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**Two Long Chain Alcohols from Leaves of *Moringa*
*stenopetala***

By
Taame Gezae
Advisor: Ermias Dagne (Professor)

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This is to certify that the thesis prepared by Taame Gezae, entitled: **Two Long Chain Alcohols from *Moringa stenopetala* Leaves** submitted in partial fulfillment of the requirements for the degree of Master of Science (Organic Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner: Dr. Estifanos Elie..... Signature: Date:

Advisor: Ermias Dagne (Professor)..... Signature: Date:

Chair of the Department or Graduate Program Coordinator

Dedicated to: my beloved family

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Abstract

Plants have been and will remain vital to mankind, animals as well as the environment. The present study focuses on the phytochemistry of the endemic food and medicinal plant species known as *Moringa stenopetala*, which is confined to the horn of Africa: Ethiopia, Kenya and Somalia. In the course of this study from the extracts of the dried leaves the well known flavonoid, rutin and two long chain alcohols were isolated and tentatively identified as 1-Heptacosanol and 14-(tridecylamino) tetradecan-1-ol. The structure elucidation of these compounds was achieved mainly by means of physical and spectroscopic analysis.

Key words: Phytochemistry, Moringa, *Moringa stenopetala*, 1-Heptacosanol, 14-(tridecylamino) tetradecan-1-ol, rutin

1. Introduction and Literature Review

1.1. Introduction

Plants have been and will remain vital to mankind, animals as well as the environment. They produce primary and secondary metabolites, which encompass a wide array of functions [1]. Humans have exploited many of these metabolites for their beneficial role in a various array of applications [2]. The most important of these bioactive constituents of plants are the secondary metabolites, which include alkaloids, phenolic compounds, tannins, phytosterols, and terpenoids. These metabolites are used for the treatment of infectious diseases, which are the leading cause of death worldwide. This is now even more relevant because efficacy of many conventional antibiotic drugs is being threatened by the emergence of drug resistant pathogens [3]. Hence, phytomedicine is popular in many countries owing to its natural origin and lesser side effects [4]. It is estimated that plant materials have provided templates for more than 50% modern drugs [5, 6]. The list of the benefits of bioactive compounds to human health is indeed long, including anti-cancer, anti-hypertension, anti-hypoglycemia, antioxidants, antimicrobial etc [7-14].

In this project, we focus on the study of the chemistry of an endemic food and medicinal plant species known as *Moringa stenopetala*, which is confined to the horn of Africa: Ethiopia, Kenya and Somalia. We shall briefly review first the literature at the genus level, with emphasis on the closely related species *Moringa oleifera* which originally occurs in the Indian subcontinent but is now cultivated in different African countries and in many other parts of the world.

1.2. On the Family Moringaceae and the Genus Moringa

The family Moringaceae is a monotypic family that contains only one genus known as Moringa. There are about 14 species of Moringa, 12 of which are in Africa and only 2 in Asia. Of those in Africa 2 are found in Madagascar, one in Angola and the rest are “endemic to the Somali-Masai floristic region” [15]. The most well known species is *M. oleifera* of Asia which is now also cultivated in many other parts of the world.

Ethnobotanical information on some members of the genus is summarized in **Table 1**. So far, there are no reported traditional uses on *M. arborea*, *M. concanensis*, *M. hildebrandtii*, *M. ovalifolia*, *M. pygmaea*, *M. rivaie* and *M. ruspoliana*.

Table 1: Occurrence and Traditional Uses of some *Moringa* species

Species Name	Origin	Traditional Uses	Ref.
<i>M. borziana</i>	Ken. & Som.	Treatment of different ailments: treatments of smudges on petal tips	[15]
<i>M. drouhardii</i>	Mad.	Scented bark used for treatment of cold and cough	
<i>M. longituba</i>	Ken., Eth. & Som.	Root extracts for treating intestinal infections of animals Kenya, Ethiopia,	
<i>M. oleifera</i>	Ind.	Antispasmodic, stimulant, expectorant and antifungal, antibacterial, as counter-irritant	[16,17]
<i>M. peregrina</i>	RS., Ara & HA.	Analgesic in the ancient world	[15]
<i>M. stenopetala</i>	Ken. & Eth.	Food and medicine	

Ara = Arabia, Eth. = Ethiopia, HA. = Horn of Africa, Ind. = India, Ken. = Kenya, Mad. = Madagascar, RS. = Red Sea, Som. = Somalia,

1.2.1. *Moringa oleifera*

M. oleifera is the best known species of the genus. The plant is known by different common names including: ben oil tree (from the name of its pressed seed oil), horseradish tree (from the taste of the roots, which resembles horseradish) and drumstick tree (from the appearance of the long, slender and triangular seedpods) [48].

M. oleifera is a deciduous tree or shrub that grows up to 10 or 12 m. It is native to the Indian sub-continent and is now naturalized and cultivated in tropical and sub-tropical areas around the world [15]. It can be grown either by use of seedlings or through utilization of hard stem cuttings. The tree grows in most types of soils, from acid to alkaline. However, the tree does not grow well in cool temperatures, low sunlight or wet conditions. The tree is drought tolerant and will grow even during 6 months of a dry season and can be found growing naturally at an elevation of up to 1000 m above sea level [18]. It also grows best in hot, semi-arid tropics with rainfalls between 250-1500 mm. Altitudes below 600 m are best for the plant, however, it grows well at altitudes up

to 1200 m in some tropical areas although it has been recorded at 2000 m. The tree takes 8 months to a year to reach flowering and can flower all year round when climatic conditions are favorable [18].

The ancient Romans, Greeks and Egyptians extracted oil from its seeds commonly known as Ben oil, which was used to make perfume and skin lotion. The name of the oil is derived from the high quantity of behenic acid. Ben oil has long shelf life and in 19th century, the oil was exported from the West Indies to Europe for making perfumes and lubricants for machinery. Commercial production of *M. oleifera* in Africa is still limited, where it is used to some extent as food supplement [18].

1.2.2. *Moringa stenopetala*

M. stenopetala is the second most important Moringa species after *M. oleifera*. It is known by different common names including: cabbage tree (English), shiferaw (Amharic) and by different vernacular names: aleko (Gamo Gofa), shalkayda (Konso), telahu (Tsemay), haleko (Burji, Derashe), and halakwa (Wollayta) [19]. It is a tree with 6-10 m tall, trunk up to 60 cm in diameter, leaf up to 55 cm long, its flowers are white and strongly scented, pods are long (ca. 19.7-50 cm), seeds are brown and spongy [15]. It is native to southern Ethiopia, northern Kenya, and Somalia [20, 21]. Similar to *M. oleifera*, it is cultivated either by direct seeding, transplanting or through utilization of hard stem cuttings [18]. It is an important food plant in South Western Ethiopia, where it is cultivated as a crop plant [17]. The yields of this plant differ, depending on the season, fertilisation and irrigation. The best yields can be realized when the plant is grown under warm, dry conditions with some supplement of irrigation. In Ethiopia, it is cultivated in many arid areas of the Southern part most extensively in Arbaminch and other areas such as Konso, Negelle, and Wollayta sodo at altitude of up to 1,800 m [19, 20]. It thrives well at mid altitude of 1100 - 1600 m above sea level. It has recently introduced to the northern part of Ethiopia especially in Wollo province [19].

1.3. Review of Chemistry of Moringa species

Table 2 and 3 shows all compounds isolated so far from the two Moringa species. To the best of our knowledge, there is no chemical analysis report on any of the other species. So far, about 65 compounds have been reported from Moringa species that belongs to the class of secondary metabolites (alkaloids, flavonoids, and fatty acids, thioglycosides, phenolics/acids and steroids). In addition to these compounds nitrile glycosides, aldehyde glycosides, carbamate glycosides and various aglycon derivatives of these compounds were reported so far. However, most of the compounds belong to the class of flavonoids and thioglycosides.

Thioglycosides are sulphurated specific group of glycosides which is also referred to as 'thiocyanate glycosides' or 'glucosinolate compounds' or isothiocyanate glycosides' in various literatures [5]. Glucosinolates are thioglycosides that contain sulfur and nitrogen which are derived from glucose and an aminoacid. Glucosinolates (**Figure 1**) contain a central carbon atom, which is bound via a sulfur atom to glucose and via a nitrogen atom to a sulfate group. In addition, the central carbon is bound to a side group (**R**). Different glucosinolates have different side groups.

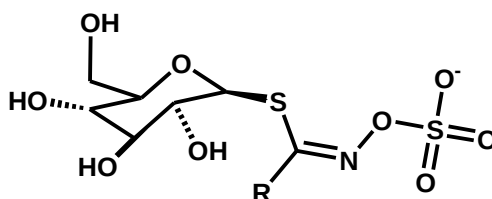


Figure 1: General Structure of Glucosinolates.

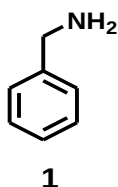
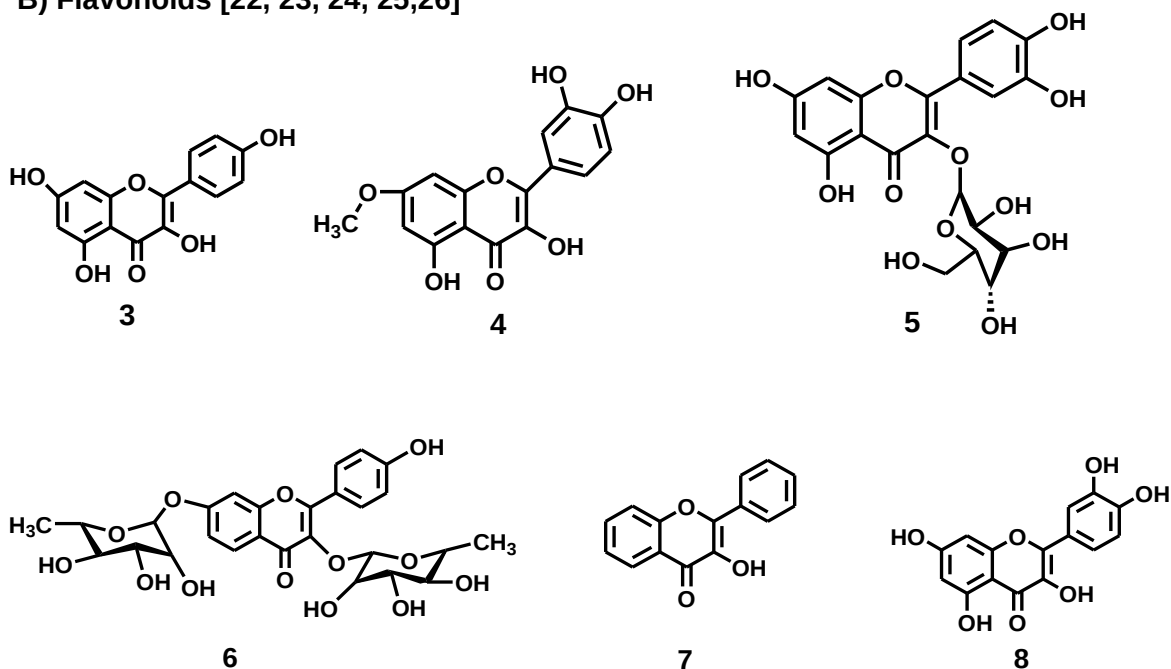
The variation of the side group is responsible for the differences in the biological activities [5].

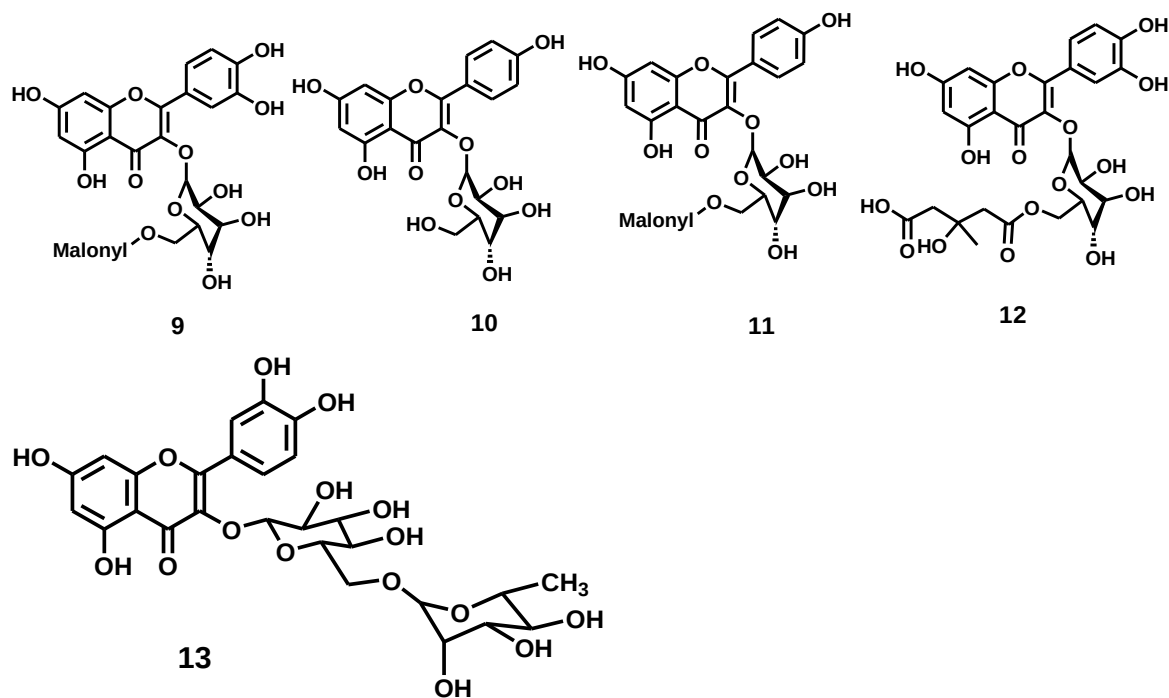
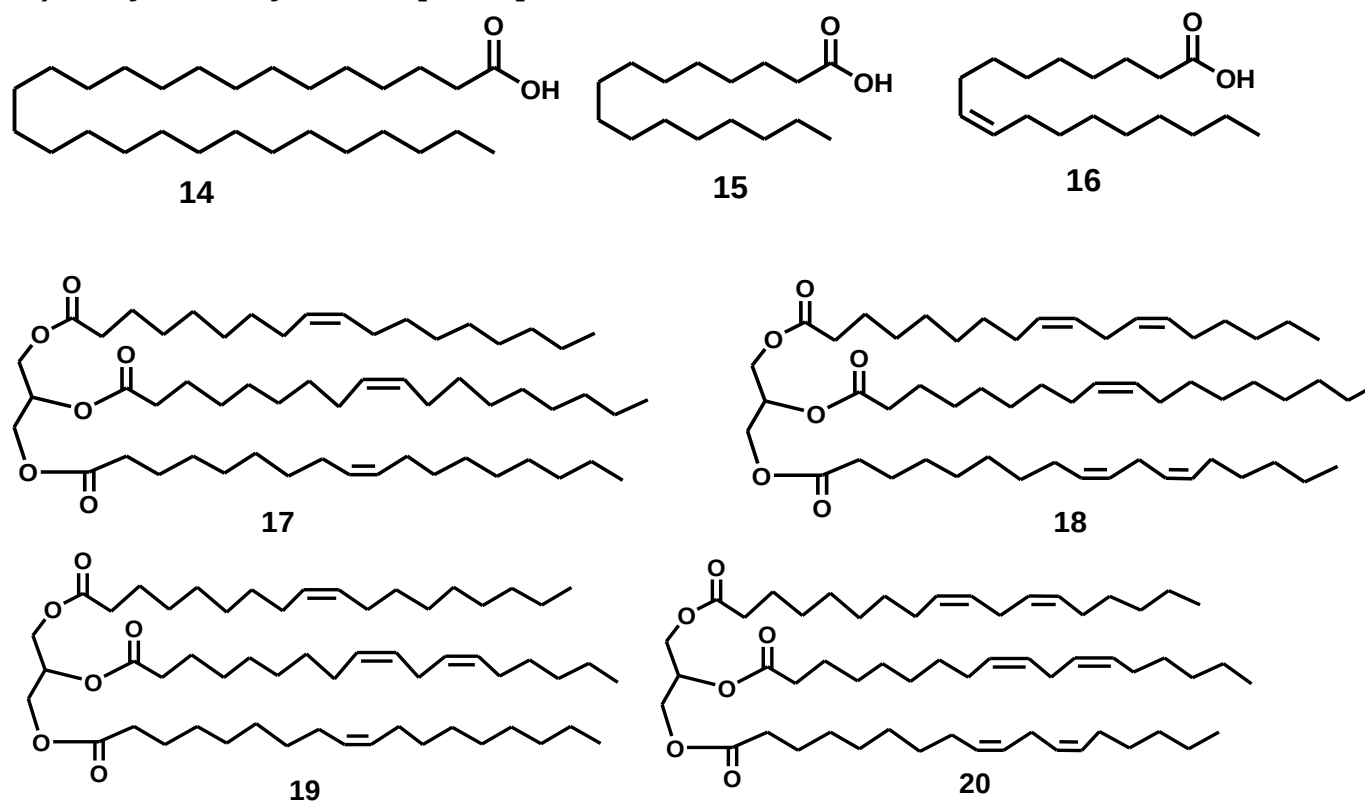
Table 2: All Compounds Isolated So Far from *M. oleifera*

Compound class	Common/IUPAC name	Structure No.	Ref.
Alkaloids	Moringine and Moringinine	(1 and 2)	[22]
Flavonoids	Kaempferol; Rhamnetin; Isoquercitrin; Kaempferitrin; 3-hydroxy-4-phenylchromen-4-one; Quercetin; D-(6''-O-malonyl)-glucoside; Astragalin; Kaempferol 3-O- β -D-(6''-O-malonyl)-glucoside and Quercetin 3-O- β -D-(6''-O-3-hydroxy-3-methylglutaryl)-glucoside	(3 -12)	[22, 23, 24, 25]
Fatty acids/ Glycerieds	Octacosanoic acid; 1,2,3-triolein; 1,3-dilinoeoyl-2-olein; 1,3-dioleoyl-2-linolein and 1,2,3-trilinoeol	(14) and (17- 20)	[16, 21]
Thioglycosides	Thiocyante; Isothiocyanate; β -Thioglucoside-N-hydroxysulphate; 4-(α -L-rhamnopyranosyloxy)benzyl glucosinolate; 4-(α -L-rhamnopyranosyloxy)benzyl glucosinolate; 4(α -L-rhamnosyloxy)-benzyl isothiocyanate; 4-(4'-O-acetyl- α -L-rhamnopyranosyloxy)benzyl isothiocyanate; 4-(β -D-glucopyranosyl-1 $\rightarrow$ 4- α -L-rhamnopyranosyloxy)-benzyl isothiocyanate; Niazinin A; Niazimicin; Niaziminin; 4'-O-(4-O-Acetyl- α -L-rhamnopyranoside) O-Me ester and Niazidin	(21 - 34)	[16, 22, 23, 28, 29, 30, 31, 32]
Phenolics/acids	Benzoic acid; Salicylic acid; Gallic acid; Cinnamic acid; Coumaric acid; Caffeic acid; Gentisic acid; Chlorogenic acid; Caffeoyl quinic acid glucosides; Chlorogenic acid; 4-O-caffeoyl quinic acid; 5-O-caffeoylquinic acid and 2-(4-hydroxyphenyl)acetic acid	(35 -47)	[22, 25,32]
Steroids	β -sitosterol; β -sitostenone; 3-O-(6'-O-Oleoyl- β -D-Glucopyranosyl)- β -sitosterol; β -sitosterol-3-O- β -D-Glucopyranoside and Linoleic sitosteroate	(48 -52)	[16, 23,37]
Alcohols	8-Heptadecanol; 8-Nonadecanol and Docosen-8-ol	(53 -55)	[33]
Miscellaneous Compounds	Niazirin; Niaziridin; O-Ethyl-4-(α -L-rhamnosyloxy) benzyl carbamate; 4-O- α -L-rhamnopyranosyloxy-N-glucopyranosyl-1 $\rightarrow$ 2-fructopyranosyloxy phenylmethaneamine; Benzylcarbamothioethionate; Benzyl thiocarbamate and Pterygospermin,	(56 - 57), (59 -60), and (62 - 64)	[16, 22, 23, 28, 29, 30, 31, 32]

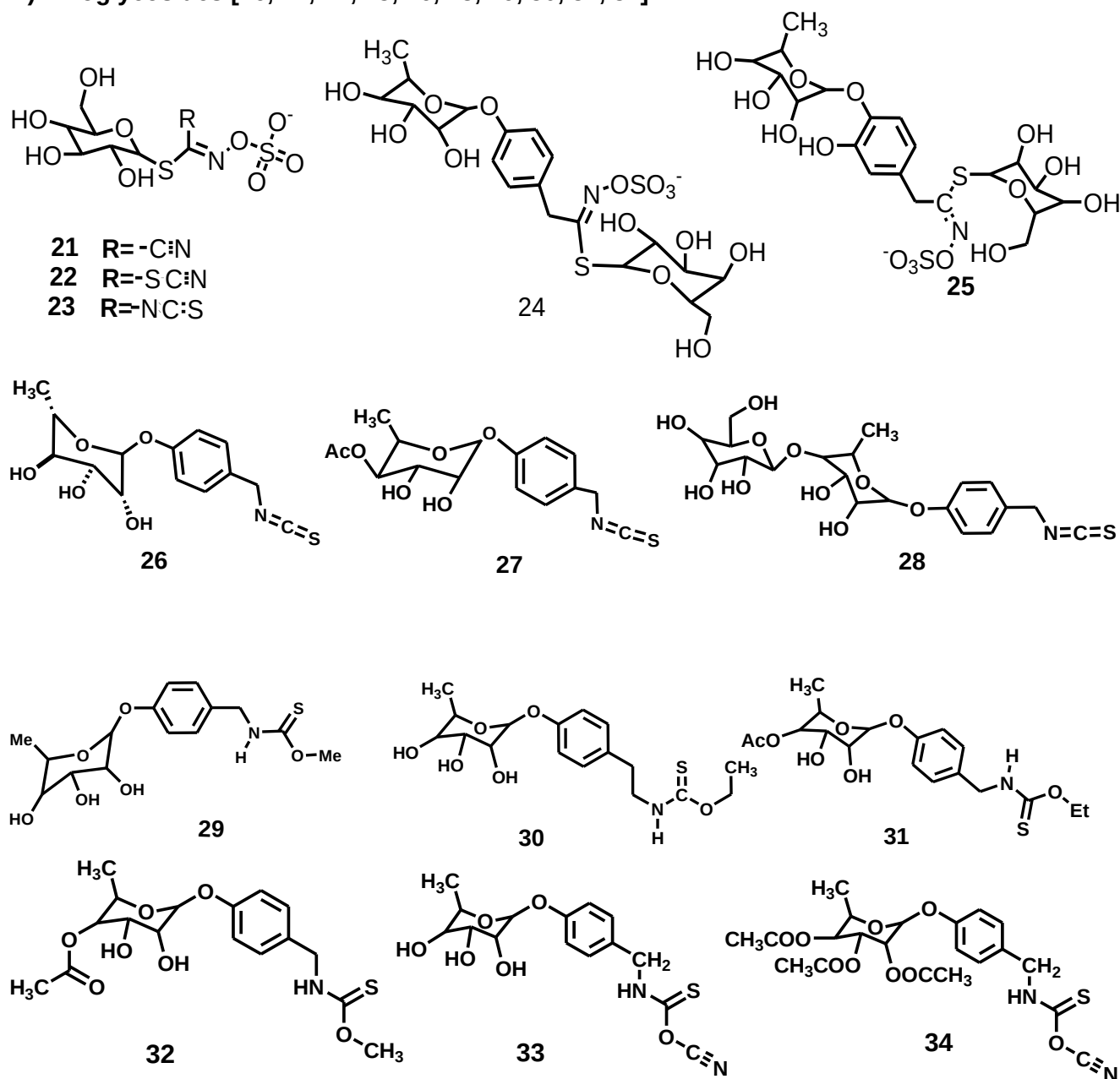
Table 3: All Compounds Isolated So Far from *M. stenopetala*

Compound class	Common/IUPAC name	Structure No.	Ref.
Alkaloids	None so far	-	-
Flavonoids	Rutin	(13)	[26]
Fatty acids/ Glycerides	Palmitic acid and Oleic acid	(15) and (16)	[16, 21]
Thioglycosides	4-(4'-O-acetyl- α -L-rhamnosyloxy)-benzylisothiocyanate	(27)	[21, 26]
Phenolics/acids	None so far	-	-
Steroids	β -sitosterol	(48)	[27]
Alcohols	None so far	-	-
Miscellaneous Compounds	Benzyl isothiocyanate and 4-(4'-O-acetyl- α -L-rhamnosyloxy)-benzaldehyde	(61) and (58)	[21, 26]

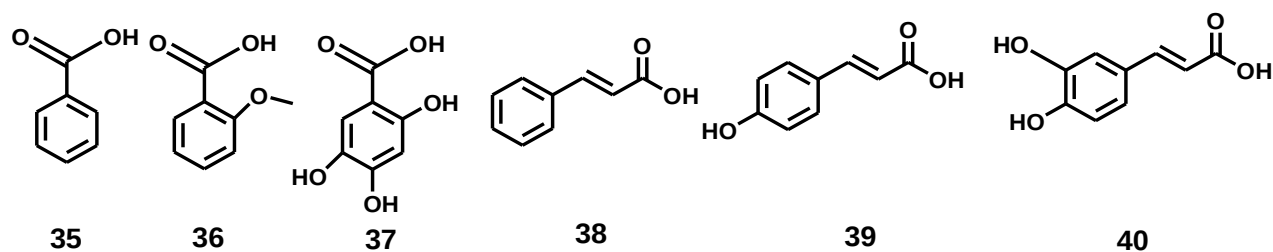
A) Alkaloids [21]**B) Flavonoids [22, 23, 24, 25,26]**

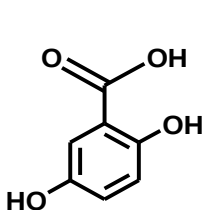
**C) Fatty acid/ Glycerides [16, 21]**

D) Thioglycosides [16, 21, 22, 23, 26, 28, 29, 30, 31, 32]

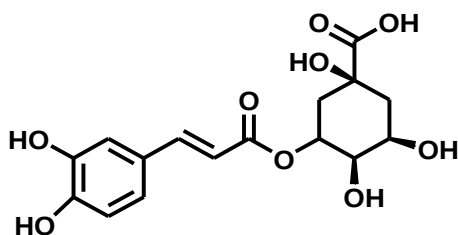


E) Phenolics/ Acids [22, 25, 32]

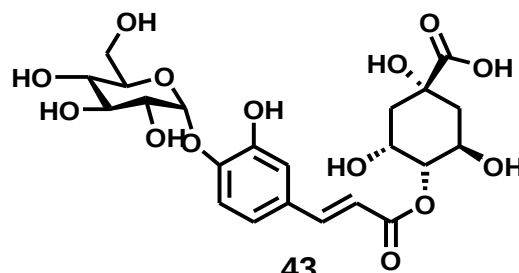




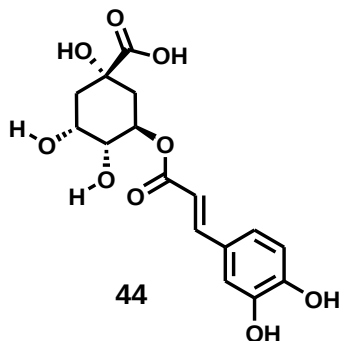
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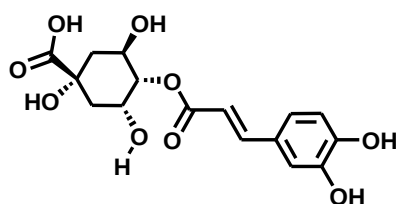
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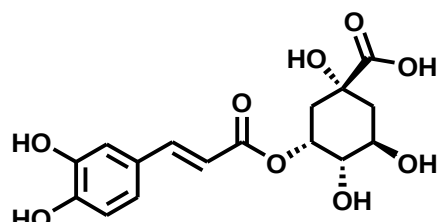
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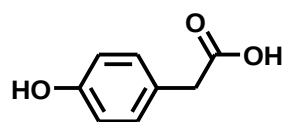
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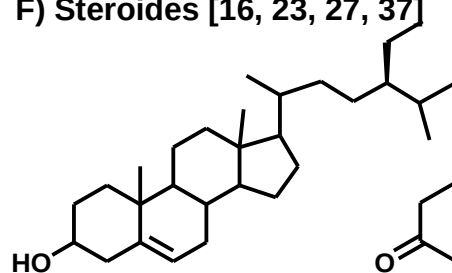


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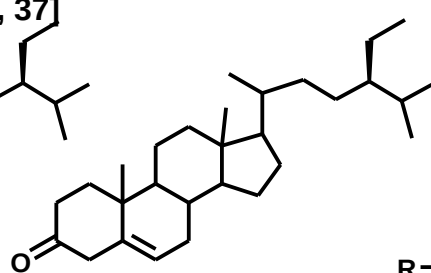


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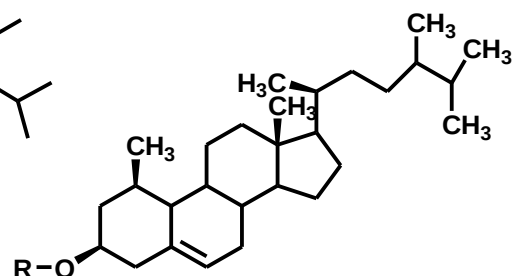
F) Steroides [16, 23, 27, 37]



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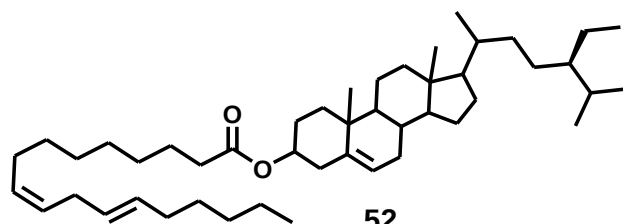


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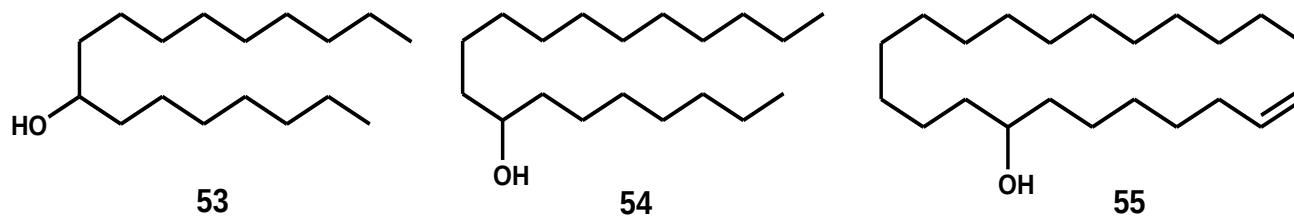
50: R= 6-O-Oleoyl-Beta-Glucopyranosyl

51: R= Beta-D-Glucopyranosyl



52

G) Alcohols [33]



H) Miscellaneous Compounds [16, 21, 22, 23, 26, 28, 29, 30, 31, 32]

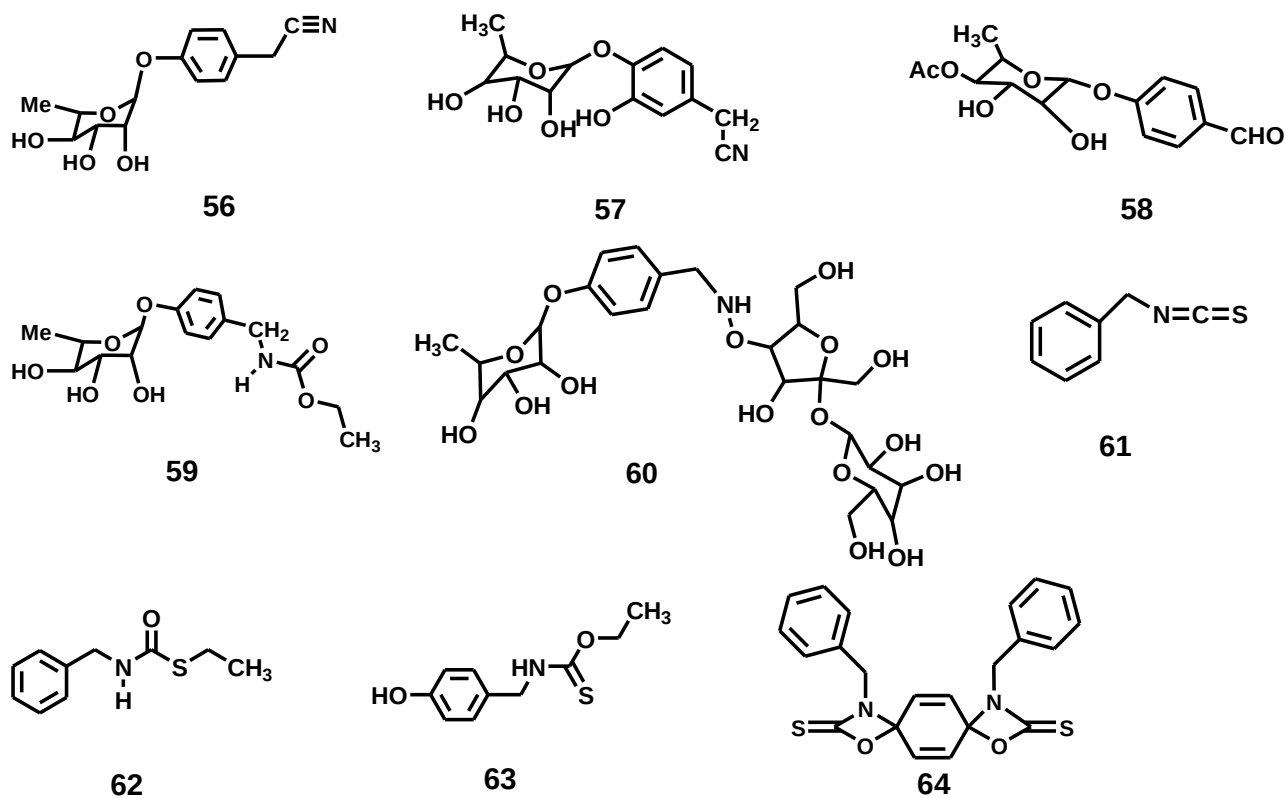
**Figure 2:** Structures of All Compounds So Far Isolated from *Moringa* species.

Table 4: Striking Similarities and differences between *M. oleifera* and *M. stenopetala*

<i>M. Oleifera</i>	<i>M. Stenopetala</i>	Ref.
“Drumstick tree”, “Horseradish tree” or the “Miracle tree”, most common species grown in many countries	“Mother’s helper” increases mother’s milk, “Cabbage tree” (English), “Shiferaw” (Amharic), rare species- Africa Ethiopia	[19, 48]
Brown colored seed with wings	Cream colored longer seeds	[15]
Easy to grow in warm weather	Harder to grow in cold weather	
It is known as slender tree	It is called bottle tree because of its shape	
It has evergreen, smaller and round leaves	Evergreen with larger longer leaves	
All parts of the tree are edible and very nutritious except roots	All parts of the tree are edible and very nutritious except roots	[38]
Used in many cooking recipes and beauty products	Used in similar way	[17,18]
Taste pretty good, similar to a mild spinach	It tastes a little better	[15]
Grows fast	Grows slow	
It flowers in 8-12 months	Needs 3 years and above to flower	
It can withstand cold weather	Will die in extreme cold weather	[17 - 19]
Grows well in bad soil, but best in rocky sandy soils	Same soil type	
It does not need a lot of water and can withstand droughts	It can withstand droughts a little better	
Its seed cake purifies polluted water	Same	[17, 41]

1.4. Biosynthesis of Long Chain Alcohols

A high diversity of fatty alcohols and their derivatives is found among organisms and plants species, in terms of both quantity and composition. They are commonly found in cuticular waxes, suberin, sporopollenin or seeds, with carbon chain length ranging from 14 to 34 carbons [49]. Long chain alcohols are biosynthetic reduction products of fatty acyl-CoA esters. The conversion of a fatty acid into a fatty alcohol is catalysed by the enzyme fatty acid reductases (FAR), which catalyze the reductions of fatty acyl-CoA/ ACPs. The reduction pathway in plants and bacteria are considered to be different [49].

The biosynthesis of fatty alcohols in bacteria prokaryotes is conducted via two-step reductions of fatty acyl-CoA/ACP. The substrate is first reduced in to a fatty aldehyde by a fatty acyl –CoA/ACP reductase (aldehyde forming FAR) and the fatty aldehyde is then reduced further into a fatty alcohol by a fatty aldehyde reductase (**Figure 3**, path B). However, in plant eukaryotes the two-step process is carried out by a single fatty acyl-CoA/ACP reductase (alcohol-forming FAR) and the free fatty aldehyde intermediate is not released (**Figure 3**, path C). Besides alcohol- forming FAR, aldehyde – forming reductase is also found in plants, although it is supposed to be involved in a different catabolism pathway, e.g. a decarbonilation pathway [49]. In addition to the fatty alcohol biosynthesis, by FAR enzymes mentioned above, some bacteria could synthesize primary fatty alcohols from n-alkane by the hydroxylation reaction catalysed by alkane hydroxylase enzymes (**Figure 3**, path A). In plants, there are also enzymes that catalyse the hydroxylation of n-alkane, but this leads to the formation of only secondary fatty alcohols [49].

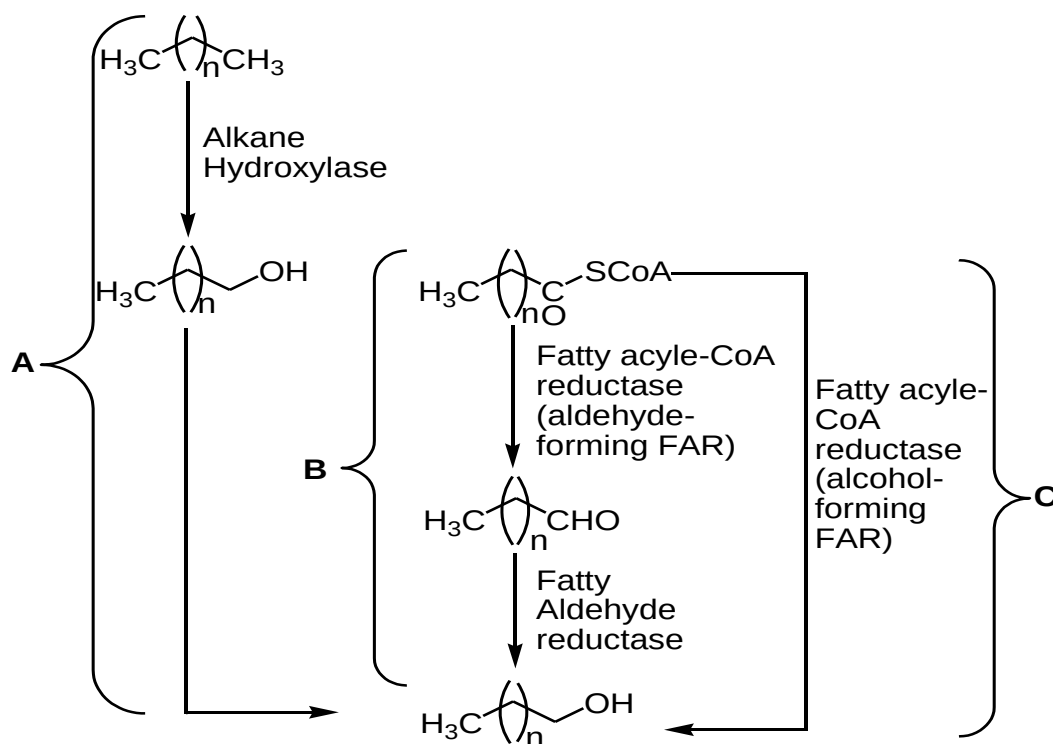


Figure 3: Biosynthesis of Long Chain Fatty Alcohols [49]

1.5. Food and Medicinal Uses of *Moringa oleifera* and *Moringa stenopetala*

Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Three non-governmental organizations in particular; Trees for Life, Church World Service and Educational Concerns for Hunger Organization have advocated *Moringa* as “natural nutrition for the tropics.” It is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce [34, 31].

***M. oleifera*:** The young leaves of *Moringa* are eaten raw, cooked like spinach or stored as dried powder for many months without refrigeration. The leaf powder can be mixed with juices, beverages using a teaspoon, vegetables or soup that is prepared for consumption. Moreover, *Moringa* powder is also sold in capsules and the daily intake is about two capsules a day. The leaves are a good source of vitamins and minerals, particularly iron and sulphur containing amino acids. The leaves of *Moringa* contain more Vitamin A, Vitamin C, calcium, iron, potassium than carrots, oranges, milk, spinach, bananas respectively and the protein quality of *Moringa* leaves rivals that of milk and eggs [31, 38].

The dried ground powder of the seeds can be used for seasoning and as vegetable cooking oil with many essential fatty acids [40]. Apart from the oil, the seeds are eaten like peanuts, as is the case in Malaysia. Flowers of *Moringa* can also be eaten raw in salads, or cooked, or can be used to make tea [18]. It is important for the dosage of *Moringa*: Leaf-10-20 ml. juice, root bark-2-5 g powder, stem bark-2-5 g powder, seed-5-10 g powder, Leaf, flower, fruit, seed, bark, root-1-3 g powder, 50-100 ml decoction [16].

Moreover, the tree is used as a potential strategy for increasing the quality and availability of feeds for smallholder ruminant animals in the dry season [18].

The most direct medicinal use of *M. oleifera* is a poultice of the leaves and bark applied directly to wounds as anti-microbial and to promote healing. The leaves and seeds of this plant had no negative effects on human health from daily consumption [18]. In India,

M. oleifera is used traditionally for treatment of anemia, asthma, bronchitis, cholera, cough, diarrhea, eye & ear infections, fever, blood pressure, pain in joints, respiratory disorders [17, 34]. Similarly, peoples in Malaysia used for intestinal worms and in Guatemala used for treatment of skin infections and sores. In Puerto Rico and Philippines, it uses for intestinal worms, glandular swelling and lactating. Furthermore, the tree continues to have an important role particularly as counter-irritant in the indigenous medicine in Asia and West Africa [17].

***M. stenopetala*:** In southern Ethiopia, around Arbaminch, Goffa, Konso, and Wollayta, the leaves and flowers are consumed in large amounts and are eaten as vegetables [39]. The local people cook the leaves of *M. stenopetala* tree and eat them with their traditional *kurkufa*, a cereal dish made with maize and sorghum. As further noted the leaves have distinctive strong, mustard-like taste, contain calcium, iron, and other trace minerals eaten as a supplement to the major staple foods [19]. The tree is also used as bee forages [20]. According to [38], no side effects has been yet found from daily consumption of Moringa leaf powder.

Local people in southern Ethiopia, traditionally use *M. stenopetala* for the treatment of malaria, leishmaniasis, hypertensionis, stomach pains, expulsion of retained placenta during birth, asthma, and diabetes [20]. People inhabiting around Lake Turkana also use its leaves for treatment of leprosy [20]. Moreover, *M. stenopetala* is used in folk medicines as antimalarial, antihypertensive, against stomach pain, antihyperglycemic [24], anticholesterol, antispasmodic and to expel retained placentae during birth [35].

To sum up, the following traditional uses of *M. oleifera* and *M. stenopetala* have been supported by scientific studies.

***M. oleifera*:** anti-hypertension [23], antimicrobial [16, 22, 36], anti-inflammatory [24, 36], antioxidant [22, 24, 36], anticancer, antifertility, hepatoprotective, antidiabetic, cardiovascular, anti-allergic [22, 23], anthelmintic, anti-ulcer, analgesic, antipyretic & wound healing, etc [16].

***M. stenopetala*:** antileishmanial [20], antibacterial [27], antihypertensive [19], antileukaemic [21], antimalarial, antidiabetic and as antispasmodic [35, 39].

Furthermore, different scholars checked biological activity of compounds isolated from moringa and is summarized in **Table 5**.

Table 5: Biologically Active Compounds Reported from *M. oleifera*

Compound name	Biological activity	Ref.
Moringine (1)	Antihypoglycemic	[22]
Kaempferol (3)	Antiinflammatory, Anti-oxidant	[24, 36]
Quercetin (8)	Anti-oxidant, Anti-microbial	[22, 36]
O-Ethyl-4-(α -L-rhamnosyloxy) benzyl carbamate (17)	Antispasmodic	[16]
4(α -L-rhamnosyloxy)-benzyl isothiocyanate (18)	Antimicrobial	[16]
Niazimicin (19)	Antihypertensive	[23]
Niazirin (20)	Bioenhancer	[30]
Isothiocyanate (22)	Anti-hypotensive	[22]
Gallic acid (47)	Anti-microbial, Anti-fungal	[22]
Cinnamic acid (48)	Anti-oxidant	[22]
β -sitosterol (59)	Antibacterial	[27, 37]

1.6. Water Purification

Water is vital to life; however, due to indiscriminate human activities its quality has deteriorated causing about 80% of diseases that plague the human race especially in many developing countries [29]. Promising water treatment techniques are far much costly and many disinfectants currently used can be harmful. For instance, introducing aluminum into the environment by the continuous use of aluminum sulphate as a coagulant in water treatment causes Alzheimer's disease due to a high level of aluminum in the brain [16, 17]. This has aggravated the search for safer organic alternatives.

Moringa seeds can be used in rural areas for clarification of drinking water to reduce turbidity. In this regard, *M. oleifera* and *M. stenopetala* seeds acts as an effective water-clarifying agent. The natural poly-electrolytes released from the crushed seed kernels function as natural flocculating agents, binding suspended particles in a colloidal suspension, forming larger sedimenting particles (flocs) in which microorganisms are generally attached. For example, 4- α -rhamnosyl oxybenzyl isothiocyanate and presently known as glucosidal mustard oil which coagulates the solid matter in water and

removes a good portion of suspended bacteria. Moringa flocculants are basic polypeptides and one seed is required for 1-liter bottle for slightly contaminated water and two seeds for very dirty water [17, 41].

1.7. Other Uses

Moringa species have bio-enhancing properties. Bio-enhancers are molecules which do not possess drug activity of their own but augment the biological activity and/or bioavailability of drugs. Niaziridin has been reported to enhance the bioactivity of rifampicin, tetracycline and ampicillin. It also facilitates the absorption of drugs, vitamins and nutrients through the gastro-intestinal membrane thus increasing their bioavailability [17]. Food protection is another potential role for Moringa seeds by inhibiting food-spoiling organisms such as *E.coli*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *S.typhimurium* and *E. aerogenes*, [26]. Moringa leaf extracts when sprayed on crops accelerate growth and increase crop yields by up to 35% [18].

The oil obtained by pressing the seed kernel of Moringa is used as a lubricant for watches and fine machinery such as timepieces, because it has a minimal tendency to deteriorate and become rancid and sticky. Moringa oil is also used in the perfume industry for stabilizing scents due to its capacity to absorb and retain volatile substances [17].

2. Objectives of this Study

It is clear that this plant species has diverse uses in the southern part of Ethiopia and various researchers put their eye on this plant material. Nevertheless, less research work on phytochemical investigation was performed on this plant species in comparison to the closely related and highly rated *Moringa* species known as *M. oleifera*. Hence, the main aim of this research is to contribute further to the study of the chemistry of the leaves of *M. stenopetala*.

3. Results and Discussion

3.1. Extractions of *M. stenopetala* Leaves

Ground dried *M. stenopetala* leaves were extracted with $\text{CHCl}_3/\text{MeOH}$ (1:1) to afford a greenish material. Likewise, the marc remained was extracted with MeOH to give yellowish solid (4%). The detailed extraction procedure was given under the experimental section.

3.2. TLC Profile of Crude Extract

The $\text{CHCl}_3/\text{MeOH}$ extracts of *M. stenopetala* leaves were analyzed on analytical TLC, the spots were visualized after spraying with vanillin in sulfuric acid and heating with hot air gun. The TLC of the crude extract (**Figure 4**) was developed using EtOAc/Hexane (3:2) as a mobile phase.

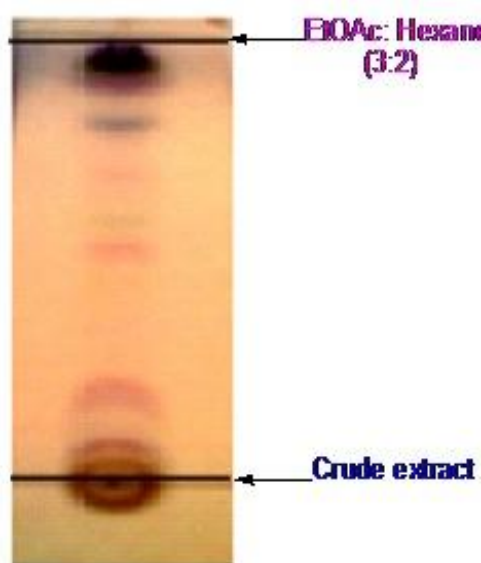


Figure 4: Analytical Thin Layer Chromatogram for $\text{CHCl}_3/\text{MeOH}$ Extracts.

Similarly, the MeOH extracts of *M. stenopetala* leaves marc were analyzed and the spots were visualized after spraying with vanillin in sulfuric acid. The TLC of the extract (**Figure 5**) was developed using EtOAc/MeOH (3:2) as a mobile phase.

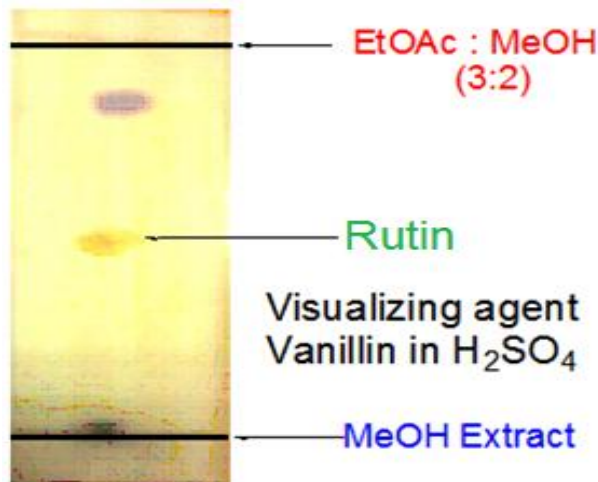


Figure 5: Analytical Thin Layer Chromatogram for MeOH Extracts *M. Stenopetala* Marc.

The MeOH extracts of *M. stenopetala* leave marc after washing with acetone were also analyzed on analytical TLC. The spots were visualized by spraying with vanillin in sulfuric acid and heating with hot air gun. The TLC of the extract (**Figure 6**) was developed using EtOAc/MeOH (4:1) as a mobile phase.

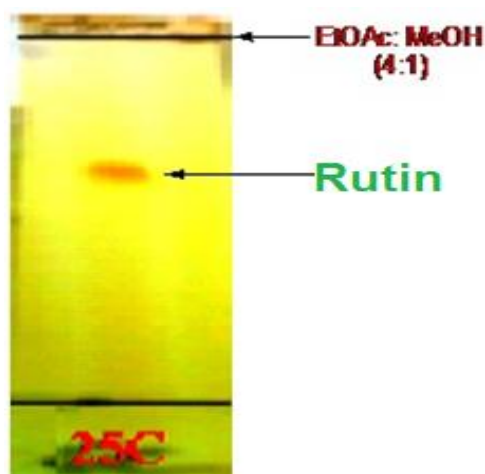


Figure 6: Analytical Thin Layer Chromatogram for Acetone Washed MeOH Extracts *M. Stenopetala* Marc.

3.3. Isolation of Compounds from *M. stenopetala* Leaves

The CHCl₃/MeOH (1:1) extract of *M. stenopetala* leaves were adsorbed on silica gel and applied on top of column chromatography packed with silica gel to furnish two compounds coded as **Ms1** and **Ms2**. Furthermore the MeOH extract of the marc of *M. stenopetala* leaves afforded a solid material which upon washing with acetone gave a pure compound rutin.

3.4. Characterization of Compounds from *M. stenopetala* Leaves

Ms1 is a white solid powder, insoluble in water but soluble in chloroform. It melts within the temperature range of 82-83°C, which is in close agreement with the literature value of 81-82°C [42]. The substance is optically inactive. From the UV-visible spectrum (**Appendix-1**), the substance does not show any absorption maxima neither in the UV region nor in the visible region. This indicates that, the substance does not belong to any of the class of flavonoids, aromatic and conjugated unsaturated hydrocarbons. However, the substance may belong to the class of saturated hydrocarbons with an auxochrome moiety that absorbs in the vacuum ultraviolet region.

Its IR spectrum (**Appendix-2**) exhibited broad absorption band at 3306 cm⁻¹, which is the characteristic band of hydroxyl group. The two strong absorption bands at 2918 and 2851 cm⁻¹ were features of the asymmetrical and symmetrical C-H stretching of the methyl group and methylene group respectively. The medium band at 1463 cm⁻¹ is most likely due to the C-H scissoring of methylene group in the structure. The two weak bands at 1267 and 1063 cm⁻¹ are due to the C-H twisting and wagging vibration of methylene group and the weak band at 720 cm⁻¹ is due to the C-H rocking vibration of the methylene group.

The ¹H NMR spectrum of **Ms1** (**Appendix-3, Table 6**) exhibited a triplet at δ 3.66 ppm (J = 6.6 Hz) assigned to two protons of methylene group attached to a heteroatom most probably oxygen. The multiplicity of this proton revealed the presence of methylene group next to it. The multiplet at δ 1.59 ppm integrated for two protons is ascribed for another methylene group flanked between two methylene groups. A broad singlet at 1.27 ppm is a characteristic signal for many overlapping methylene protons in the

substance and assigned for forty-eight protons. An up field triplet signal at 0.90 ppm ($J = 6.7$ Hz) indicates a more shielded aliphatic proton and integrated for three protons which is most likely for the proton signal of terminal methyl group in the substance. The ^{13}C NMR (**Appendix-4, Table 6**) spectrum with the aid of DEPT-135 showed seven signals at δ 63.1, 32.8, 31.9, 29.7-29.7, 25.7, 22.7 and 14.1ppm. The down field signal at δ 63.1 ppm corresponds for an oxygenated aliphatic carbon and the strong signal in the range δ 29.7-29.4 ppm is assigned for the many overlapping carbons. The DEPT-135 NMR spectrum of **Ms1** revealed the presence of six methylene and one methyl carbons (**Appendix-5, Table 6**). The carbon resonance at δ 14.1 ppm is characteristic signal for a terminal carbon of methyl group.

Therefore, based on the above spectroscopic analysis and physical properties along with literature value, the structure of compound **Ms1** is most likely shown in **Figure 7** below. The name of the compound was found to be 1-Heptacosanol (**65**).

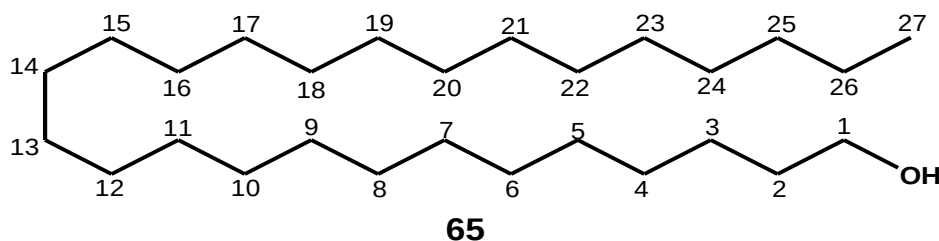


Figure 7: Structure of 1-Heptacosanol.

Table 6 : The NMR Spectral Data for **Ms1**

Observed NMR spectral data			Literature value [42]	
$^1\text{H-NMR}$ (δ in ppm)	$^{13}\text{C-NMR}$ (δ in ppm)	DEPT-135 (δ in ppm)	$^1\text{H-NMR}$ (δ in ppm)	$^{13}\text{C-NMR}$ (δ in ppm)
3.66 (2H, t, $J = 6.6$ Hz, H-1)	63.1 (C-1)	63.1 (CH_2)	3.64 (2H, t, $J = 6.3$ Hz, H-1)	63.12 (C-1)
1.59 (2H, m, H-2)	32.8 (C-2)	32.8 (CH_2)	1.57 (2H, m, H-2)	32.82 (C-2)
1.27(48H, br s, H-3-26)	31.9 (C-3)	31.9 (CH_2)	1.25(45H, br s, H-3-26)	31.92 (C-3)
	29.7-29.4 (C-4-24)	29.7-29.7 (CH_2)		29.71-29.36 (C-4-24)
	25.7 (C-25)	25.7 (CH_2)		25.74 (C-25)
	22.7 (C-26)	22.7 (CH_2)		22.69 (C-26)
0.90(3H, t, $J = 6.7$ Hz, H-27)	14.1 (C-27)	14.1 (CH_3)	0.88 (3H, t, $J = 6.6$ Hz, H-27)	14.11(C-27)

s= singlet, br s= broad singlet, t=triplet, m= multiplet

Ms2 is white solid crystalline substance soluble in CHCl_3 but insoluble in water. The UV-visible spectrum (**Appendix-7**) of **Ms2** displayed no absorption maxima in the UV-Visible region. Hence, the substance is most probably categorized to the class of saturated hydrocarbons.

It is clear that secondary amines show a single weak band near 3350 cm^{-1} and these bands are shifted to longer wavelength by hydrogen bonding. The associated N-H bands are weaker and frequently sharper than the corresponding O-H bands [43]. Thus, the IR spectrum (**Appendix-8**) of **Ms2** exhibited a broad and weaker absorption band at 3428 cm^{-1} , which is the characteristic band of secondary amines. The two strong absorption bands appeared at 2918 and 2848 cm^{-1} were the features of the asymmetrical and symmetrical C-H stretching of the methyl group and methylene group respectively. The medium band at 1473 cm^{-1} and 1463 cm^{-1} is mostly due to various N-H and C-H modes of vibration in the structure respectively. The weak bands at 1065 cm^{-1} are due to the C-H wagging vibration of methylene group and the weak band at 720 cm^{-1} is due to the C-H rocking vibration of the methylene group.

The ^1H NMR spectrum (**Appendix-9, Table 7**) of **Ms2** shows a triplet at $\delta\ 3.66\text{ ppm}$ ($J = 6.6\text{ Hz}$) integrated for two protons of methylene group attached to a heteroatom most likely oxygen and methylene on the other side. The triplet at $\delta\ 2.40\text{ ppm}$ ($J = 7.4\text{ Hz}$) integrated for four protons is ascribed for another methylene group attached to an electronegative atom which is most likely nitrogen. The multiplet at $\delta\ 1.58\text{ ppm}$ ($J = 6.7\text{ Hz}$) integrated for two protons is assigned for another methylene group aligned between two methylene groups, one which is bearing oxygen. A broad singlet at $\delta\ 1.33\text{--}1.27\text{ ppm}$ is a characteristic signal for many overlapping methylene protons in the compound and integrated for forty-four protons. An up field triplet signal at $\delta\ 0.90\text{ ppm}$ ($J = 6.7\text{ Hz}$) indicates a more shielded aliphatic proton and integrated for three protons which is most likely a terminal methyl group in the compound.

The ^{13}C NMR (**Appendix-10, Table 7**) shows thirteen major signals at $\delta\ 63.1, 42.8, 32.8, 31.9, 29.7\text{--}29.3, 25.7, 23.9, 22.7$ and 14.1 ppm . The down field signal at $\delta\ 63.1\text{ ppm}$ corresponds to terminal oxygenated aliphatic carbon and the weak signal at $\delta\ 42.8\text{ ppm}$ indicates the presence of methylene carbon directly attached to a heteroatom,

which is most likely a nitrogen atom appearing in the center of the skeleton. The strong signal in the range δ 29.7-29.3 ppm were designated for many overlapping carbons. The emerged weak signal at δ 23.9 ppm was principally assigned for the methylene carbon of the far non-polar end of the skeleton. This shift is most likely due to the effect of the central heteroatom in the substance. Finally, an up field signal at δ 14.1 ppm is ascribed for the terminal methyl group of the compound.

Correspondingly, there are twelve negative and one positive major signals appeared in the DEPT-135 NMR spectrum (**Appendix-11, Table 7**). The one positive signal at δ 14.1 ppm confirms the presence of methyl carbon in the substance. The series of negative signals in the spectrum indicates the presence of methylene carbon atoms throughout the skeleton. Hence, based on the above spectroscopic analysis and physical properties, the structure of compound **Ms2** was proposed as **Figure 8** below. The name of the compound was found as 14-(tridecylamino) tetradecan-1-ol (**66**).

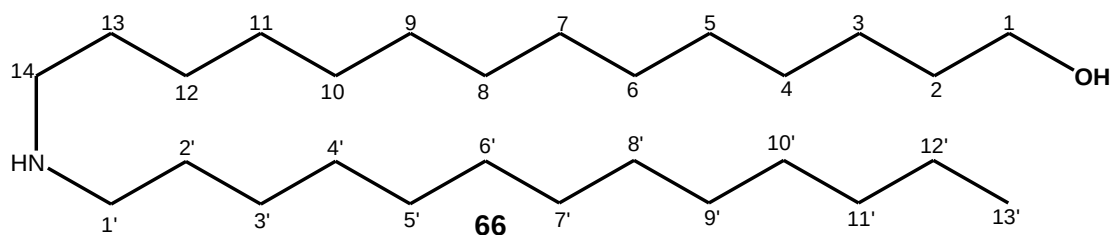


Figure 8: Structure of 14-(tridecylamino) tetradecan-1-ol

Table 7 : The NMR Spectral Data for Ms2

Observed NMR spectra		
$^1\text{H-NMR}$ (δ in ppm)	$^{13}\text{C-NMR}$ (δ in ppm)	DEPT-135 (δ in ppm)
3.66 (2H, t, J=6.6 Hz, H-1)	63.1 (C-1)	63.1 (CH_2)
2.40 (4H, t, J= 7.4 Hz, H-14,H-1')	42.8 (C-14,1')	42.8 (CH_2)
1.58 (2H, m, H-2)	32.8 (C-2)	32.8 (CH_2)
1.33- 1.27 (44H, br.s, H-3-13, H-2'-12')	31.9 (C-3)	31.9 (CH_2)
	29.7-28.6 (C-5-13,C-2'-10')	29.7-28.6 (CH_2)
	25.7 (C-4)	25.7 (CH_2)
	23.9 (C-11')	23.9 (CH_2)
	22.7 (C-12')	22.7 (CH_2)
0.90 (3H, t, J= 6.7 Hz, H-13')	14.1 (C-13')	14.1 (CH_3)

br s= broad singlet, t=triplet, m= multiplet

Rutin is a yellowish solid, whose TLC chromatogram changes to deep yellow up on spraying with vanillin in H_2SO_4 . The compound melts in the temperature range of 168-170°C, which is comparable to the literature value, 173-175 [44]. The compound is optically active with optical rotation $[\alpha]_{\text{D}}^{25} = 3.50$, this indicated that the compound possess chiral carbon. The UV spectrum (**Appendix-16**) of this compound showed two major absorption bands at λ_{Max} . 357 nm and 260 nm which indicate the presence of flavonoid skeleton. The UV (MeOH) absorption maximum at 357 nm is a characteristic band of a flavonols with 3-OH substituted. The absorption maximum at 260 nm is most likely due to π - π^* transition in the benzene system of ring A.

The IR spectrum (**Appendix-17**) shows a broad band at 3426 cm^{-1} which is the unique feature of hydrogen bonded hydroxyl group. The weak band at 2935 cm^{-1} is due to the C-H stretching and the medium band at 1653 cm^{-1} is due to the carbonyl (C=O) stretching. The medium band at 1509 cm^{-1} is the characteristic band of the C=C stretch of the aromatic structure and the series of bands at 1365, 1297, 1205, 1063 and 1015 cm^{-1} is due to the various C-O modes of vibration. The medium band at 808 cm^{-1} is a finest indicative of a substituted aromatic moiety in the structure of the substance.

From the ^1H NMR spectrum, a sharp singlet at 12.60 ppm integrating for one hydrogen indicates the signal of a hydrogen bonded proton of 5-OH group. The ^1H NMR spectrum also showed an overlapping doublet at 7.54 ppm ($J = 7.8\text{ Hz}$), these were apparently assigned for two hydrogen, H-2' and H-6'. The doublet at 6.84 ppm ($J = 8.3\text{ Hz}$) corresponds to the one proton, H-5' while the two doublets at 6.40 ppm ($J = 2.1\text{ Hz}$) and 6.20 ppm ($J = 2.1\text{ Hz}$) were assigned for the meta related H-8 (1H) and H-6 (1H) protons respectively. The two doublet at 5.35 ppm ($J = 7.8\text{ Hz}$) and 4.38 ppm were assigned for the protons of the glucose, H-1'' and rhamnose H-1''' moiety respectively. The appearance of the broad singlet at around 3.47 ppm indicates the presence of a sugar/ rhamnose moiety and assigned for seven protons. The two doublet at 3.70 ppm ($J = 10.8\text{ Hz}$) and 3.05 ppm assigned for the two diastereotopic hydrogen, Hb-6'' and Ha-6'' of the glucose moiety respectively. The doublet of doublet signal at 3.22 ppm was assigned for the one proton of the glucose moiety, H-5''. The signal at 0.97 ppm (d, 3H,

$J = 6.1$ Hz) indicates the presence of shielded methyl protons which is ascribed to H-6'''. This is most likely due to the protons on methyl group of rhamnose.

The molecular ion peak appeared at m/z 611.1 $[M+H]^+$ in the mass spectrum, indicating that the molecular weight of the compound is 610. Furthermore, the base peak appeared at m/z 300.4 in the 30 ev negative ion CID mass spectrum, corresponds to the aglycon, quercetin after the loss of two protons. The medium peak emerged at m/z 299.5 was due to quercetin moiety after departure of one proton. The various peaks at m/z 270.6, 254.5, 179.2 and 150.9 were the characteristic fragment ions of the quercetin moiety. The 15 ev positive ion CID mass spectrum, further confirms the presence of sugar moiety in the main skeleton of the compound. The medium peak at m/z 465 was a good confirmation for the loss of the rhamnosyl moiety. The various peaks at m/z 449, 303, 302 and 146 were the characteristic fragment ions of the sugar moiety.

The physical properties (melting point and solubility), spectral data (UV, IR, 1D NMR and EIMS) generated and comparison made with literature value are consistent with the structure of rutin (**13**), **Figure 9** [26, 44].

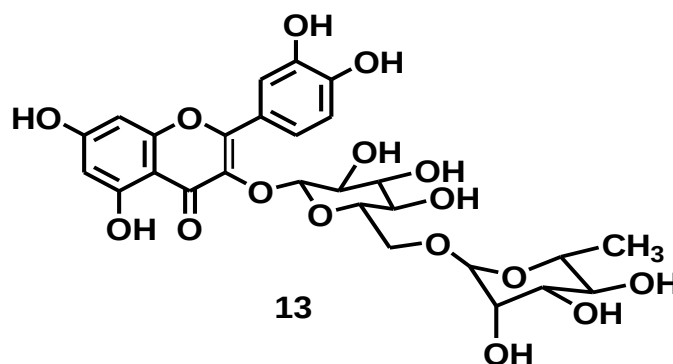


Figure 9: Structure of Rutin.

4. Experimental

4.1. Plant Material

The dried leaves of *M. stenopetala* were obtained from trees growing in Arbaminch Town and collected by prof. Ermias Dagne in Jan 2013, Addis Ababa University, College of Natural Science, Aratkilo, Addis Ababa, Ethiopia.

4.2. Instruments

Melting point was determined in capillary tube with a digital electrothermal melting point apparatus. Analytical TLC was run on a 0.25 mm thick layer of silica gel GF254 (Merck) on aluminum plate. Spots were detected by observation under UV light (254 nm) and spraying with Vanillin in H₂SO₄ and by heating with hot air gun. Column chromatography was performed using silica gel (230-400 mesh) Merck. Samples were applied on the top of the column by adsorbing on silica gel. Solvent was freed using rotavapor BUCHI, RE 121.

¹H and ¹³C NMR spectra in CDCl₃/DMSO-d₆ were recorded using the solvent peak as reference. ¹H, and ¹³C NMR were obtained on Bruker Avance instrument NMR machine (Bruker Avance 400 NMR spectrometer), with TMS and solvent as internal standards and δ values are given in ppm relative to TMS internal standard.

UV-Vis spectra were recorded using T60 UV-Visible spectrophotometer (PG instrument, England). Optical rotations were measured using Autopol[®]IV Automatic polarimeter. The IR spectra were also recorded using a Perkin-Elmer BX Spectrometer (400-4000 cm⁻¹) in KBr.

4.3. Extraction

To the dried and powdered *M. stenopetala* leaves (20 g), was added CHCl₃/MeOH (1:1, 100 mL) and placed on steam bath (30 min), shaker (4 h). The mixture was filtered, concentrated under reduced pressure to give a greenish viscous residue (2 g, 10%). The greenish extract was adsorbed on silica gel (230-400 mesh) and chromatographed over silica gel column packed in petrol. The column was eluted successively with

dichloromethane (100%), mixture of dichloromethane and ethyl acetate (9:1, 4:1, 1:1), ethyl acetate (100%), mixture of ethyl acetate and methanol (9:1, 4:1, 1:1) and finally with methanol (100%). Various fractions were collected and combined based on their TLC profiles. Out of 32 major fractions(**Appendix-18**), fraction 7 was recrystallized from ethyl acetate to give **Ms1** (5 mg). Likewise fraction 10 & 11 were found to be a pure compound labeled as **Ms2** (3 mg).

After the preliminary extraction of *M. stenopetala* leaves using MeOH/CHCl₃, further extraction on the marc (15 g) using MeOH (100 mL) was carried out by placing on shaker for 6 h. After filtering the mixture and evaporating the solvent under reduced pressure, a black solid residue (0.6 g) was obtained. This was washed with acetone (3x5 mL) to afford a yellowish solid insoluble in acetone (200 mg) identified as rutin.

Ms1: white solid; soluble in CHCl₃; insoluble in water; mp 82-83°C; optically inactive; UV (CHCl₃ in MeOH, **Appendix-1**) : no observable band; IR (KBr, **Appendix-2**) $\nu_{\max}$: 3306, 2918, 2851, 1737, 1463, 1267, 1063, and 720 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, **Appendix-3**), chemical shift δ in ppm, coupling constant J in Hz: δ_{H} 3.66 (2H, t, J = 6.6 Hz, H-1), 1.59 (2H, m, H-2), 1.27 (48H, br s, H-3-26), 0.90 (3H, t, J = 6.7 Hz, H-27); ¹³C NMR (101 MHz, CDCl₃, **Appendix-4**): δ_{C} 63.1 (C-1), 32.8 (C-2), 31.9 (C-3), 29.7-29.4 (C-4-24), 25.7 (C-25), 22.7 (C-26), 14.1 (C-27); DEPT-135 NMR (101 MHz, CDCl₃, **Appendix-5**): δ 63.1 (CH₂), 32.8 (CH₂), 31.9 (CH₂), 29.7-29.7 (CH₂), 25.7 (CH₂), 22.7 (CH₂), 14.1 (CH₃).

Ms2: white solid; soluble in CHCl₃, insoluble in water; optically inactive; UV (CHCl₃ in MeOH, **Appendix-7**) : no observable band, IR (KBr, **Appendix-8**) $\nu_{\max}$: 3428, 2918, 2848, 1473, 1463, 1065, and 720 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, **Appendix-9**), chemical shift δ in ppm, coupling constant J in Hz: δ 3.66 (2H, t, J = 6.6 Hz, H-1), 2.40 (4H, t, J = 7.4 Hz, H-14, H-1'), 1.58 (2H, m, H-2), 1.33- 1.27 (44H, br.s, H-3-13, H-2'-12'), 0.90 (3H, t, J = 6.7 Hz, H-13'); ¹³C NMR (400 MHz, CDCl₃, **Appendix-10**) δ 63.1, 42.8, 32.8, 31.9, 29.7-28.6, 25.7, 23.9, 22.7, 14.1; DEPT-135 NMR (400 MHz, CDCl₃, **Appendix-11**) δ 63.1, 42.8, 32.8, 31.9, 29.7-28.6, 25.7, 23.9, 22.7, 14.1.

Rutin: yellow solid; soluble in water; R_f 0.64; mp 168-170°C; $[\alpha]_{D}^{21} 3.50$; UV (MeOH, **Appendix-16**) λ_{max} : 357, 260 nm; IR (KBr, **Appendix-17**) ν_{max} : 3426, 2935, 1653, 1509, 1365, 1297, 1205, 1063, 1015, 808, and 598 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6), chemical shift δ in ppm, coupling constant J in Hz: δ 7.54 (2H, d , $J=7.8$ Hz, H-2', H-6'), 6.84 (1H, d , $J = 8.3$ Hz, H-5'), 6.40 (1H, d , $J = 2.1$ Hz, H-8), 6.2 (1H, d , $J=2.1$ Hz, H-6), 5.35 (1H, d , $J=7.8$ Hz, H-1''), 4.38 (1H, $br.s$, H-1'''), 3.22 (1H, $br.s$, H-5''), 3.47 (7H, $br.s$, sugar), 3.70 (1H, d , $J = 10.8$ Hz, Hb-6''), 3.05 (1H, m , Ha-6''), 0.97 (3H, d , $J = 6.1$ Hz, CH_3 -6''); EIMS m/z 611 $[\text{M}+\text{H}]^+$, 609 $[\text{M}-\text{H}]^-$, 343, 301, 300, 299, 270, 254, 179 and 150.

5. Conclusions and Recommendations

The present investigation on the dried leaves of *Moringa stenopetala* led to the isolation of two compounds coded as **Ms1** and **Ms2**. These compounds were not yet reported from the genus *Moringa*. Furthermore, one known compound, rutin, was also isolated in the course of this work. These compounds were partially characterized using different spectroscopic analysis and physical properties. Therefore, the first two compounds were tentatively identified as 1-Heptacosanol and 14-(tridecylamino)tetradecan-1-ol respectively.

Further data from soft mass spectrometric method is required to fully confirm the proposed structures of these long chain alcohols.

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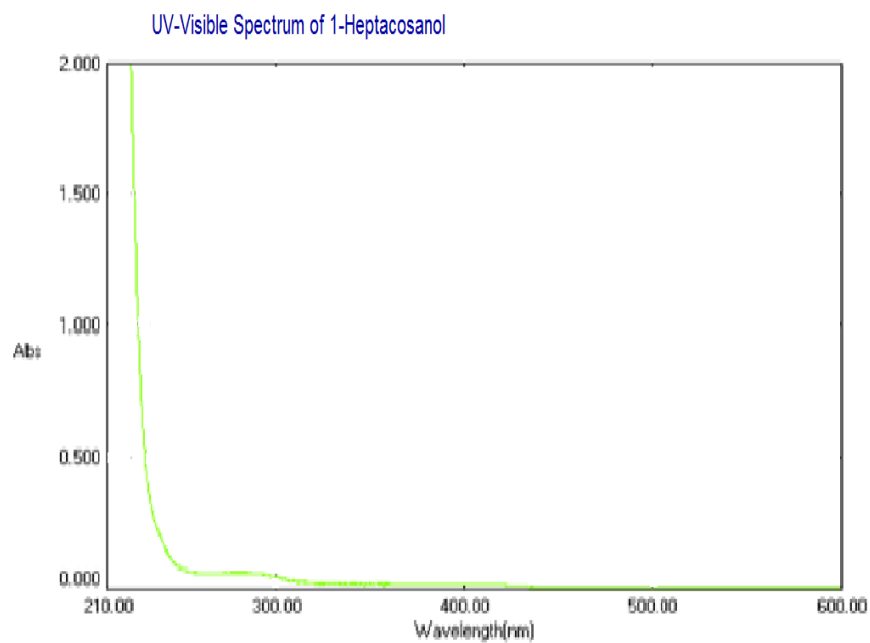
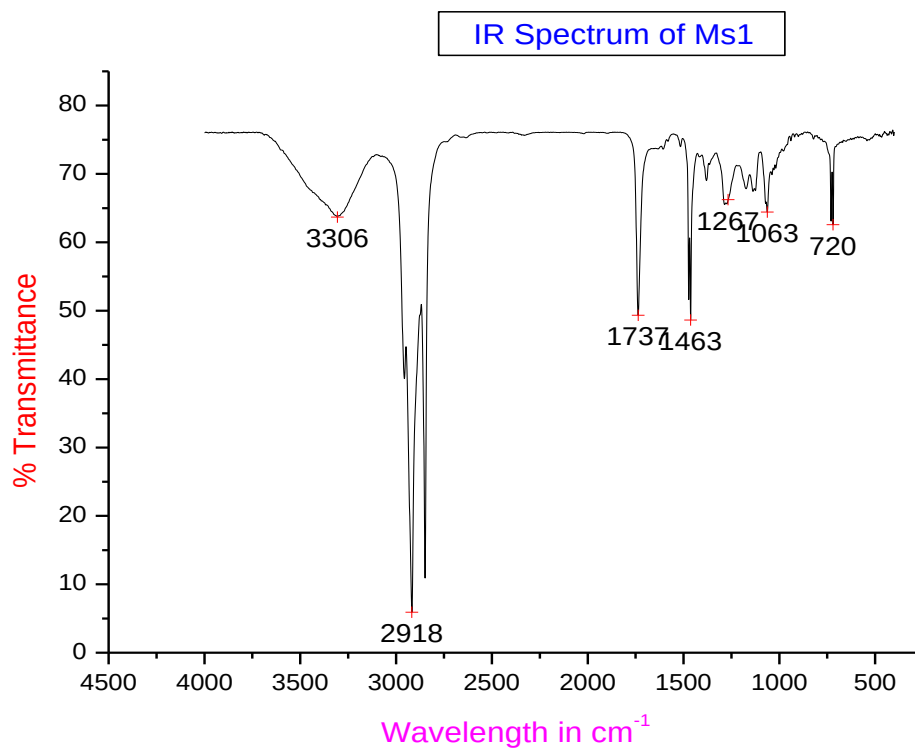
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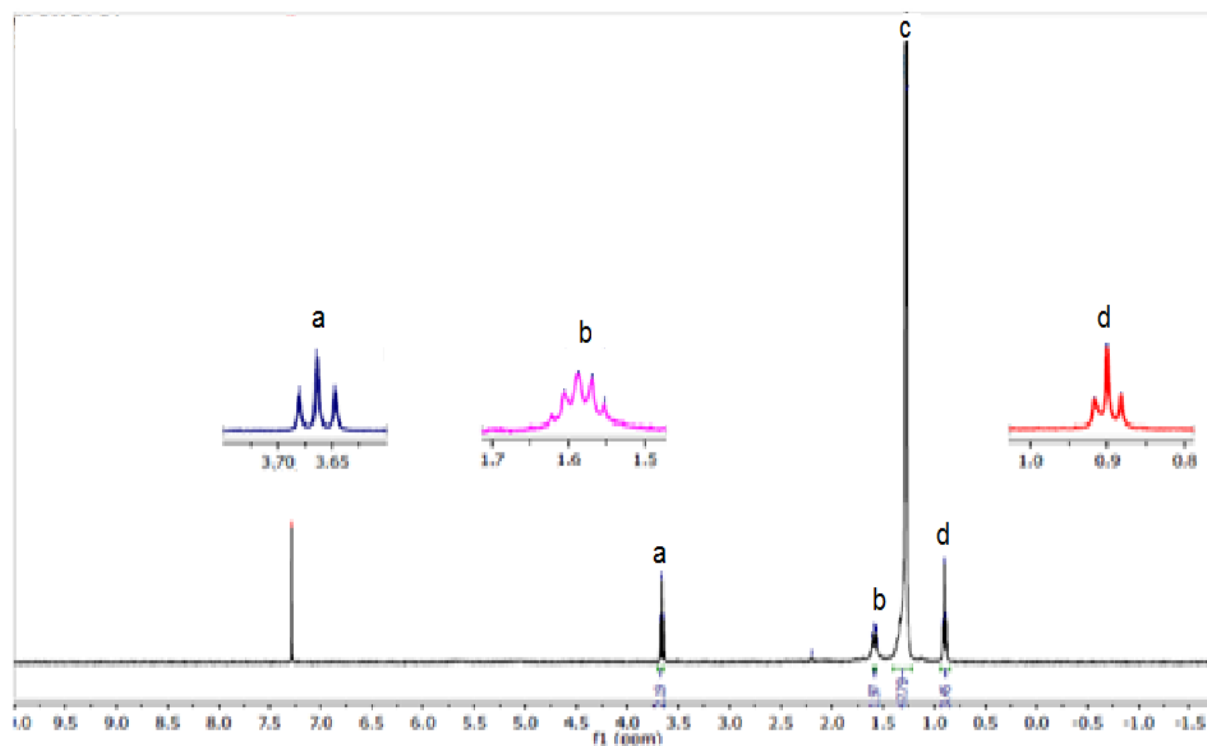
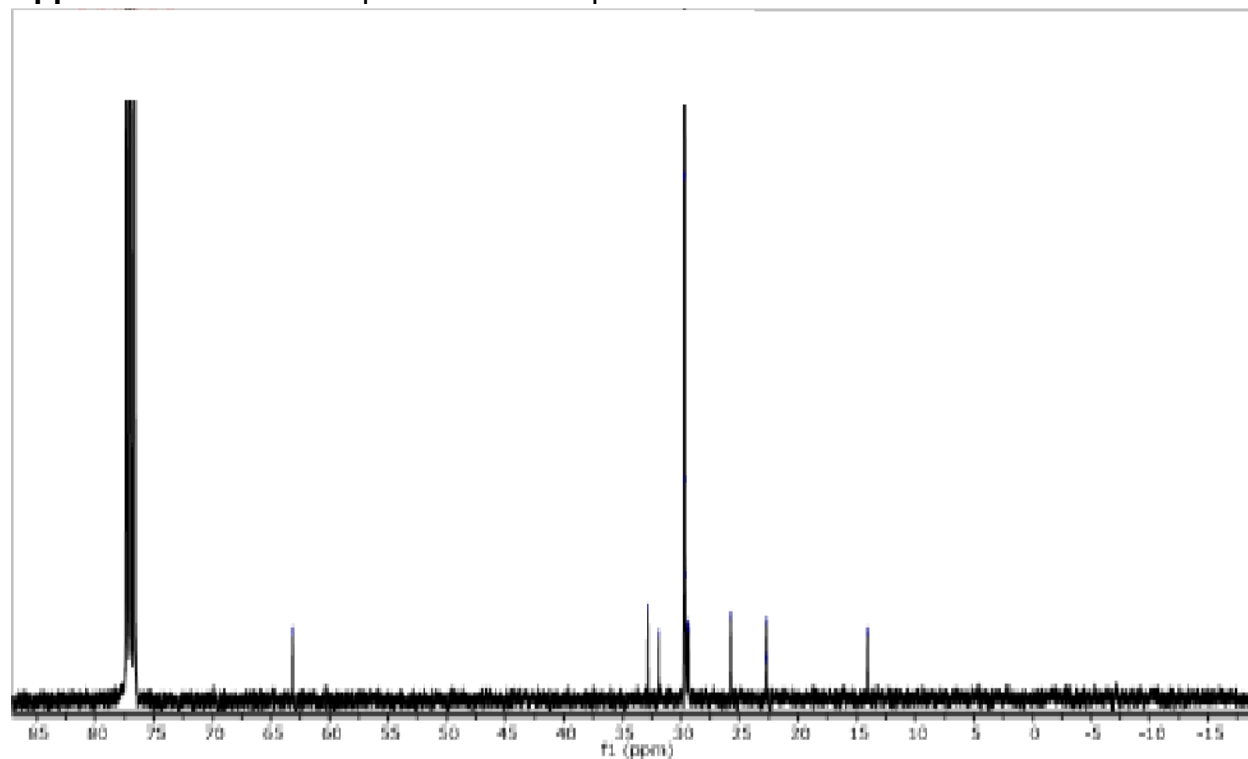
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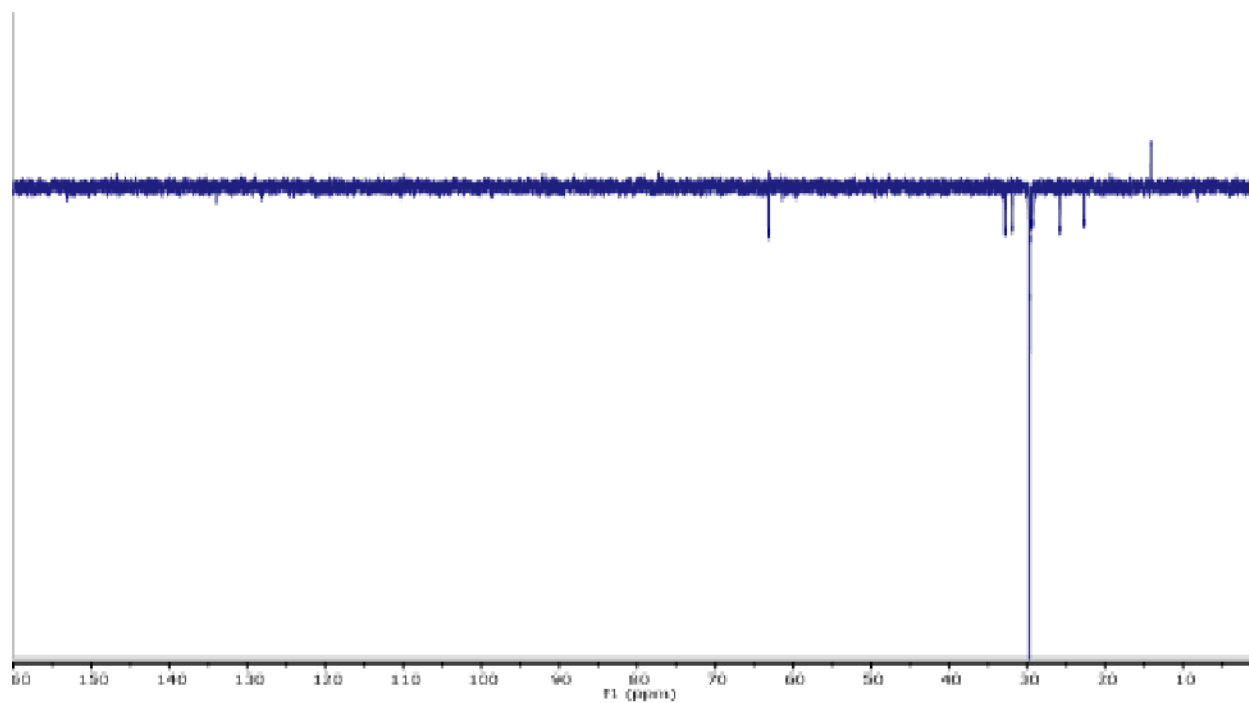
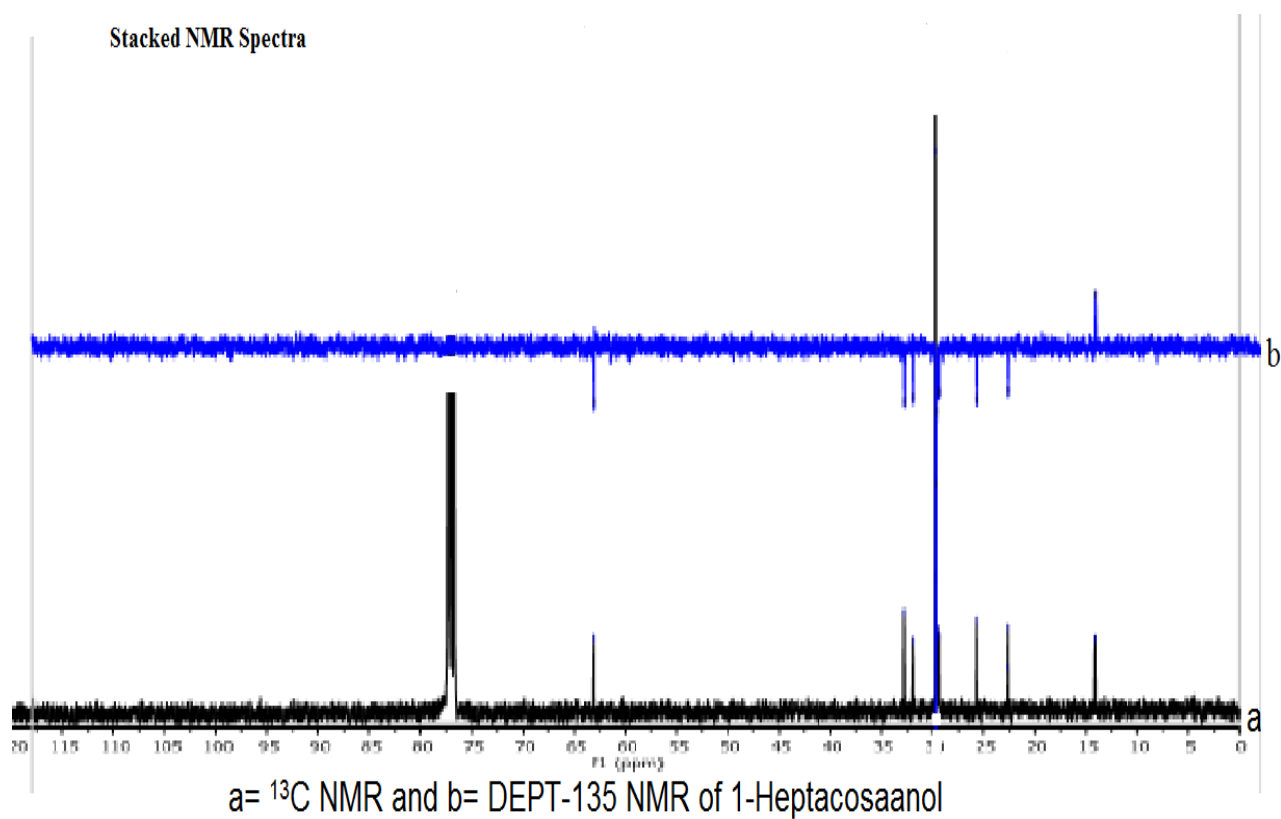
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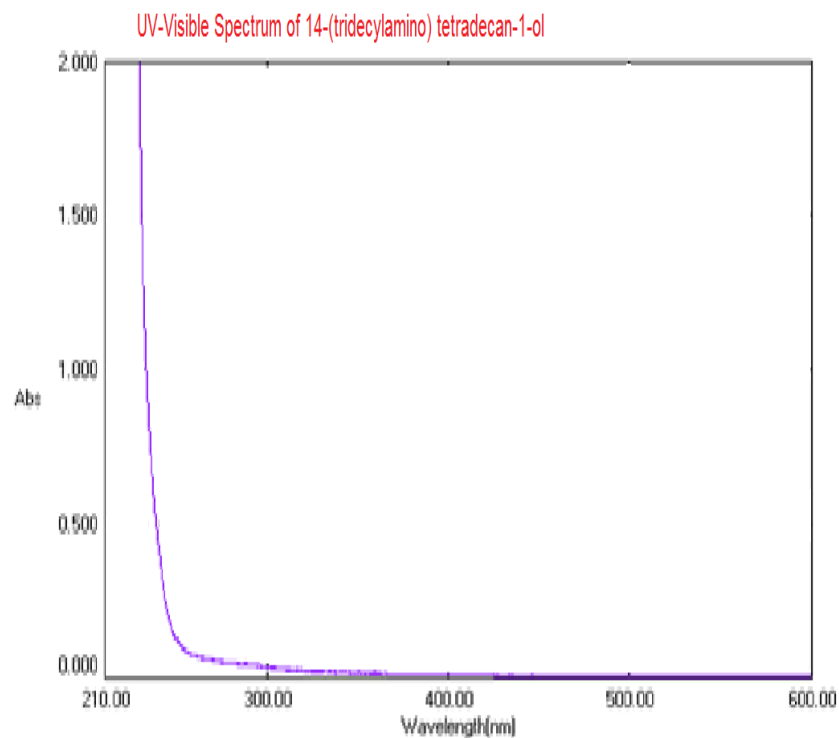
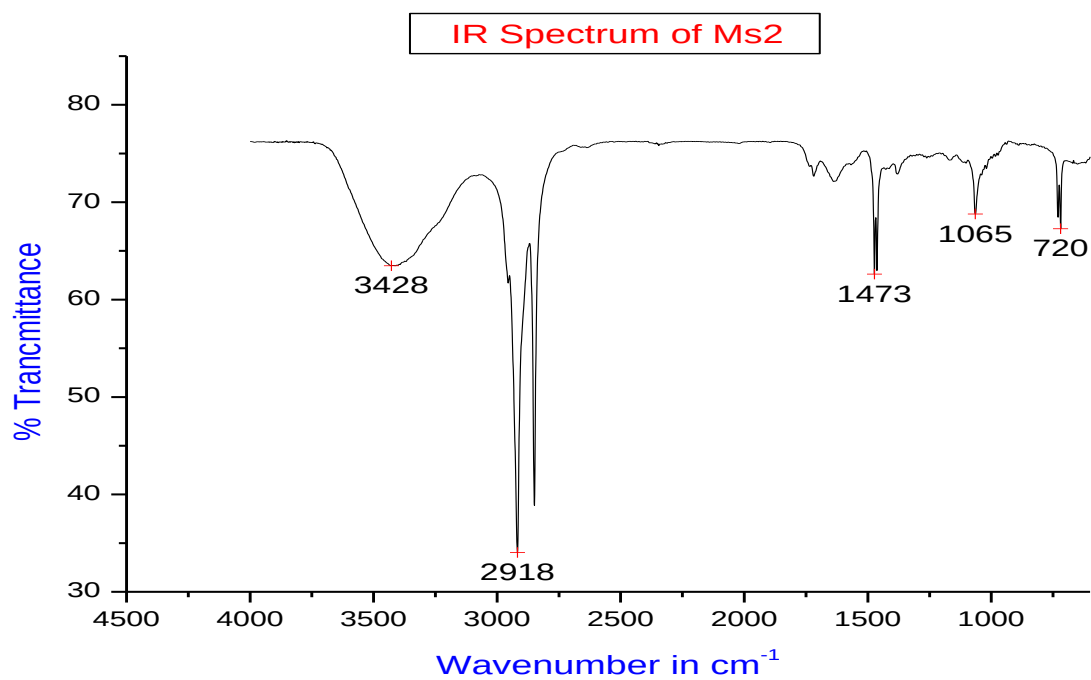
*Ref Man ID refers to ALNAP Literature Database, which is a collection of references in print and in electronic version.

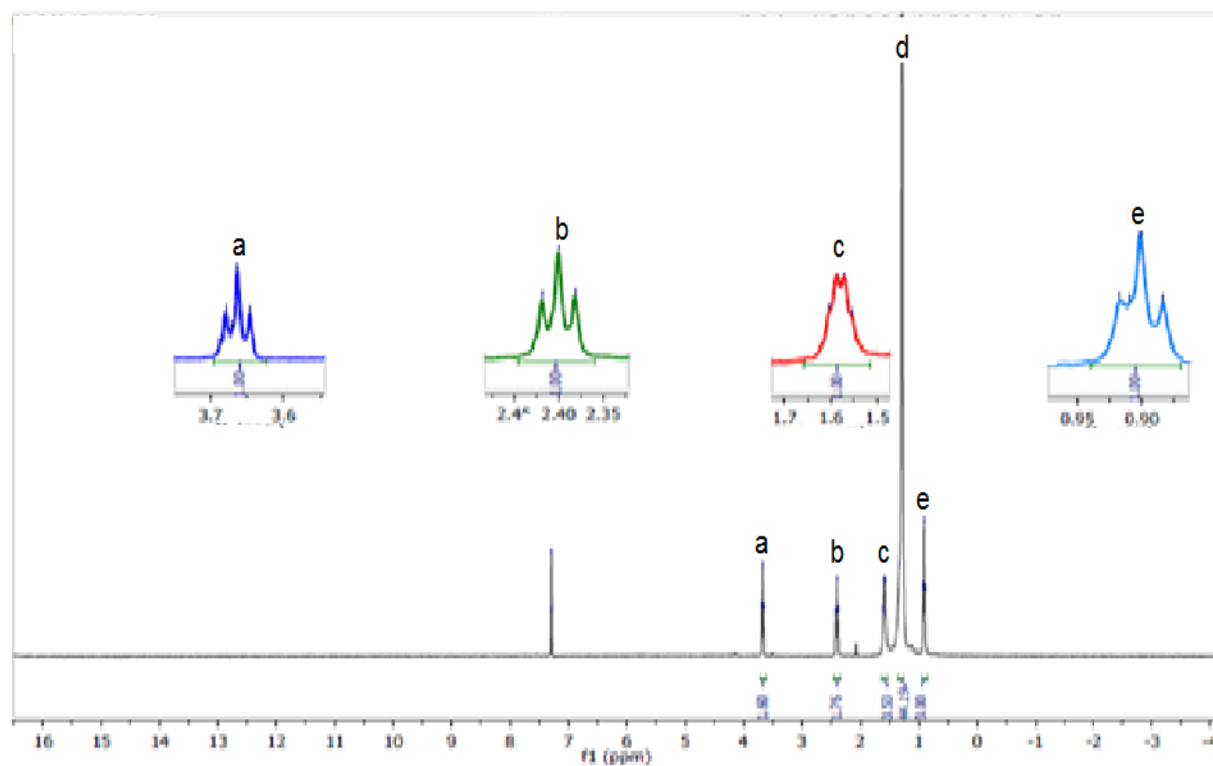
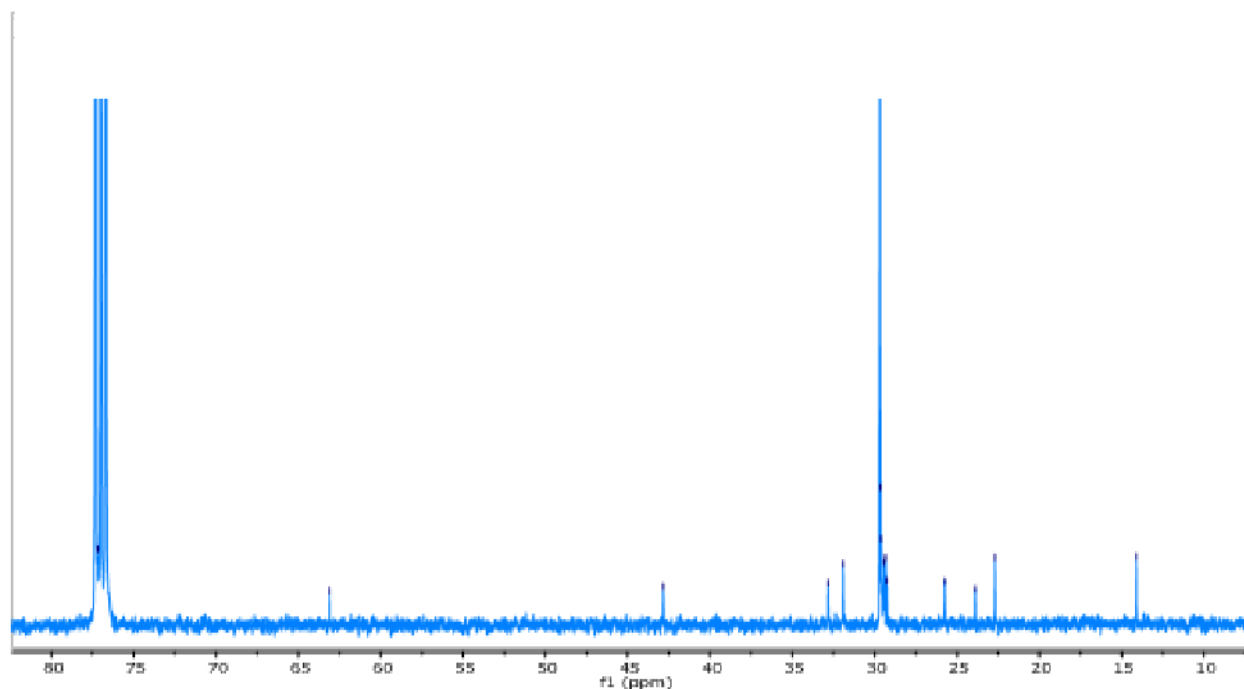
APPENDICES

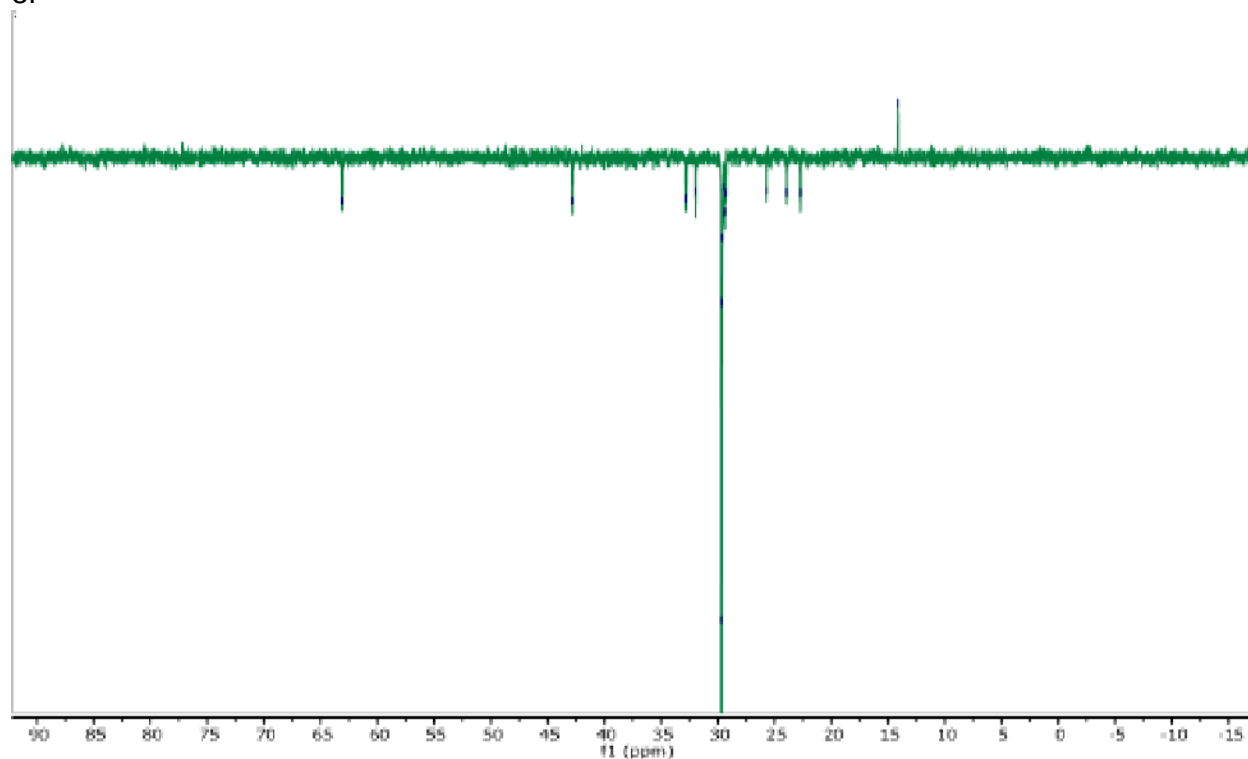
Appendix-1: UV-Visible Spectroscopy of 1-Heptacosanol**Appendix-2: IR Spectrum of 1- Heptacosanol**

Appendix-3: ^1H NMR Spectrum of 1-Heptacosanol**Appendix-4:** ^{13}C NMR Spectrum of 1-Heptacosanol

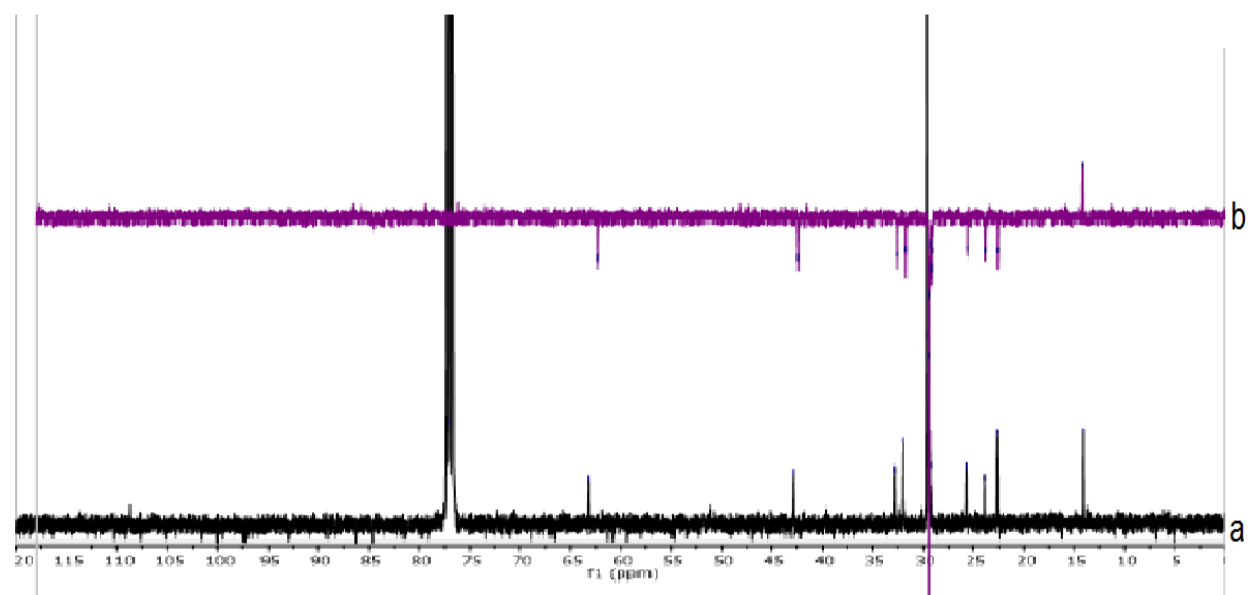
Appendix-5: DEPT-135 NMR Spectrum of 1-Heptacosanol**Appendix-6: Stacked NMR Spectrum of 1-Heptacosanol**

Appendix-7: UV-Visible Spectroscopy of 14-(tridecylamino) tetradecan-1-ol**Appendix-8: IR Spectrum of 14-(tridecylamino) tetradecan-1-ol**

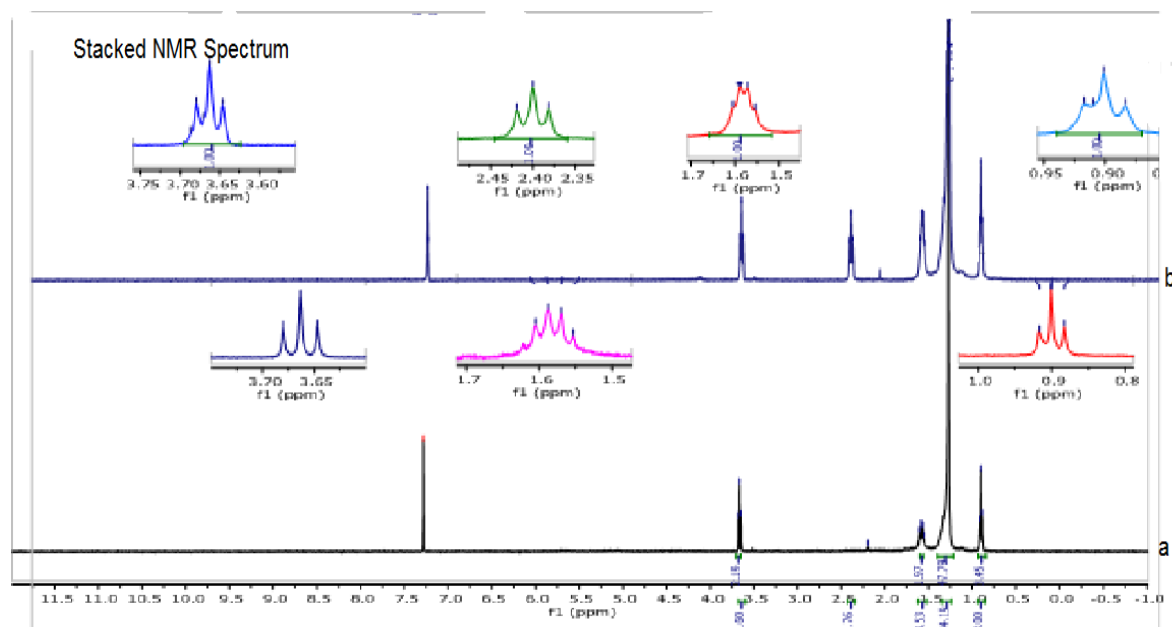
Appendix-9: ^1H NMR Spectrum of 14-(tridecylamino) tetradecan-1-ol**Appendix-10:** ^{13}C NMR Spectrum of 14-(tridecylamino) tetradecan-1-ol

Appendix-11: DEPT-135 NMR Spectrum of 14-(tridecylamino) tetradecan-1-ol**Appendix-12:** Stacked NMR Spectrum of 14-(tridecylamino) tetradecan-1-ol

Stacked NMR Spectrum

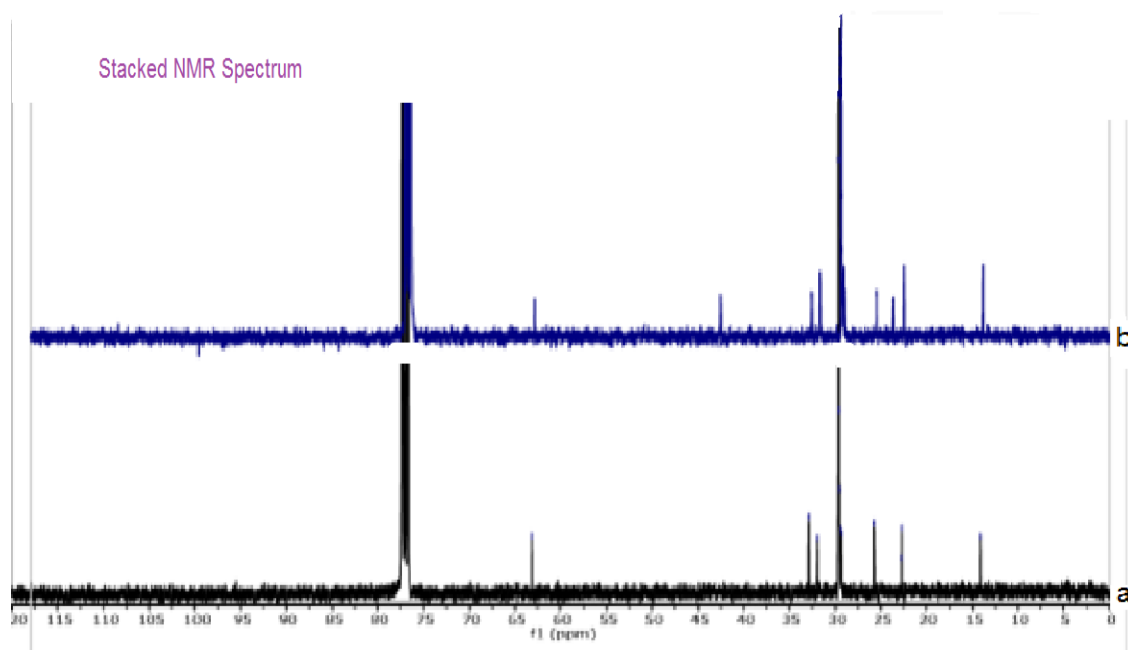
 $a = ^{13}\text{C}$ NMR and $b = \text{DEPT-135 NMR}$ of 14-(tridecylamino) tetradecan-1-ol

Appendix-13: Stacked ^1H NMR of 1-Heptacosanol (a) and 14-(tridecylamino) tetradecan-1-ol (b)



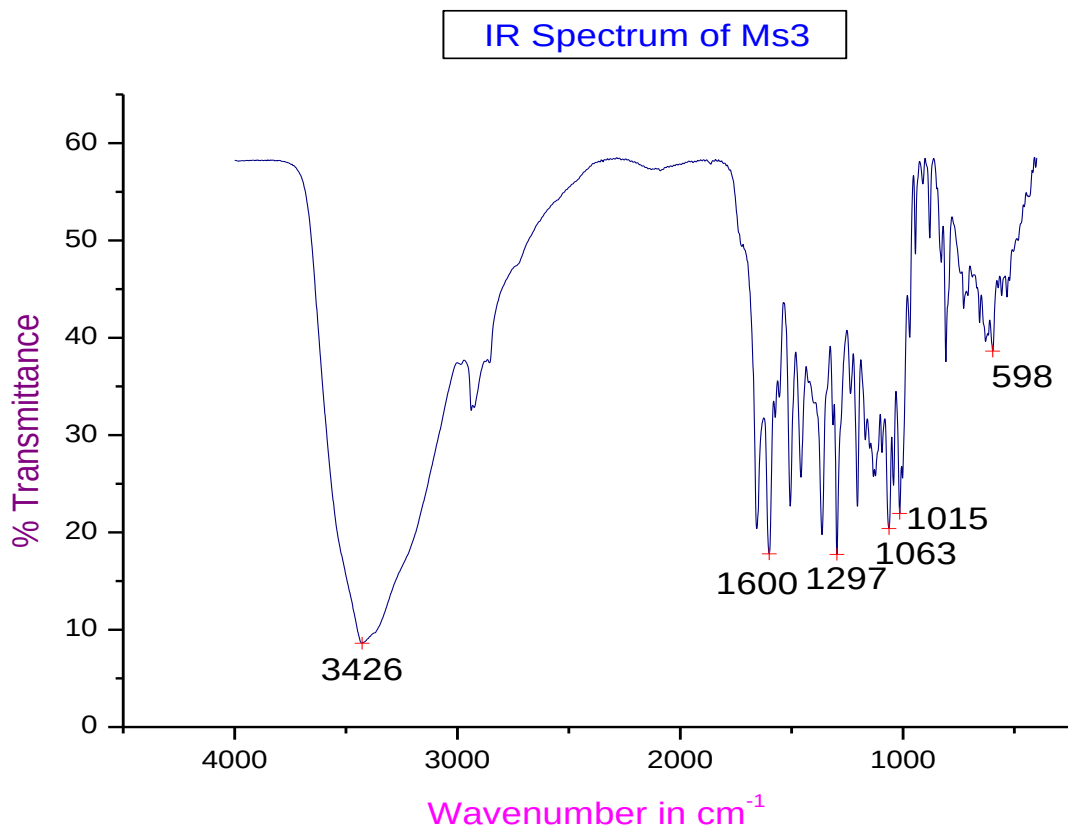
a= ^1H NMR of 1-Heptacosanol and b= ^1H NMR of 14-(tridecylamino)tetradecan-1-ol

Appendix-14: Stacked ^{13}C NMR of 1-Heptacosanol (a) and 14-(tridecylamino) tetradecan-1-ol (b)



a= ^{13}C NMR of 1-Heptacosanol and b= ^{13}C NMR of 14-(tridecylamino) tetradecan-1-ol

Appendix-17: IR Spectrum of Rutin

Appendix-18: Column Chromatographic Fractionation of *M. stenopetala* Leave Extract.

Fraction No	Eluted with	Volume collected (mL)	Amount obtained (mg)	Fellow Up
99-26fr ₁	CH ₂ Cl ₂	10	2	
99-26fr _{2&3}	"	20	4	
99-26fr _{4&5}	"	"	7	
99-26fr ₆	"	"	12	
99-26fr ₇	"	"	5	Supplied to NMR, spectra in file
99-26fr _{8&9}	"	"	88	
99-26fr _{10&11}	"	30	3	Supplied to NMR, spectra in file
99-26fr ₁₂	"	20	26	
99-26fr ₁₃	"	20	51	
99-26fr ₁₄	CH ₂ Cl ₂ : EtOAc (9:1)	30	27	

99-26fr ₁₅	“ “	“	38	
99-26fr ₁₆	“ (4:1)	“	8	
99-26fr ₁₇	“ “	“	10	
99-26fr ₁₈	“ (1:1)	“	9	
99-26fr ₁₉	EtOAc	“	11	
99-26fr ₂₀	“	“	22	
99-26fr ₂₁	EtOAc : MeOH (9:1)	“	51	
99-26fr ₂₂	“ (4:1)	“	26	
99-26fr ₂₃	“ “	40	19	
99-26fr ₂₄	“ (1:1)	“	27	
99-26fr ₂₅	“ “	“	26	
99-26fr ₂₆	“ “	“	10	Supplied to NMR, spectra in file
99-26fr ₂₇	“ “	“	19	
99-26fr ₂₈	“ “	“	141	
99-26fr ₂₉	“ “	“	144	
99-26fr ₃₀	MeOH	“	20	
99-26fr ₃₁	“	30	60	
99-26fr ₃₂	“	“	30	
99-26fr ₃₃	“	“	71	
99-26fr ₃₄	“	“	53	
99-26fr ₃₅	“	“	32	Supplied to NMR, spectra in file
99-26fr ₃₆	“	“	80	

Appendix-19: Abbreviations

- **IR-** Infrared
- **Ms-** *Moringa stenopetala*
- **NMR-** Nuclear Magnetic Resonance
- **TLC-** Thin Layer Chromatography
- **UV-** Ultraviolet
- **¹H NMR-** Proton Nuclear Magnetic Resonance
- **¹³C NMR-** Carbon-13 Nuclear Magnetic Resonance

Appendix-20: Submitted Materials

- 20.1. References: of the 49 cited references, 49 are submitted in soft copy; of these 32 are entered in the Reference Manager Database.
- 20.2. Soft copies of NMR fid data with description.
- 20.3. Soft copy of power point presentation
- 20.4. Soft copy of Abstracts and Ref ID of all papers downloaded on moringa
- 20.4. Thesis in both hard and soft copy
- 20.5. Isolated compounds submitted; Ms1 (-), Ms2 (-) and Rutin (-).
- 20.6. Box file submitted includes; NMR, UV-Vis and IR spectra, Literature papers on Moringa, potato and banana.
- 20.7. Lab note book № 99.