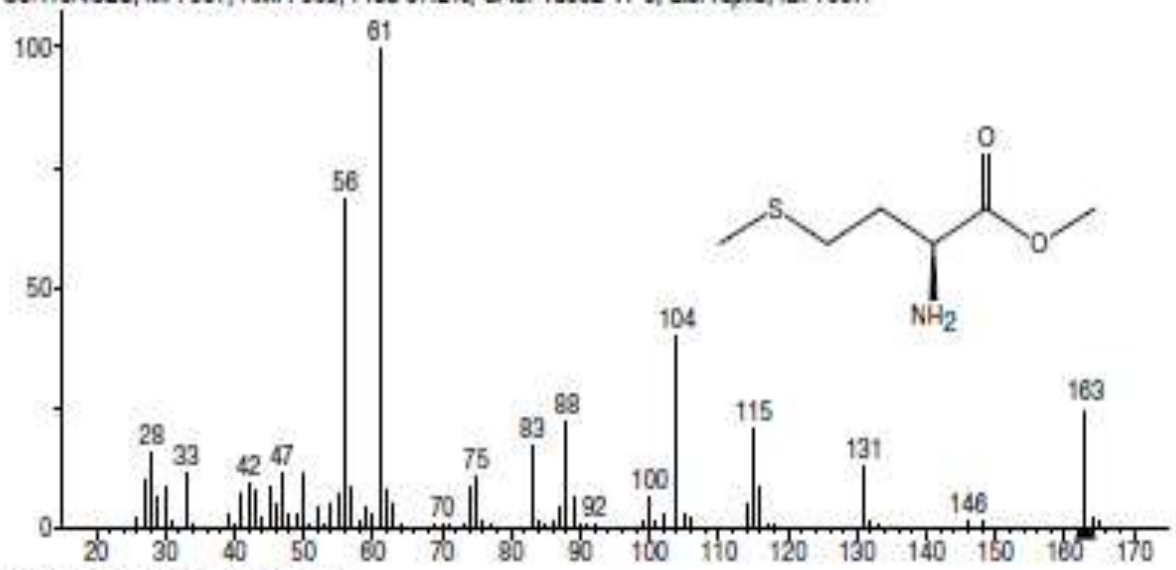
(replib) L-Methionine, methyl ester



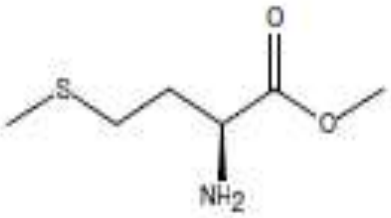
Name: L-Methionine, methyl ester
Formula: $C_6H_{13}NO_2S$
MW: 163 Exact Mass: 163.0667 CAS#: 10332-17-9 NIST#: 332764 ID#: 7638 DB: replib
Other DBs: EINECS
Contributor: NIST Mass Spectrometry Data Center
InChIKey: UIHPNZDZCOEZEN-YFKPBYRVSA-N Non-stereo
10 largest peaks:
61 999 | 56 699 | 104 360 | 88 231 | 115 197 | 163 171 | 83 150 | 28 138 | 131 133 | 57 103 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1290±N/A (1)

Hit 3 : L-Methionine, methyl ester
C6H13NO2S; MF: 937; RMF: 939; Prob 67.2%; CAS: 10332-17-9; Lib: replib; ID: 7637.



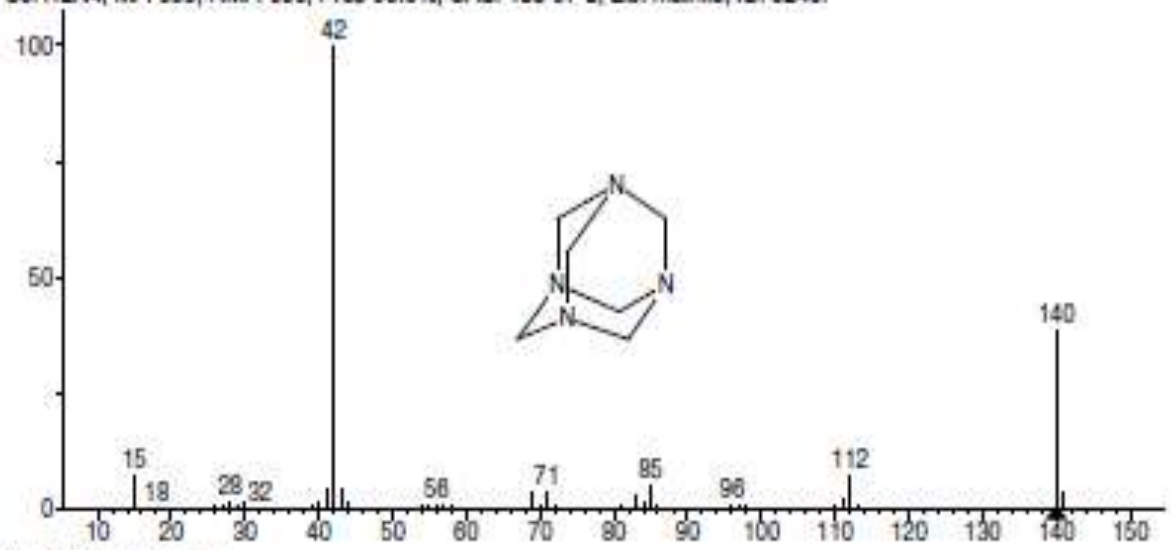
(replib) L-Methionine, methyl ester



Name: L-Methionine, methyl ester
Formula: $C_6H_{13}NO_2S$
MW: 163 Exact Mass: 163.0667 CAS#: 10332-17-9 NIST#: 221074 ID#: 7637 DB: replib
Other DBs: EINECS
Contributor: Chemical Concepts
InChIKey: UIHPNZDZCOEZEN-YFKPBYRVSA-N Non-stereo
10 largest peaks:
61 999 | 56 686 | 104 403 | 163 249 | 88 227 | 115 211 | 83 171 | 28 159 | 131 132 | 33 118 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1290±N/A (1)

Hit 1 : Methenamine
C6H12N4; MF: 999; RMF: 999; Prob 95.6%; CAS: 100-97-0; Lib: mainlib; ID: 5248.

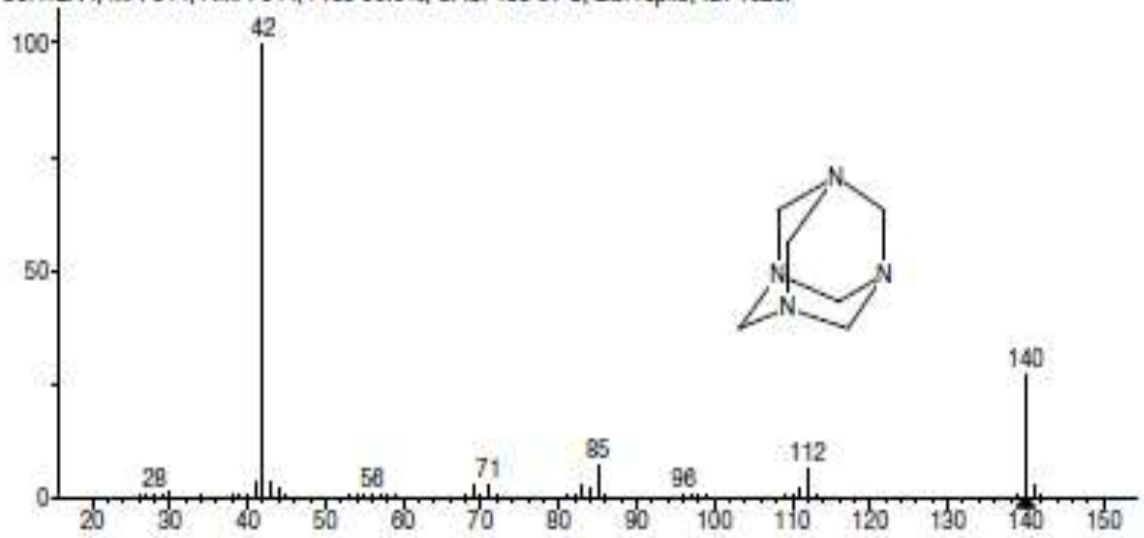


(mainlib) Methenamine

Name: Methenamine
Formula: $C_6H_{12}N_4$
MW: 140 Exact Mass: 140.106196 CAS#: 100-97-0 NIST#: 228325 ID#: 5248 DB: mainlib
Other DBs: Fine, TSCA, RTECS, USP, HODOC, NIH, EINECS, IRDB
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-1244
Related CAS#: 56549-34-9; 74734-16-0; 91773-48-7; 11103-67-6; 15978-33-3
InChIKey: VKYKSIONXSXAKP-UHFFFAOYSA-N Non-stereo
10 largest peaks:
42 999 | 140 385 | 112 76 | 15 73 | 85 54 | 71 38 | 41 36 | 43 36 | 141 31 | 69 30 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1205±1 (3)
Standard non-polar: 1210±N/A (1)
Polar: 2014±0 (2)

Hit 2 : Methenamine
C6H12N4; MF: 944; RMF: 944; Prob 95.6%; CAS: 100-97-0; Lib: replib; ID: 1626.

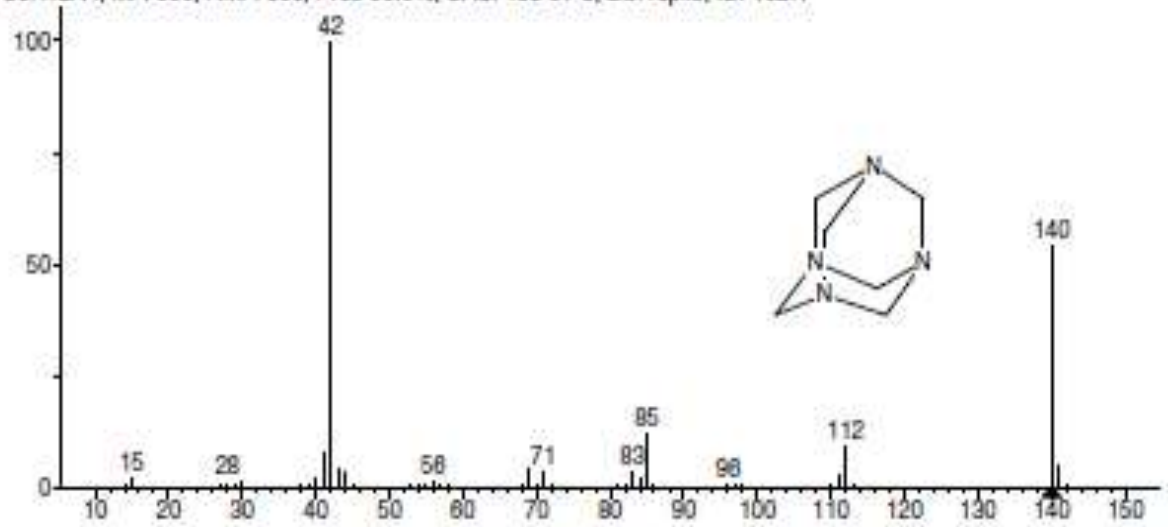


(replib) Methenamine

Name: Methenamine
Formula: $C_6H_{12}N_4$
MW: 140 Exact Mass: 140.106196 CAS#: 100-97-0 NIST#: 153442 ID#: 1626 DB: replib
Other DBs: Fine, TSCA, RTECS, USP, HODOC, NIH, EINECS, IRDB
Contributor: Chemical Concepts
Related CAS#: 56549-34-9; 74734-16-0; 91779-48-7; 11103-67-6; 15979-33-3
InChIKey: VKYKSIONXSXAKP-UHFFFAOYSA-N Non-stereo
10 largest peaks:
42 999 | 140 274 | 85 72 | 112 67 | 43 33 | 71 32 | 41 31 | 69 28 | 141 25 | 83 23 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1205±1 (3)
Standard non-polar: 1210±N/A (1)
Polar: 2014±0 (2)

Hit 3 : Methenamine
C6H12N4; MF: 936; RMF: 936; Prob 95.6%; CAS: 100-97-0; Lib: replib; ID: 1627.

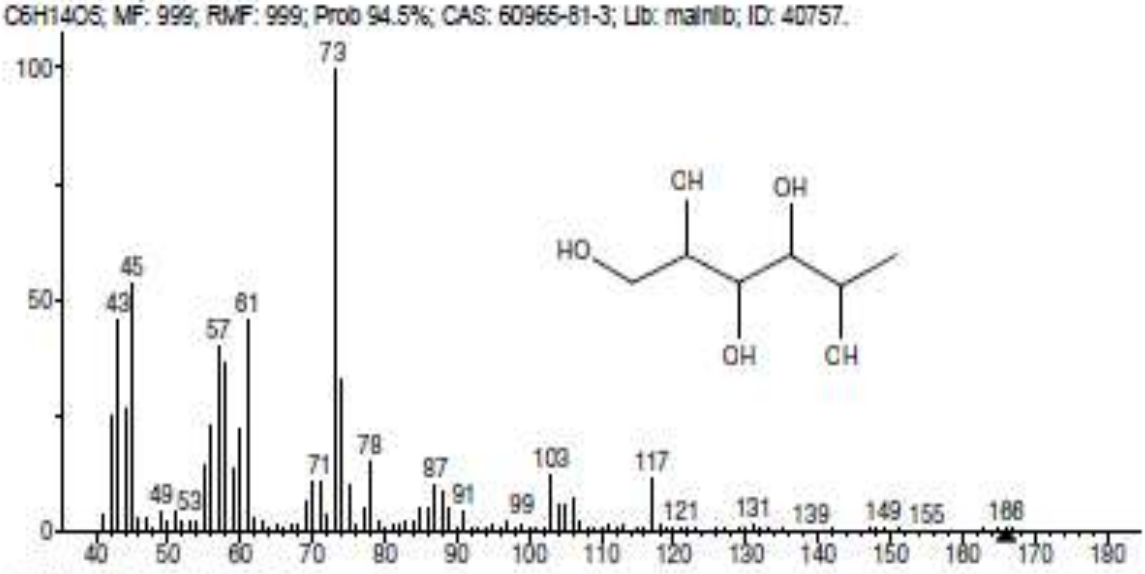


(replib) Methenamine

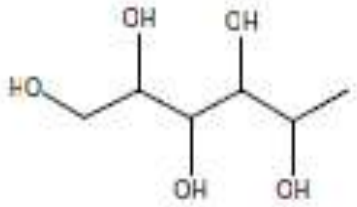
Name: Methenamine
Formula: $C_6H_{12}N_4$
MW: 140 Exact Mass: 140.106196 CAS#: 100-97-0 NIST#: 290732 ID#: 1627 DB: replib
Other DBs: Fine, TSCA, RTECS, USP, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center, 1998.
Related CAS#: 56549-34-9; 74734-16-0; 91773-48-7; 11103-67-6; 15979-33-3
InChIKey: VKYKSIONXSXAKP-UHFFFAOYSA-N Non-stereo
10 largest peaks:
42 999 | 140 544 | 85 125 | 112 95 | 41 75 | 141 45 | 43 42 | 71 42 | 83 40 | 69 38 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1205±1 (3)
Standard non-polar: 1210±N/A (1)
Polar: 2014±0 (2)

Hit 1 : 1-Deoxy-d-mannitol
C6H14O5; MF: 999; RMF: 999; Prob 94.5%; CAS: 60965-81-3; Lib: mainlib; ID: 40757.

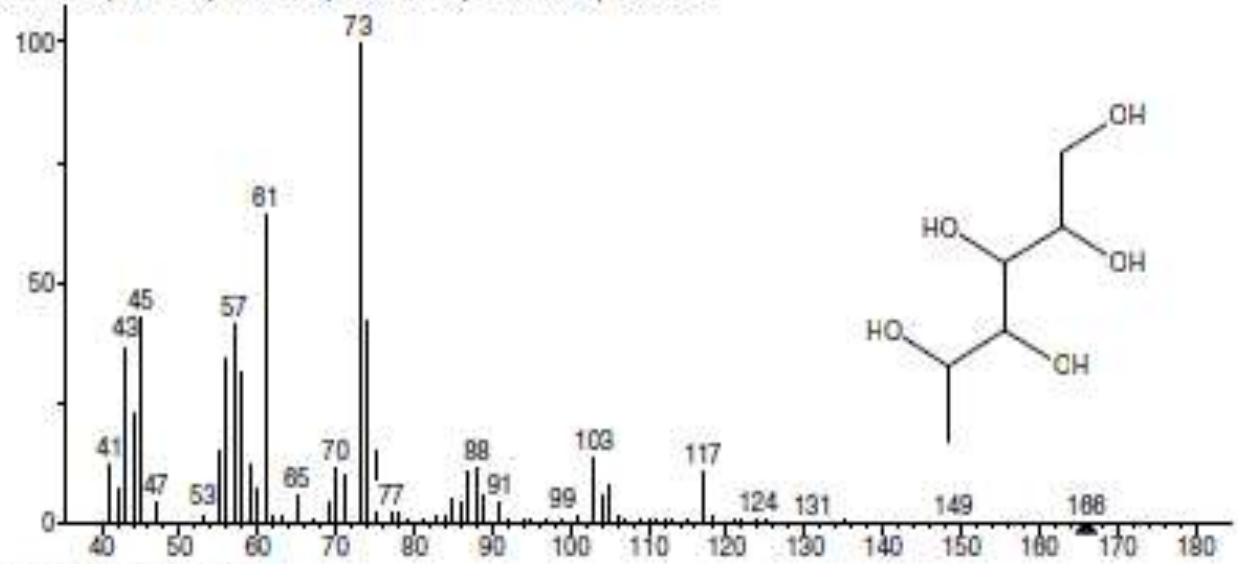


(mainlib) 1-Deoxy-d-mannitol

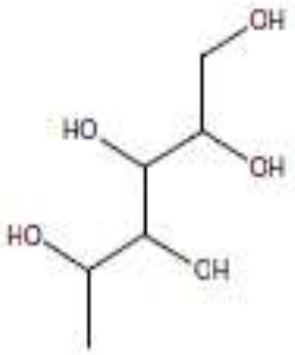


Name: 1-Deoxy-d-mannitol
Formula: $C_6H_{14}O_5$
MW: 166 Exact Mass: 166.084124 CAS#: 60965-81-3 NIST#: 124797 ID#: 40757 DB: mainlib
Other DBs: None
Contributor: H. Zissis, LC, NIDDK, NIH, Bethesda, MD 20892
InChIKey: SKCKOFZKJLZSFA-UHFFFAOYSA-N Non-stereo
10 largest peaks:
73 999 | 45 537 | 43 462 | 61 458 | 57 404 | 59 360 | 74 327 | 44 262 | 42 249 | 56 225 |

Hit 2 : 6-Desoxy-l-gulitol
C6H14O5; MF: 844; RMF: 869; Prob 2.43%; Lib: mainlib; ID: 41159.

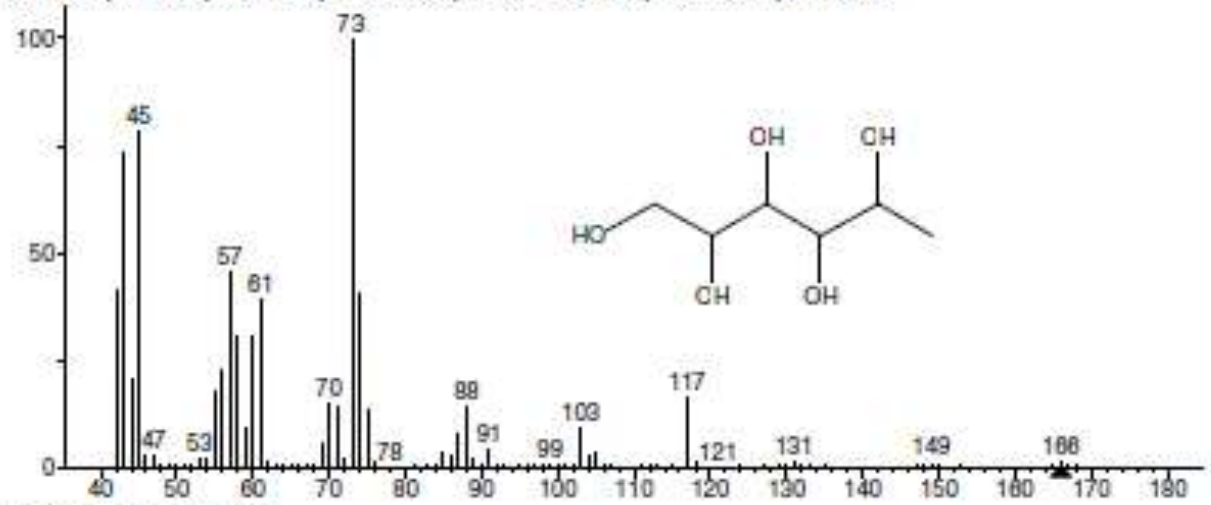


(mainlib) 6-Desoxy-l-gulitol

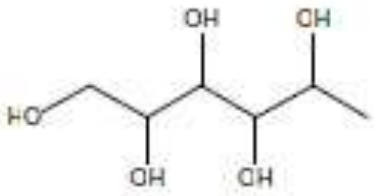


Name: 6-Desoxy-l-gulitol
Formula: $C_6H_{14}O_5$
MW: 166 Exact Mass: 166.084124 NIST#: 130019 ID#: 41159 DB: mainlib
Contributor: LAC, NIDDK, NIH, Bethesda, MD 20892
InChIKey: SKCKOFZKJLZSFA-UHFFFAOYSA-N Non-stereo
10 largest peaks:
73 999 | 61 646 | 45 432 | 57 418 | 74 415 | 43 370 | 56 338 | 58 309 | 44 223 | 55 148 |

Hit 3 : 1-Deoxy-d-altritol
C6H14O5; MF: 841; RMF: 849; Prob 2.15%; CAS: 68832-18-8; Lib: mainlib; ID: 40758.

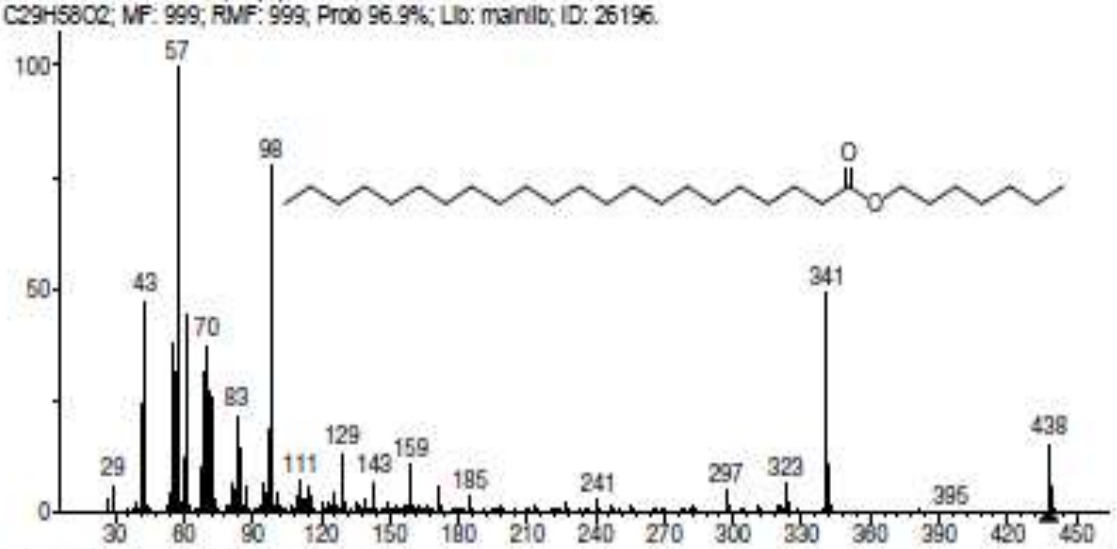


(mainlib) 1-Deoxy-d-altritol

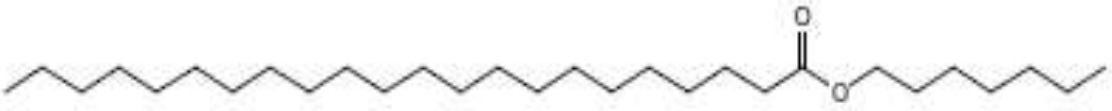


Name: 1-Deoxy-d-altritol
Formula: $C_6H_{14}O_5$
MW: 166 Exact Mass: 166.084124 CAS#: 68832-18-8 NIST#: 125000 ID#: 40758 DB: mainlib
Other DBs: None
Contributor: E. Zissis, LC, NIDDK, NIH, Bethesda, MD 20892
InChIKey: SKCKOFZKJLZSFA-UHFFFAOYSA-N Non-stereo
10 largest peaks:
73 999 | 45 799 | 43 733 | 57 462 | 42 413 | 74 401 | 61 395 | 58 304 | 60 301 | 56 226 |

Hit 1 : Docosanoic acid, heptyl ester
C29H58O2; MF: 999; RMF: 999; Prob 96.9%; Lib: mainlib; ID: 26196.



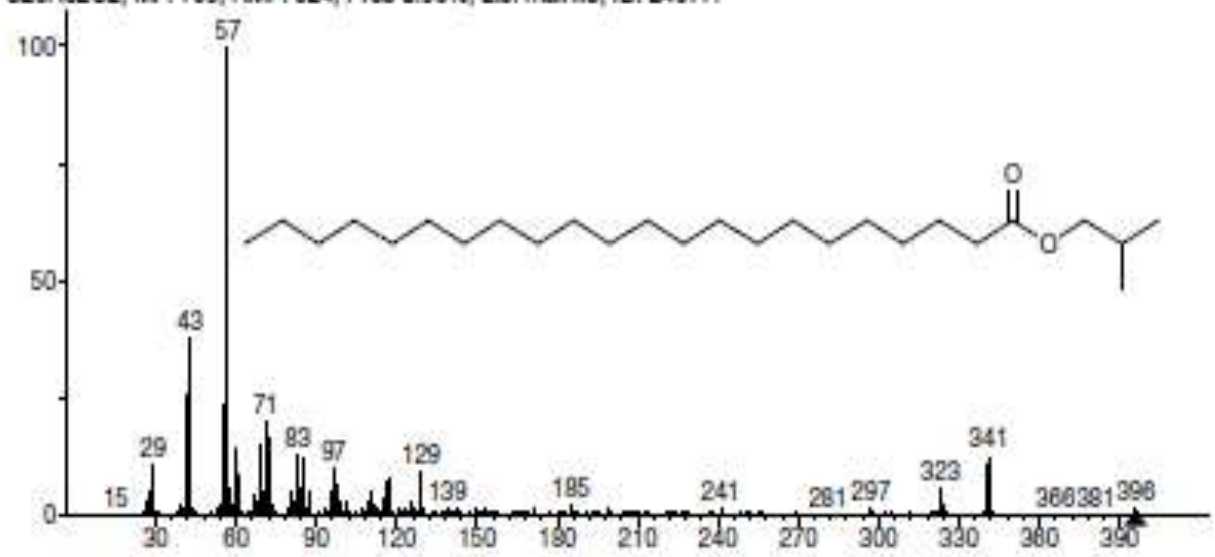
(mainlib) Docosanoic acid, heptyl ester



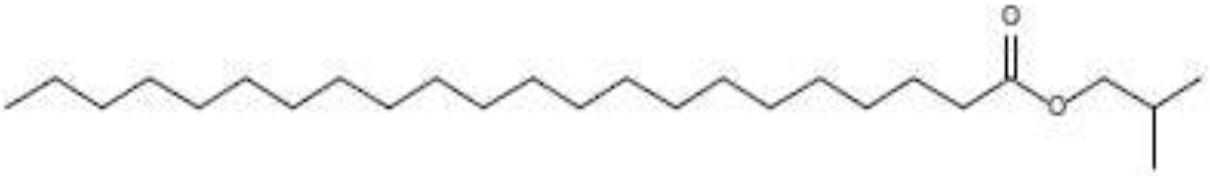
Name: Docosanoic acid, heptyl ester
Formula: $C_{29}H_{58}O_2$
MW: 438 Exact Mass: 438.443682 NIST#: 405218 ID#: 26196 DB: mainlib
Contributor: V.G. Zaikin, R.S.Borisov, TIPS RAS, Moscow, Russia
InChIKey: IAJAWAXYUAWNTH-UHFFFAOYSA-N Non-stereo
10 largest peaks:

57 999	98 777	341 492	43 477	81 440	55 377	70 377	56 308	69 307	71 286

Hit 2 : Docosanoic acid, isobutyl ester
C26H52O2; MF: 769; RMF: 624; Prob 0.96%; Lib: mainlib; ID: 24877.



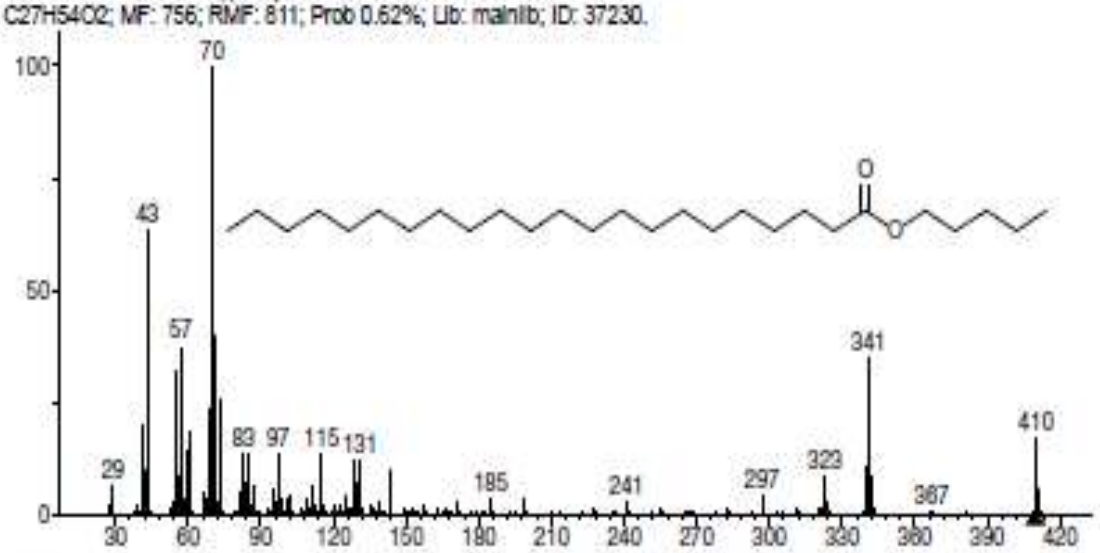
(mainlib) Docosanoic acid, isobutyl ester



Name: Docosanoic acid, isobutyl ester
Formula: $C_{26}H_{52}O_2$
MW: 396 Exact Mass: 396.39873 NIST#: 405171 ID#: 24877 DB: mainlib
Contributor: V.G. Zaikin, R.S.Borisov, TIPS RAS, Moscow, Russia
InChIKey: MLOOWFAQLIBHEP-UHFFFAOYSA-N Non-stereo
10 largest peaks:
57 999 | 56 774 | 43 383 | 41 254 | 55 235 | 71 201 | 73 163 | 69 147 | 60 141 | 83 130 |

Experimental RI median±deviation (#data)
Standard non-polar: 2724±N/A (1)

Hit 3 : Docosanoic acid, pentyl ester
C27H54O2; MF: 756; RMF: 811; Prob 0.62%; Lib: mainlib; ID: 37230.

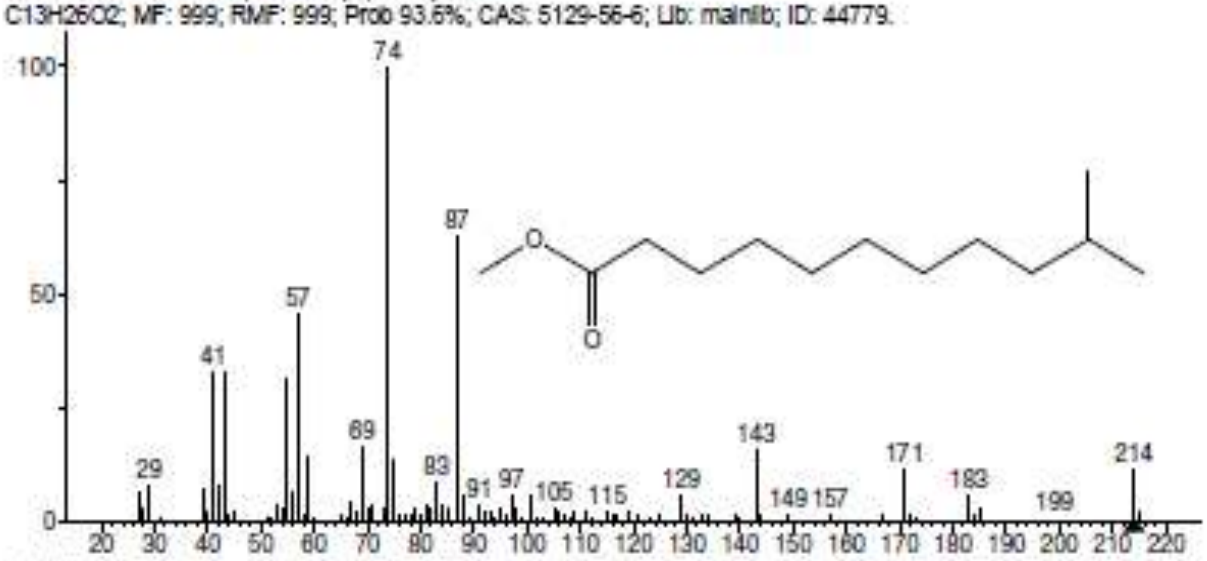


(mainlib) Docosanoic acid, pentyl ester

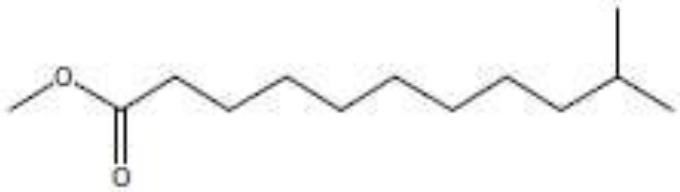
Name: Docosanoic acid, pentyl ester
Formula: $C_{27}H_{54}O_2$
MW: 410 Exact Mass: 410.41238 NIST#: 405195 ID#: 37230 DB: mainlib
Contributor: V.G. Zaikin, R.S.Borisov, TIPS RAS, Moscow, Russia
InChIKey: UPGXFTXMGCZBRA-UHFFFAOYSA-N Non-stereo
10 largest peaks:

70 999	43 839	71 394	57 377	341 353	55 315	73 253	69 233	41 199	61 194

Hit 1 : Undecanoic acid, 10-methyl-, methyl ester
C13H26O2; MF: 999; RMF: 999; Prob 93.6%; CAS: 5129-56-6; Lib: mainlib; ID: 44779.



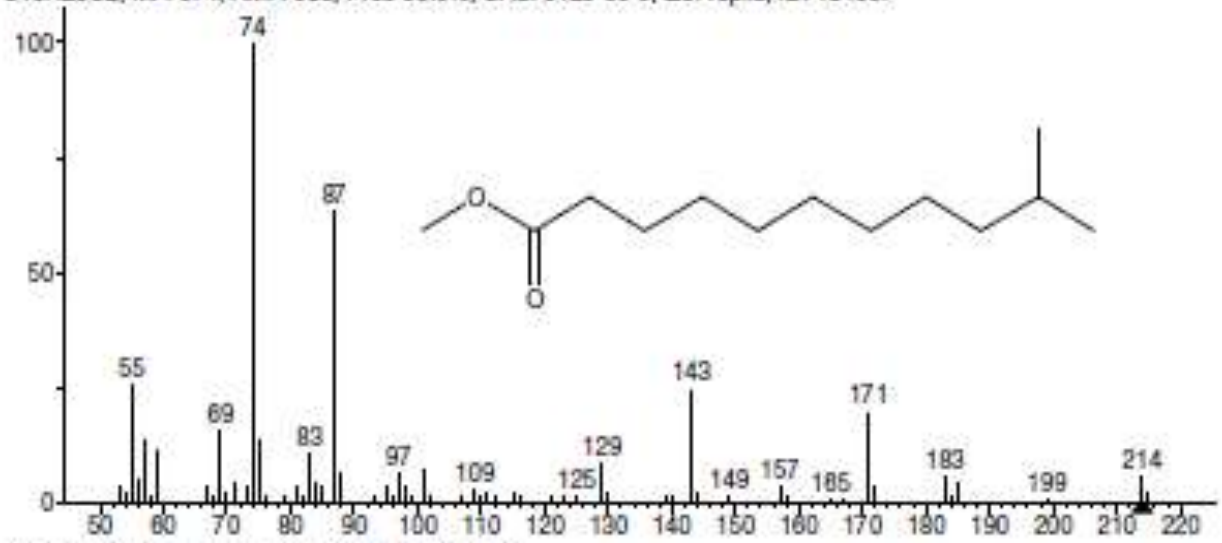
(mainlib) Undecanoic acid, 10-methyl-, methyl ester



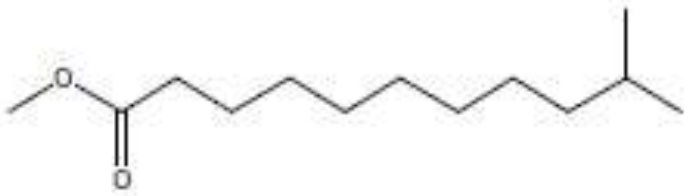
Name: Undecanoic acid, 10-methyl-, methyl ester
Formula: $C_{13}H_{26}O_2$
MW: 214 Exact Mass: 214.19328 CAS#: 5129-56-6 NIST#: 35795 ID#: 44779 DB: mainlib
Other DBs: None
Contributor: R.T.HOLMAN,UNIVERSITY OF MINNESOTA
InChIKey: XPVCTJYIICVJOE-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 830 | 57 480 | 41 330 | 43 330 | 55 310 | 69 170 | 143 160 | 59 140 | 75 130 |

Experimental RI median±deviation (#data)
Standard non-polar: 1471±2 (2)

Hit 2 : Undecanoic acid, 10-methyl-, methyl ester
C13H26O2; MF: 874; RMF: 905; Prob 93.6%; CAS: 5129-56-6; Lib: replib; ID: 10466.



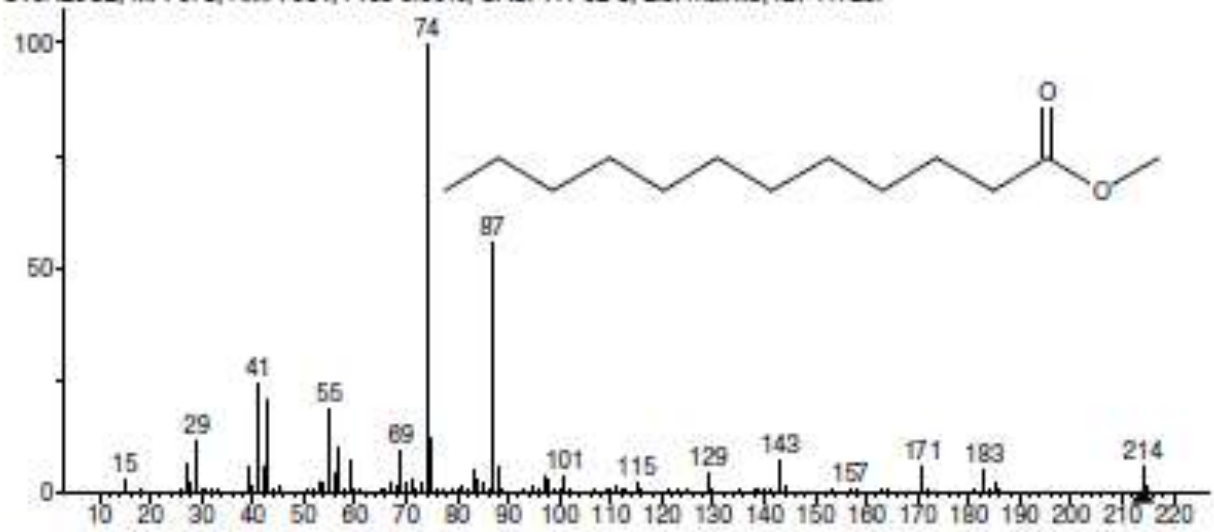
(replib) Undecanoic acid, 10-methyl-, methyl ester



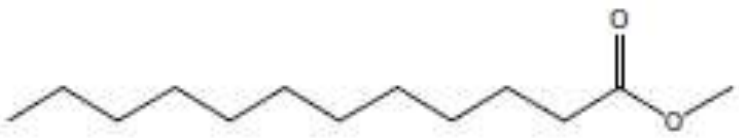
Name: Undecanoic acid, 10-methyl-, methyl ester
Formula: $C_{13}H_{26}O_2$
MW: 214 Exact Mass: 214.19328 CAS#: 5129-56-6 NIST#: 336211 ID#: 10466 DB: replib
Other DBs: None
Contributor: William W. Christie, Mylnefield Lipid Analysis, Invergowrie, Dundee, Scotland, UK
InChIKey: XPVCTJYIICVJOE-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 639 | 55 259 | 143 249 | 171 199 | 69 159 | 57 129 | 75 129 | 59 109 | 83 109 |

Experimental RI median±deviation (#data)
Standard non-polar: 1471±2 (2)

Hit 3 : Dodecanoic acid, methyl ester
C13H26O2; MF: 870; RMF: 901; Prob 3.88%; CAS: 111-82-0; Lib: mainlib; ID: 44725.



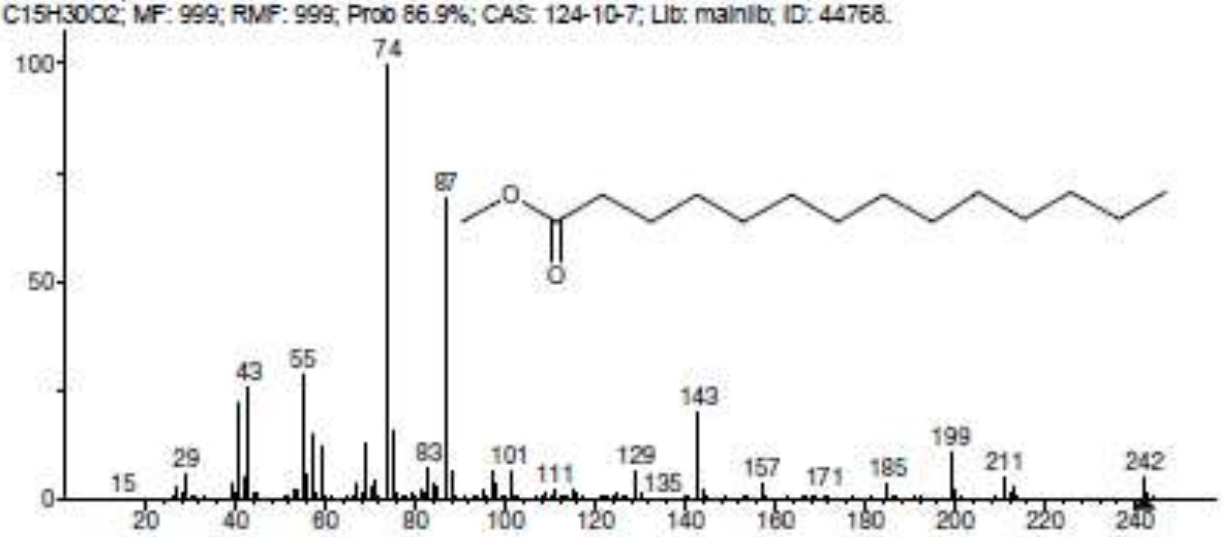
(mainlib) Dodecanoic acid, methyl ester



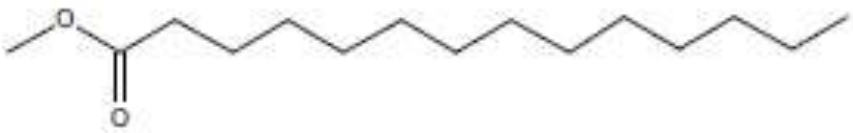
Name: Dodecanoic acid, methyl ester
Formula: $C_{13}H_{26}O_2$
MW: 214 Exact Mass: 214.19328 CAS#: 111-82-0 NIST#: 229476 ID#: 44725 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-1738
InChIKey: UQDUPQYQJKYHQI-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 557 | 41 245 | 43 204 | 55 191 | 29 120 | 75 119 | 57 98 | 69 98 | 143 77 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1528±2 (36)
Standard non-polar: 1508±2 (37)
Polar: 1804±7 (40)

Hit 1 : Methyl tetradecanoate
C15H30O2; MF: 999; RMF: 999; Prob 86.9%; CAS: 124-10-7; Lib: mainlib; ID: 44768.



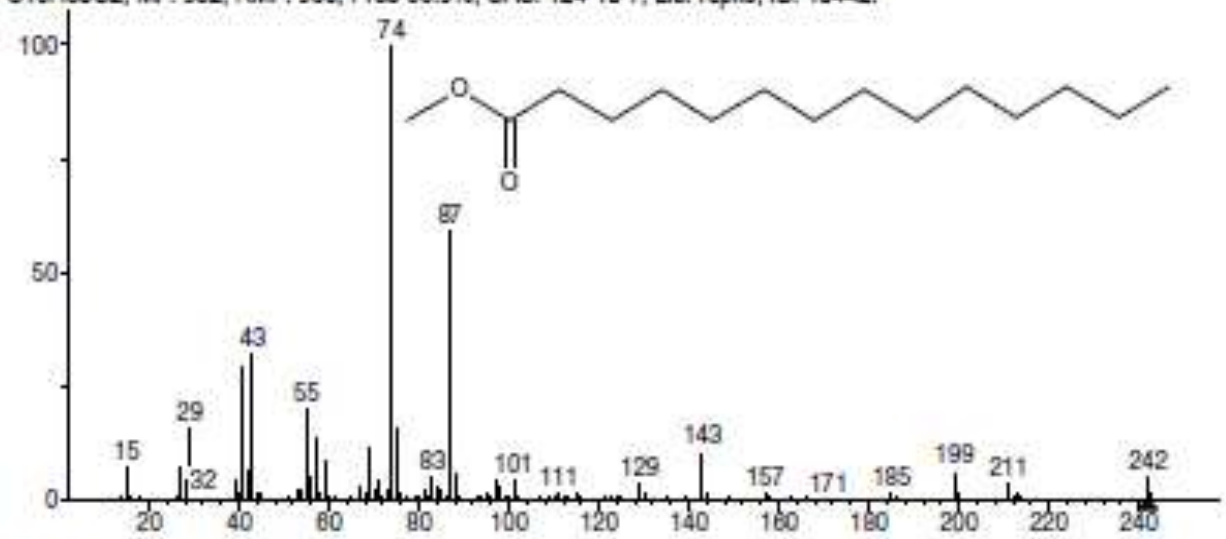
(mainlib) Methyl tetradecanoate



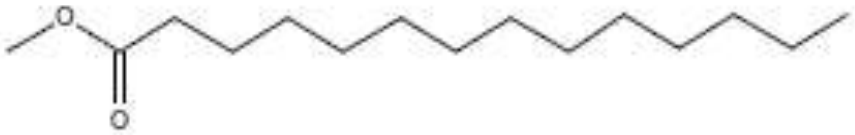
Name: Methyl tetradecanoate
Formula: $C_{15}H_{30}O_2$
MW: 242 Exact Mass: 242.22458 CAS#: 124-10-7 NIST#: 333719 ID#: 44768 DB: mainlib
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center
InChIKey: ZAZKJZBWRNNLDS-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 691 | 55 289 | 43 280 | 41 214 | 143 205 | 75 155 | 57 144 | 69 126 | 59 115 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1725±2 (48)
Standard non-polar: 1708±2 (43)
Polar: 2005±15 (31)

Hit 2 : Methyl tetradecanoate
C15H30O2; MF: 932; RMF: 935; Prob 86.9%; CAS: 124-10-7; Lib: replib; ID: 10442.



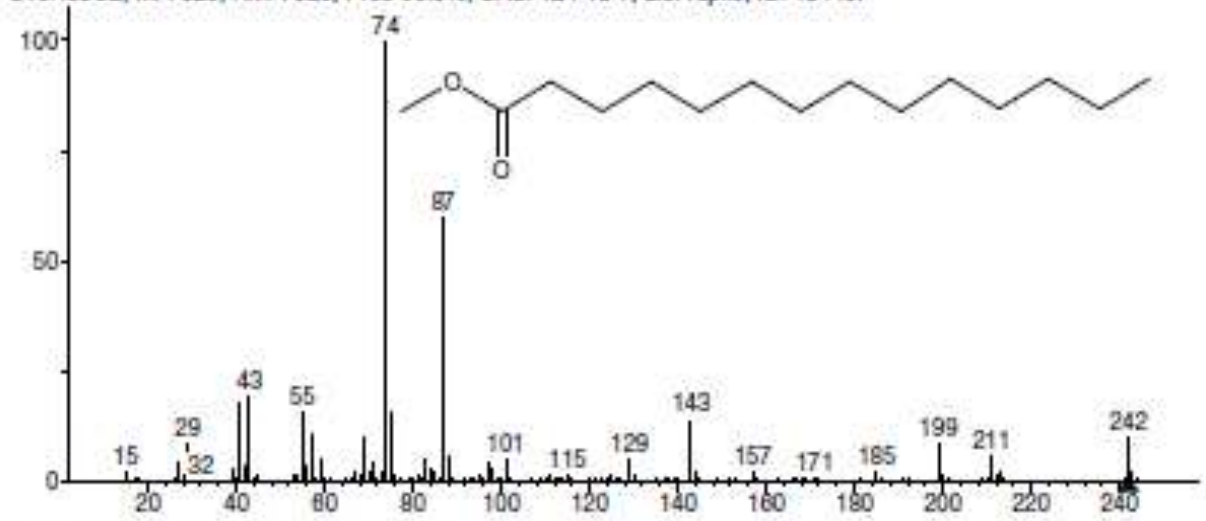
(replib) Methyl tetradecanoate



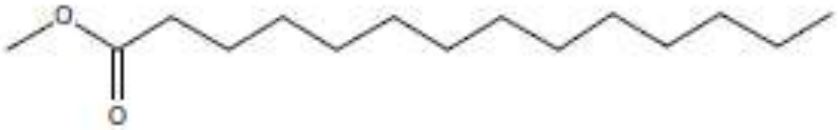
Name: Methyl tetradecanoate
Formula: $C_{15}H_{30}O_2$
MW: 242 Exact Mass: 242.22458 CAS#: 124-10-7 NIST#: 79125 ID#: 10442 DB: replib
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB
Contributor: O A MAMER, MCGILL UNIVERSITY, MONTREAL, HEM. P
InChIKey: ZAZKJZBWRNNLDS-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 595 | 43 321 | 41 286 | 55 204 | 29 158 | 75 153 | 57 131 | 69 109 | 143 107 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1725±2 (48)
Standard non-polar: 1708±2 (43)
Polar: 2005±15 (31)

Hit 3 : Methyl tetradecanoate
C15H30O2; MF: 929; RMF: 929; Prob 86.9%; CAS: 124-10-7; Lib: replib; ID: 10443.



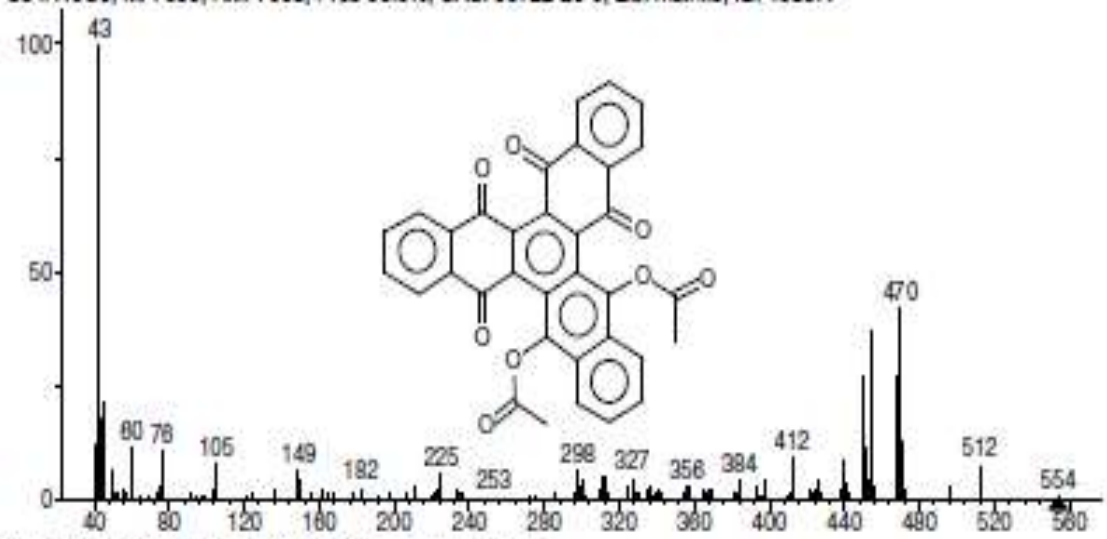
(replib) Methyl tetradecanoate



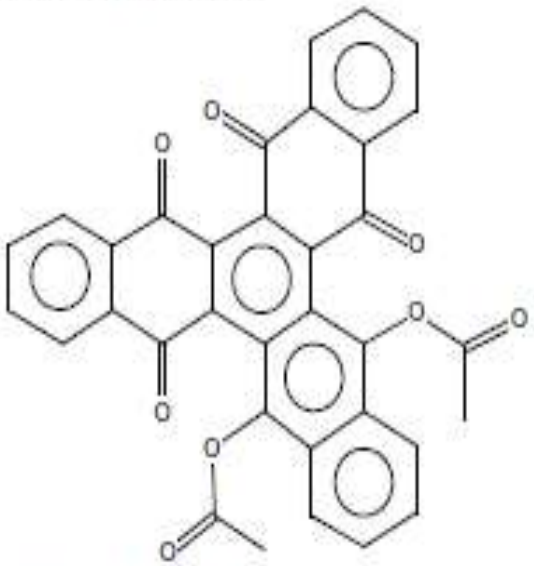
Name: Methyl tetradecanoate
Formula: $C_{15}H_{30}O_2$
MW: 242 Exact Mass: 242.22458 CAS#: 124-10-7 NIST#: 227991 ID#: 10443 DB: replib
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-1513
InChIKey: ZAZKJZBWRNNLDS-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 606 | 43 196 | 41 177 | 55 158 | 75 154 | 143 142 | 242 104 | 57 101 | 69 96 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1725±2 (48)
Standard non-polar: 1709±2 (43)
Polar: 2005±15 (31)

Hit 1 : 12,17-Diacetoxy-5,6,11,18-trinaphthylenetetrone
C34H18O8; MF: 999; RMF: 999; Prob 96.8%; CAS: 96722-26-8; Lib: mainlib; ID: 15057.

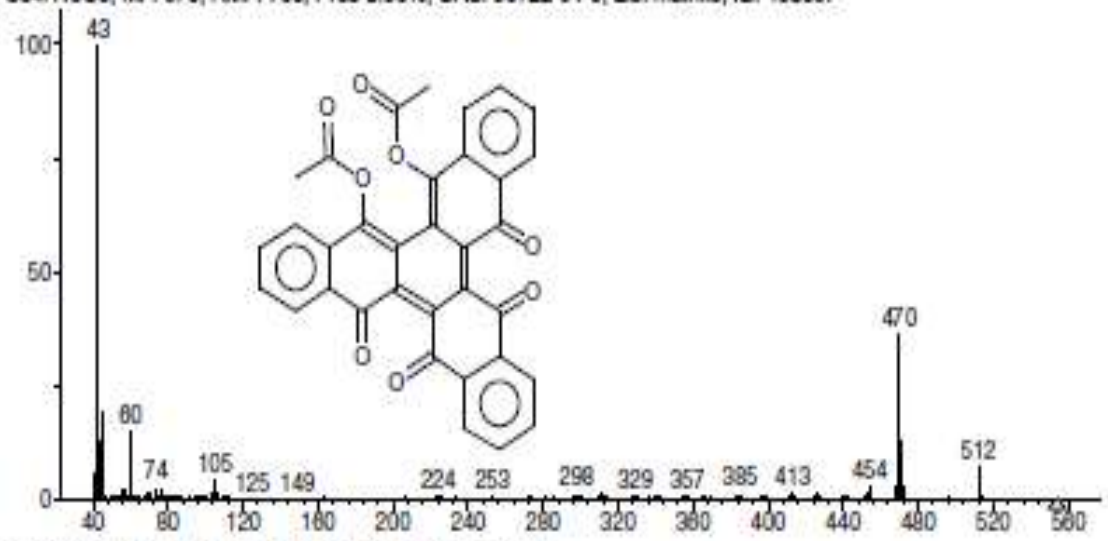


(mainlib) 12,17-Diacetoxy-5,6,11,18-trinaphthylenetetrone

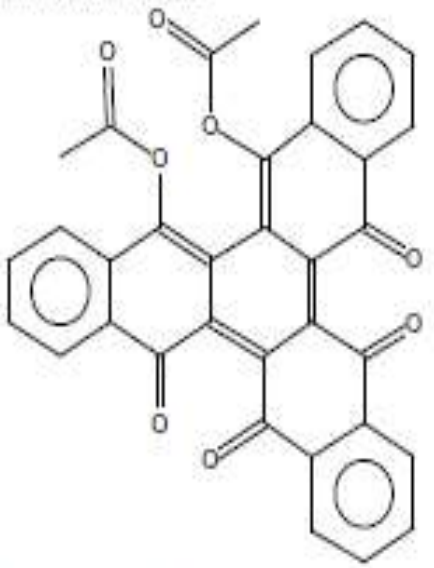


Name: 12,17-Diacetoxy-5,6,11,18-trinaphthylenetetrone
Formula: $C_{34}H_{18}O_8$
MW: 554 Exact Mass: 554.100166 CAS#: 96722-26-8 NIST#: 100423 ID#: 15057 DB: mainlib
Other DBs: None
Contributor: H.LAATSCH,INST. ORG. CHEM.,GEORG-AUGUST-UNIV. GOTTINGEN,W.GER.
InChIKey: QHYIBAPOJHXLKZ-UHFFFAOYSA-N Non-stereo
10 largest peaks:
43 999 | 470 423 | 454 370 | 468 265 | 450 264 | 45 211 | 42 206 | 44 176 | 469 151 | 471 124 |

Hit 2 : 5,8-Diacetoxy-11,12,17,18-trinaphthylenetetrone
C34H18O8; MF: 673; RMF: 763; Prob 0.98%; CAS: 96722-31-5; Lib: mainlib; ID: 15056.

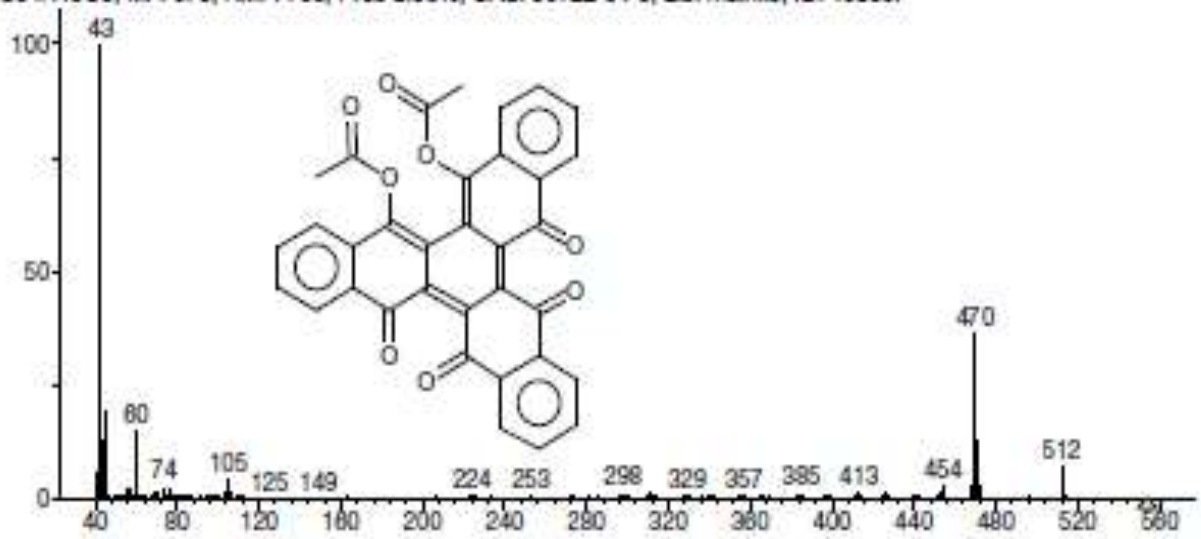


(mainlib) 5,8-Diacetoxy-11,12,17,18-trinaphthylenetetrone

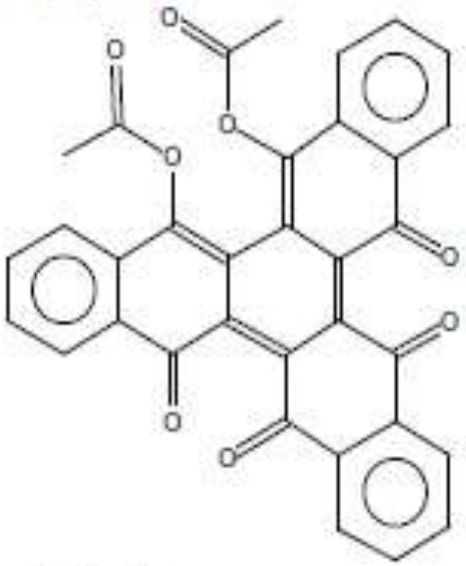


Name: 5,8-Diacetoxy-11,12,17,18-trinaphthylenetetrone
Formula: $C_{34}H_{18}O_8$
MW: 554 Exact Mass: 554.100166 CAS#: 96722-31-5 NIST#: 100425 ID#: 15056 DB: mainlib
Other DBs: None
Contributor: H.LAATSCH,INST. ORG. CHEM.,GEORG-AUGUST-UNIV. GOTTINGEN,W.GER.
InChIKey: BXGDTGBEVCYXRM-UHFFFAOYSA-N Non-stereo
10 largest peaks:
43 999 | 470 389 | 45 198 | 60 150 | 42 147 | 44 122 | 471 121 | 469 101 | 512 72 | 41 52 |

Hit 2 : 5,8-Diacetoxy-11,12,17,18-trinaphthylenetetrone
C34H18O6; MF: 673; RMF: 763; Prob 0.98%; CAS: 96722-31-5; Lib: mainlib; ID: 15056.

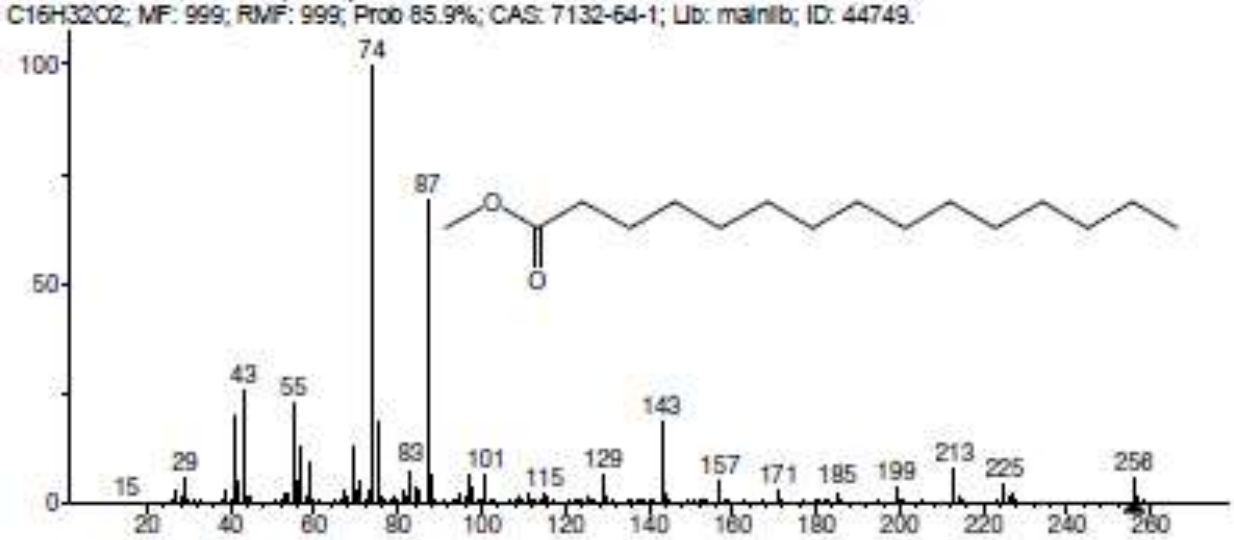


(mainlib) 5,8-Diacetoxy-11,12,17,18-trinaphthylenetetrone



Name: 5,8-Diacetoxy-11,12,17,18-trinaphthylenetetrone
Formula: $C_{34}H_{18}C_8$
MW: 554 Exact Mass: 554.100166 CAS#: 96722-31-5 NIST#: 100425 ID#: 15056 DB: mainlib
Other DBs: None
Contributor: H.LAATSCH,INST. ORG. CHEM.,GEORG-AUGUST-UNIV. GOTTINGEN,W.GER.
InChIKey: BXGDTGBEVCYXRM-UHFFFAOYSA-N Non-stereo
10 largest peaks:
43 999 | 470 369 | 45 198 | 60 150 | 42 147 | 44 122 | 471 121 | 469 101 | 512 72 | 41 52 |

Hit 1 : Pentadecanoic acid, methyl ester
C16H32O2; MF: 999; RMF: 999; Prob 85.9%; CAS: 7132-64-1; Lib: mainlib; ID: 44749.

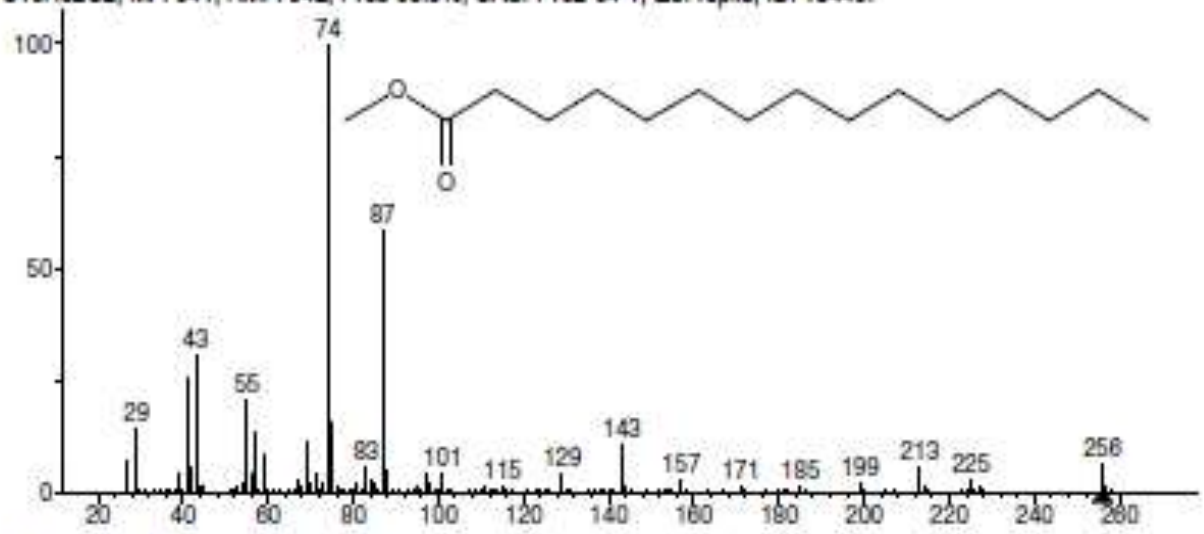


(mainlib) Pentadecanoic acid, methyl ester

Name: Pentadecanoic acid, methyl ester
Formula: $C_{16}H_{32}O_2$
MW: 256 Exact Mass: 256.24023 CAS#: 7132-64-1 NIST#: 352552 ID#: 44749 DB: mainlib
Other DBs: Fine, TSCA, HODOC, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center
InChIKey: XIUXKAZJZFLLDQ-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 895 | 43 262 | 55 230 | 41 194 | 143 187 | 75 181 | 57 128 | 69 122 | 59 91 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1820±4 (16)
Standard non-polar: 1808±2 (23)
Polar: 2108±6 (10)

Hit 2 : Pentadecanoic acid, methyl ester
C16H32O2; MF: 941; RMF: 942; Prob 85.9%; CAS: 7132-64-1; Lib: replib; ID: 10449.

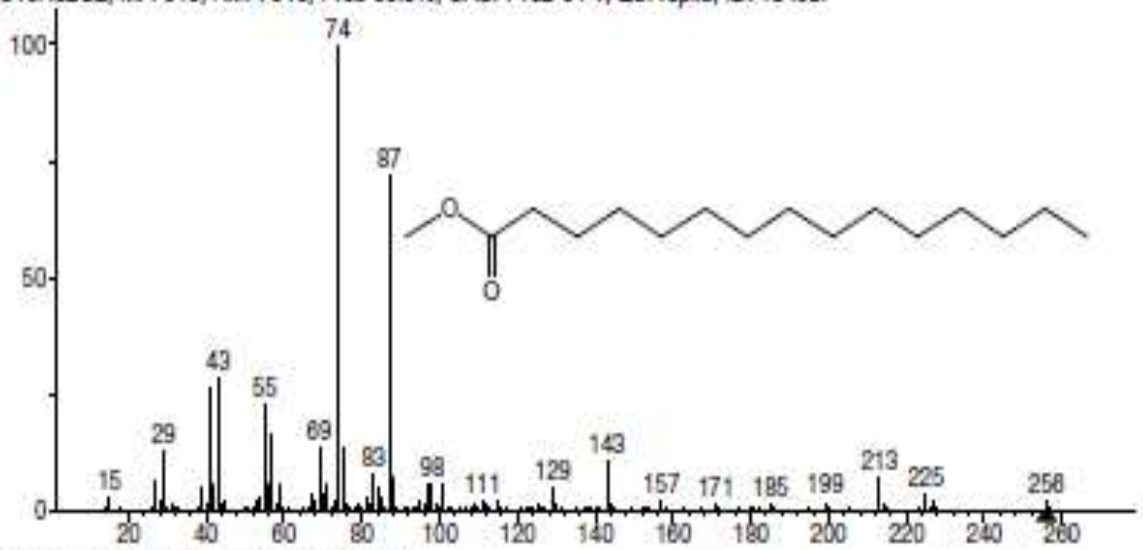


(replib) Pentadecanoic acid, methyl ester

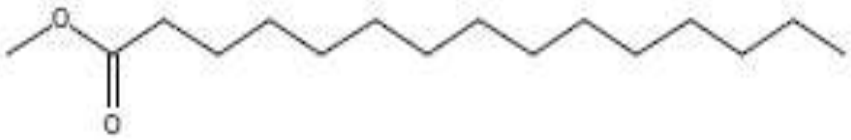
Name: Pentadecanoic acid, methyl ester
Formula: $C_{16}H_{32}O_2$
MW: 256 Exact Mass: 256.24023 CAS#: 7132-64-1 NIST#: 151142 ID#: 10449 DB: replib
Other DBs: Fine, TSCA, HODOC, EINECS, IRDB
Contributor: Chemical Concepts
InChIKey: XIUXKAZJZFLLDQ-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 585 | 43 307 | 41 256 | 55 212 | 75 155 | 29 145 | 57 130 | 69 109 | 143 108 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1820±4 (16)
Standard non-polar: 1909±2 (23)
Polar: 2108±6 (10)

Hit 3 : Pentadecanoic acid, methyl ester
C16H32O2; MF: 918; RMF: 918; Prob 65.9%; CAS: 7132-64-1; Lib: replib; ID: 10450.



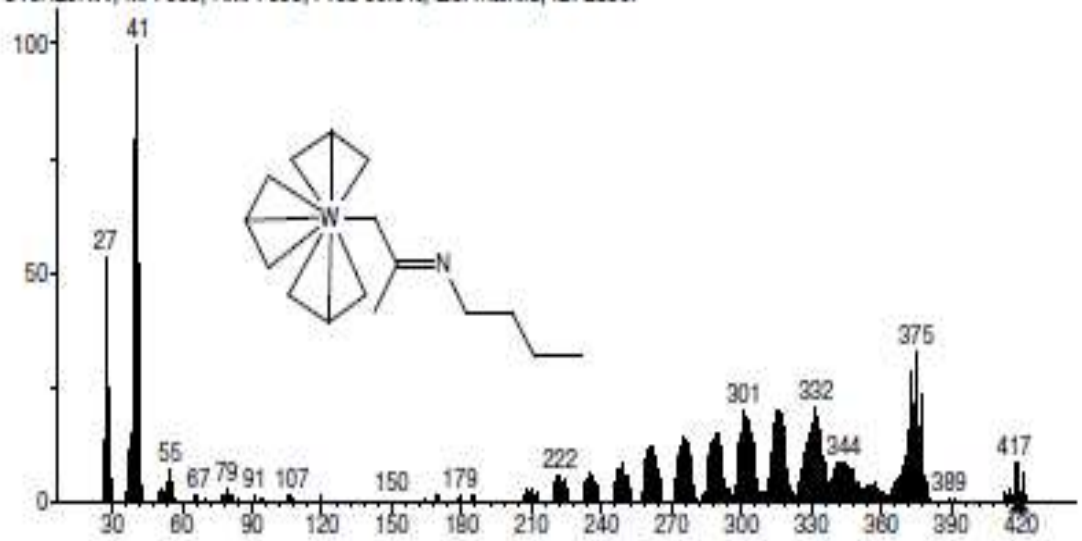
(replib) Pentadecanoic acid, methyl ester



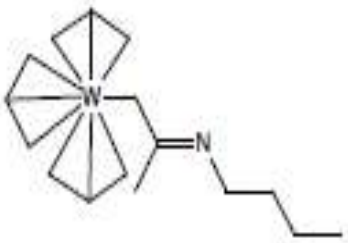
Name: Pentadecanoic acid, methyl ester
Formula: $C_{16}H_{32}O_2$
MW: 256 Exact Mass: 256.24023 CAS#: 7132-64-1 NIST#: 233107 ID#: 10450 DB: replib
Other DBs: Fine, TSCA, HODOC, EINECS, IRDB
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-5303
InChIKey: XIUXKAZJZFLLDO-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 724 | 43 289 | 41 262 | 55 230 | 57 157 | 69 139 | 29 130 | 75 130 | 143 109 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1820±4 (16)
Standard non-polar: 1808±2 (23)
Polar: 2108±6 (10)

Hit 1 : Tungsten, tris(η-3-allyl)-1-(butylimido)propyl)
C16H29NW; MF: 999; RMF: 999; Prob 98.8%; Lib: mainlib; ID: 2098.

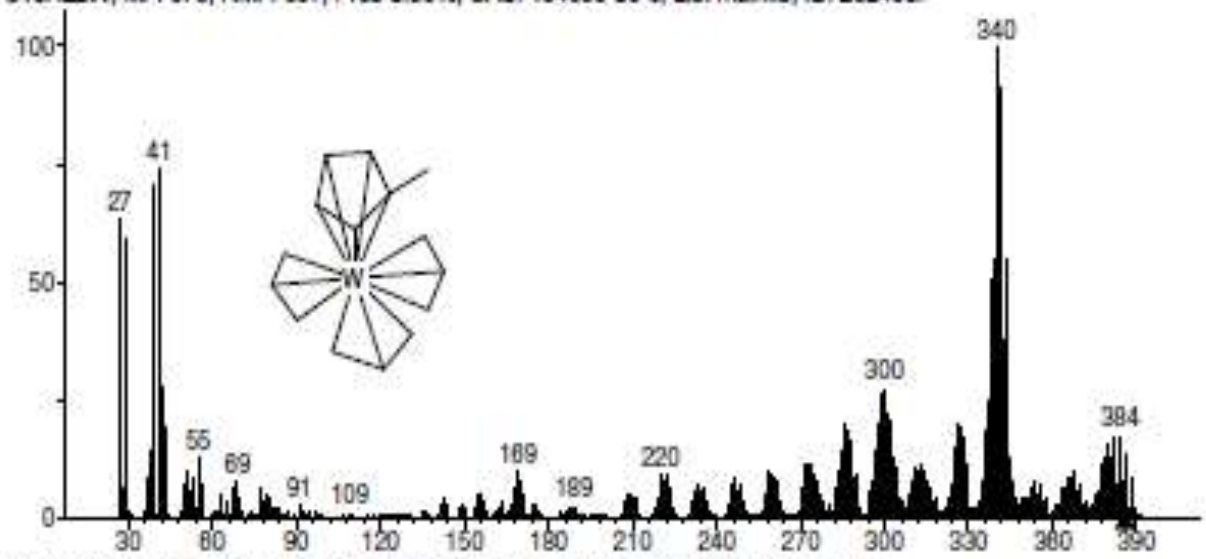


(mainlib) Tungsten, tris(η-3-allyl)-1-(butylimido)propyl)



Name: Tungsten, tris(η-3-allyl)-1-(butylimido)propyl)
Formula: $C_{16}H_{29}NW$
MW: 419 Exact Mass: 419.180931 NIST#: 156859 ID#: 2098 DB: mainlib
Contributor: Chemical Concepts
InChIKey: FAVSEIGMDYDRSN-LIIRSGIESA-N Non-stereo
10 largest peaks:
41 999 | 39 784 | 27 540 | 42 516 | 375 329 | 373 282 | 29 243 | 377 232 | 332 210 | 374 209 |

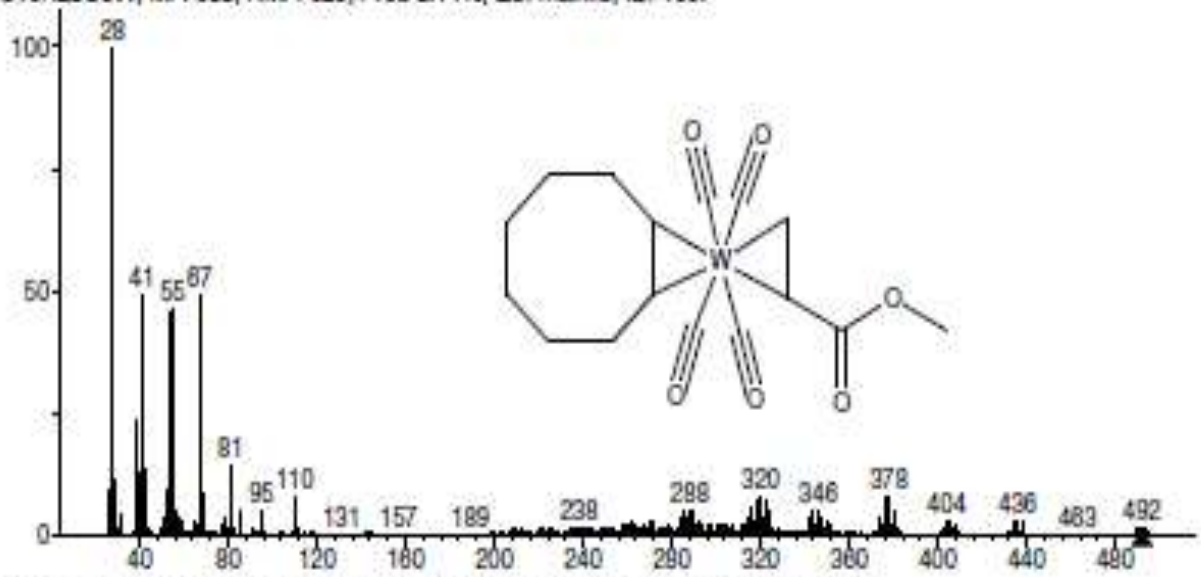
Hit 2 : Tungsten, [(1,2,3,4,5-η)-1-methyl-2,4-cyclopentadien-1-yl]tris(η3-2-propenyl)
C15H22W; MF: 673; RMF: 687; Prob 0.98%; CAS: 134385-08-3; Lib: mainlib; ID: 232485.



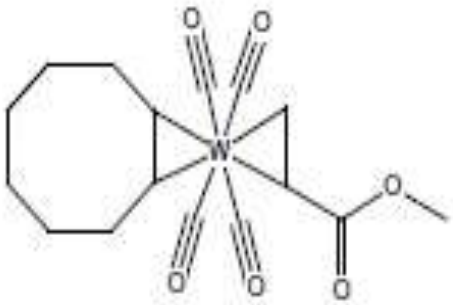
(mainlib) Tungsten, [(1,2,3,4,5-η)-1-methyl-2,4-cyclopentadien-1-yl]tris(η3-2-propenyl)

Name: Tungsten, [(1,2,3,4,5-η)-1-methyl-2,4-cyclopentadien-1-yl]tris(η3-2-propenyl)
Formula: $C_{15}H_{22}W$
MW: 386 Exact Mass: 386.123081 CAS#: 134385-08-3 NIST#: 156809 ID#: 232485 DB: mainlib
Other DBs: None
Contributor: Chemical Concepts
InChIKey: RQKFAIAJFXQPIJ-UHFFFAOYSA-N Non-stereo
10 largest peaks:
340 999 | 342 912 | 41 745 | 39 704 | 341 682 | 27 639 | 29 591 | 339 547 | 344 542 | 338 499 |

Hit 3 : Tungsten(0), η-2-acrylic acid, methyl ester-η-2-E-cyclooctene-tetracarbonyl-
C16H20O6W; MF: 605; RMF: 629; Prob 0.14%; Lib: mainlib; ID: 165.

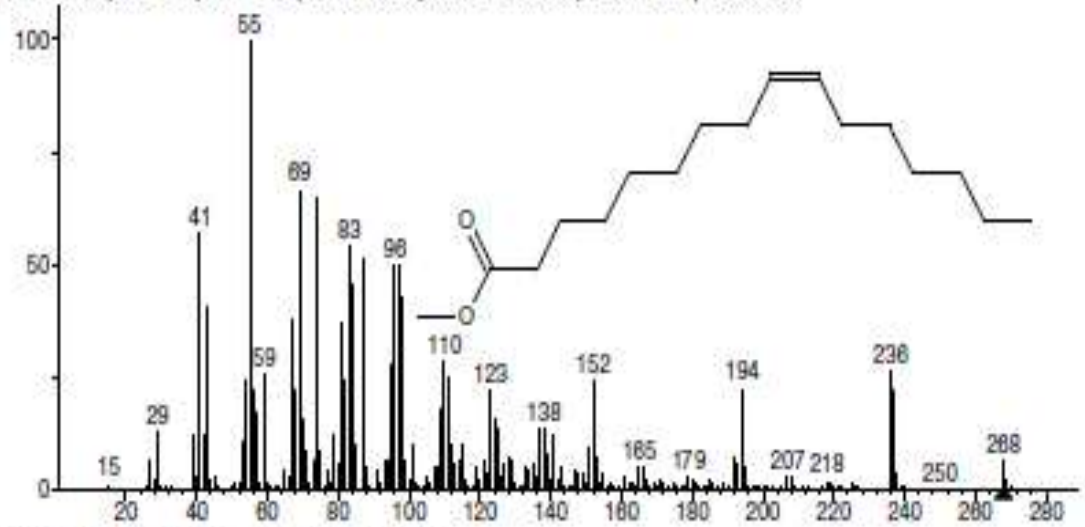


(mainlib) Tungsten(0), η-2-acrylic acid, methyl ester-η-2-E-cyclooctene-tetracarbonyl-

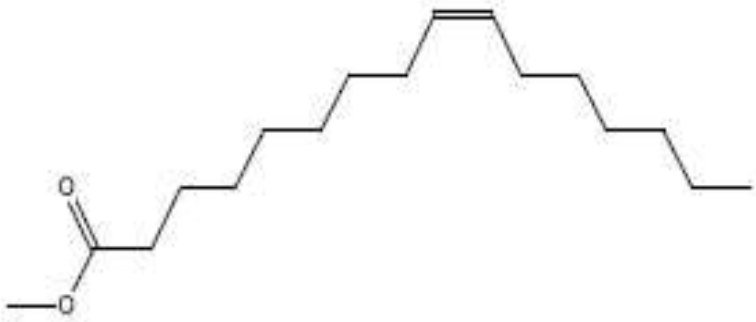


Name: Tungsten(0), η-2-acrylic acid, methyl ester-η-2-E-cyclooctene-tetracarbonyl-
Formula: $C_{16}H_{20}O_6W$
MW: 492 Exact Mass: 492.07692 NIST#: 154528 ID#: 165 DB: mainlib
Contributor: Chemical Concepts
InChIKey: MFPFMTRCAWPJBA-UHFFFAOYSA-N Non-stereo
10 largest peaks:
28 999 | 41 497 | 67 492 | 55 463 | 27 459 | 54 451 | 39 235 | 81 148 | 82 144 | 68 136 |

Hit 1 : 9-Hexadecenoic acid, methyl ester, (Z)-
C17H32O2; MF: 999; RMF: 999; Prob 69.7%; CAS: 1120-25-8; Lib: mainlib; ID: 20213.



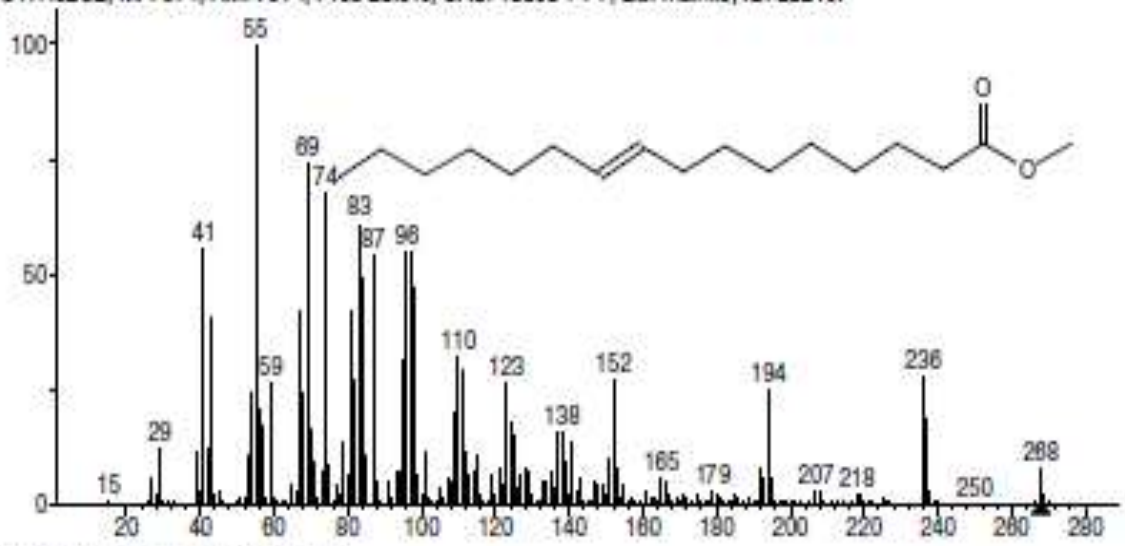
(mainlib) 9-Hexadecenoic acid, methyl ester, (Z)-



Name: 9-Hexadecenoic acid, methyl ester, (Z)-
Formula: $C_{17}H_{32}O_2$
MW: 268 Exact Mass: 268.24023 CAS#: 1120-25-8 NIST#: 333193 ID#: 20213 DB: mainlib
Other DBs: Fine, EINECS
Contributor: NIST Mass Spectrometry Data Center
InChIKey: IZFGRAGOVZCUFB-HJWRWDBZSA-N Non-stereo
10 largest peaks:
55 999 | 69 669 | 74 643 | 41 573 | 83 547 | 87 510 | 96 505 | 97 495 | 84 454 | 98 425 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1899±11 (8)
Standard non-polar: 1987±3 (10)
Polar: 2240±5 (6)

Hit 2 : Methyl hexadec-9-enoate
C17H32O2; MF: 974; RMF: 974; Prob 20.9%; CAS: 10030-74-7; Lib: mainlib; ID: 20216.

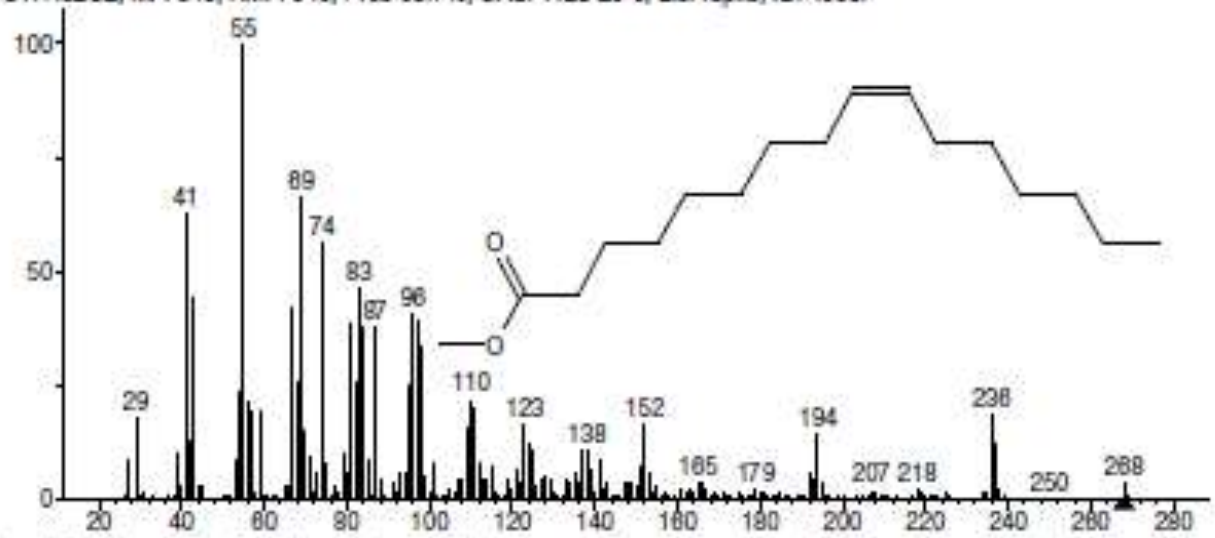


(mainlib) Methyl hexadec-9-enoate

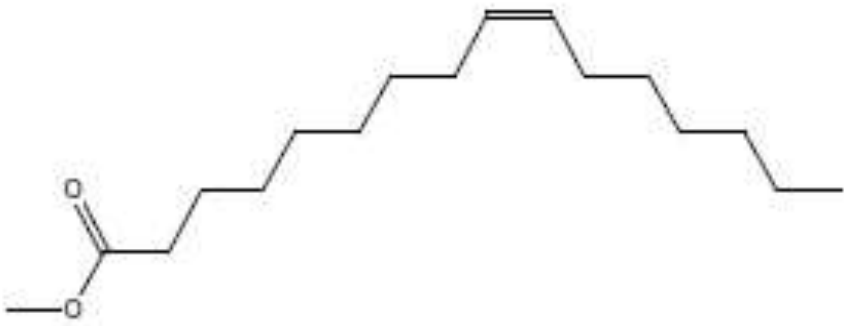
Name: Methyl hexadec-9-enoate
Formula: $C_{17}H_{32}O_2$
MW: 268 Exact Mass: 268.24023 CAS#: 10030-74-7 NIST#: 333999 ID#: 20216 DB: mainlib
Other DBs: None
Contributor: NIST Mass Spectrometry Data Center
InChIKey: IZFGRAGOVZCUFB-CMDGGOBGSA-N Non-stereo
10 largest peaks:
55 999 | 69 745 | 74 679 | 83 612 | 41 559 | 96 552 | 97 552 | 87 544 | 84 487 | 98 486 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1904±6 (6)
Standard non-polar: 1892±0 (2)
Polar: 2238±1 (2)

Hit 3 : 9-Hexadecenoic acid, methyl ester, (Z)-
C17H32O2; MF: 945; RMF: 946; Prob 69.7%; CAS: 1120-25-8; Lib: replib; ID: 4955.



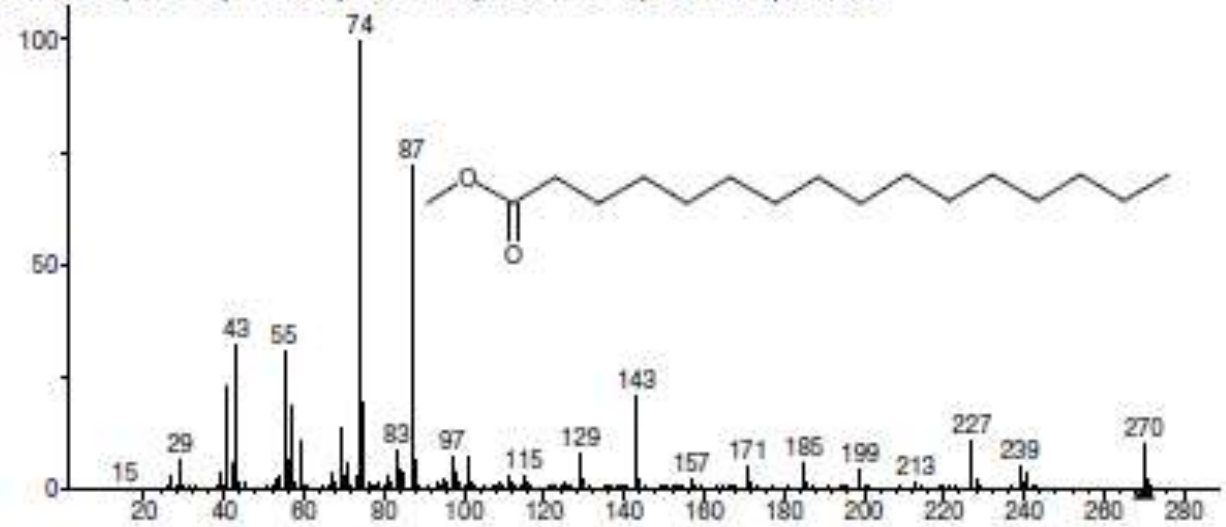
(replib) 9-Hexadecenoic acid, methyl ester, (Z)-



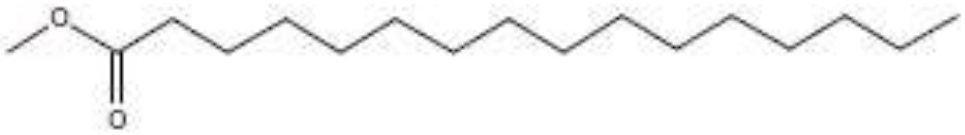
Name: 9-Hexadecenoic acid, methyl ester, (Z)-
Formula: $C_{17}H_{32}O_2$
MW: 268 Exact Mass: 268.24023 CAS#: 1120-25-8 NIST#: 157575 ID#: 4955 DB: replib
Other DBs: Fine, EINECS
Contributor: Chemical Concepts
InChIKey: IZFGRAGOVZCUFB-HJWRWDBZSA-N Non-stereo
10 largest peaks:
55 999 | 69 869 | 41 631 | 74 568 | 83 467 | 43 437 | 67 414 | 96 412 | 97 387 | 81 390 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1899±11 (8)
Standard non-polar: 1887±3 (10)
Polar: 2240±5 (6)

Hit 1 : Hexadecanoic acid, methyl ester
C17H34O2; MF: 999; RMF: 999; Prob 92.1%; CAS: 112-39-0; Lib: mainlib; ID: 44729.



(mainlib) Hexadecanoic acid, methyl ester



Name: Hexadecanoic acid, methyl ester
Formula: $C_{17}H_{34}O_2$
MW: 270 Exact Mass: 270.25588 CAS#: 112-39-0 NIST#: 333716 ID#: 44729 DB: mainlib
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS
Contributor: NIST Mass Spectrometry Data Center
InChIKey: FLIACVVQZYBSBS-UHFFFAOYSA-N Non-stereo
10 largest peaks:
74 999 | 87 720 | 43 325 | 55 310 | 41 229 | 143 209 | 75 188 | 57 183 | 69 132 | 227 110 |

Experimental RI median±deviation (#data)
Semi-standard non-polar:1926±2 (136)
Standard non-polar: 1909±2 (89)
Polar: 2208±10 (64)