CHEMICAL INVESTIGATION OF THE LEAVES OF *MORINGA STENOPETALA*

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BY

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Dedicated

to

my wife, Tialu Mullualem

and

my daughters, Hirut and Beteleliam Alemayehu,

for tlieir unfailing affection, patience and positive contributions to my education.

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ABSTRACT

CHEMICAL INVESTIGATION OF THE LEAVES OF *MORINGA STENOPETALA*

BY

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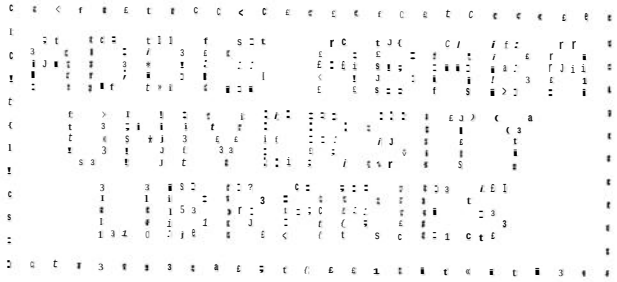
Moringa stenopetala is a fast growing tree that grows abundantly in the southern part of Ethiopia, especially in the Konso area. It is widely used in Ethiopian traditional medicine for the control of diabetes, malaria and hypertension. The seeds are used traditionally as water clarifying agent.

The ethanol extract of the leaves of *M. stenopetala* led to the isolation of three medicinally useful glycosides. The compounds were identified to be rutin, 4-(4'-O-acetyl-a-L-rhamnosyloxy)benzylisothiocyanate and 4-(4'-O-acety!-a-L-rhamnosyloxy)benzaidehyde. 4-(4'-O-acetyl-a-L-rhamnosyloxy)benzylisothiocyanate and 4-(4'-O-acetyl-a-L-rhamnosyloxy)benzaldehyde are reported for the first time from *M. stenopetala.* Structure determination was accomplished by means of spectroscopic methods (UV, IR, ^{1}H NMR, MS) and chemical studies.



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1.0 INTRODUCTION

1.1 MORINGACEAE

Moringaceae is a monogeneric family with 14 xerophytic known species, which are indigenous to Africa (9), Madagascar (2), Arabia (2) and India (1). Half of them are relatively common and are sporadically cultivated. All of them are fast growing ornamental trees [1, 2, 3, 4]. They have swollen trunks and their woods are mostly brittle with resinous or gummy barks. Their stems are often thickened with myrosin cells. Only *Moringa oleifera*, because of its many uses, is planted in the whole tropical belt. *M. oleifera* is easily cultivable, grows easily even in pots in red, loamy and black soils and the yield is bountiful. The plant starts flowering in 3.5- 6 months' time, and the fruits are fit for harvesting. The second flowering occurs with an interval of three months with the result that two crops per year are possible [2].

1.2 Chemical constituents and medicinal uses

Moringa oleifera is a smali-to-medium sized tree with multiple uses. Its different parts are reputed to be used in folk medicine for the treatment of a variety of human ailments, such as chronic rheumatism, paralytic infections, epilepsy, tetanus and ascites. The seeds are the source of oil and the leaves, flowers and fruits are eaten as vegetables. The root is also used as a horseradish substitute. In addition, almost every part of the plant is used as a vegetable. Moreover, seeds of the plant are traditionally utilized for water purification because of its strong coagulating properties for sedimentation of suspended mud and turbidity, and its disinfectant effect against pathogens [5]. During some coagulation experiments, a slight decrease of total bacterial count of the purified water was observed indicating the presence of antimicrobial substances, such as benzyl isothiocyanate [6, 7, 8]. It removes more than 90% of *Schistosoma mansoni* cercariae.

Because of the flocculating property of its seeds, *M. oleifera* is also known as "clarifier tree" [9,10,11]. Natural coagulants were detected by laboratory studies in seeds of

seven different *Moringa* species: *M. oleifera* (Sudan), *M. peregrina* (Egypt), *M. stenopetala* (Kenya), *M. longituba* (Somalia), *M. ovalifolia* (Namibia), *M. drouhardii* (Madagascar) and *M. concanensis* (India and Pakistan) [1]. The active substances which could be isolated from these species consisted of water soluble basic polypeptides (proteins) with molecular masses of 6,000-16,000 Dalton. For instance, their number amounted to six in *M. oleifera* and nine in *M. stenopetala.* It seems likely that the rare Moringaceae also contain natural coagulants, but this is not of practical interest [6, 7, 8].

The purified proteins are more effective coagulants than alum. As a coagulant, *Moringa* is nontoxic and biodegradable. Unlike alum, it does not significantly affect the pH. Sludge from coagulation with *Moringa* is 4-5 times less in volume than the chemical sludge produced by alum coagulation (12, 13, 14]. A flocculating protein from the seeds was isolated by extraction with phosphate buffers followed by cation exchange chromatography. The amino acids were analyzed and sequencing showed high contents of glutamine, arginine, and proline and a total of 60 residues. Proline in *M. oleifera* was less than in *M. stenopetala.*

The crushed seed powder, when mixed with water, yields water soluble proteins that possess a net positive charge. The solution acts as a neutral cationic polyelectrolyte during water treatment. The positively charged coagulant causes compression on the double layer of the colloids and provides the neutralization of the electrostatic surface potential of the particles. The particles are destabilized and stick together when contact is made [4]. In general colloidal particles, suspended in raw water, carry negative electrical charge. These particles are surrounded by an electrical double layer and thus, they can't get in contact with each other. It can be assumed, therefore, that most of the *Moringa* seed powder will be trapped in the sediment, only water soluble ingredients will remain in the supernatant water [4].

Seed powders from *M. drouhardii* and *M. ovalifolia* could be used in almost equal concentration to those of *M. oleifera* but more plant material was needed from *M.*

peregrina. However clarification was achieved with lower doses of seed powder from *M. longituba* and in particular *M. stenopetala*. At the present time *M. stenopetala* is considered the most suitable species to replace *M. oleifera* because of its bigger seeds and the presence of more flocculating agents. If *M. stenopetala* seeds were planted, the number of trees required to clarify a given water supply could be 3-4 times less. The average mass of their white kernel is 300-310 mg. Thus optimum doses required for the treatment of water were up to 2-3 times lower than for *M. oleifera*. For example, to clarify 20 L of White Nile water with low turbidities (50-75 FTU) requires 18- 19 average seeds of *M. oleifera* but only 4-6 seeds of *M. stenopetala* were needed. Therefore, the later species is more economical in the long term as far as cultivation costs, soil and water requirements and time for cleaning the seeds are concerned. *M. stenopetala* has the disadvantage of taking several (2.5-3) years to flower and fruit for the first time. The quantity of fruits seems at least as high as in the case of fully grown *M. oleifera*. After 4 years the annual seed production can reach 250-500 pods per tree, with an average of about 100 seeds per pod. A rough estimate reveals that five trees would produce seeds to purify 200 L water per day for a whole year. Number of seeds per tree also varies with age and with the height of the tree [1, 4, 8].

Although the use of *Moringa* seeds had no major effect on the quality of the treated water, there was a considerable increase in the concentration of organic matter in the treated water with increased dosage of *Moringa* seeds [15]. Furthermore, the regrowth of bacteria after a certain time-lag (about 24 hrs) is a rather negative impact. This is because the activity of antimicrobial substances decreases with time. Purified water has to be consumed within 24 hours after treatment. With regard to possible toxicological effects of the coagulants, *Moringa* species was found to be nontoxic during an acute toxicity study [4].

On account of its cheapness, local availability, demand of unskilled manpower, and above ail its simplicity to carry out at household level, researchers in developing countries are engaged in investigating methods for the utilization and optimization of these cheap, native flocculants [4].

Furthermore, it was found in India that plant extracts from *M. oleifera* showed antiviral activity against ranikhet disease (identical with new castle disease virus) strains. This activity appears to be carried out by protein molecules biologically resembling interferon [7, 14).

M. oleifera is widely cultivated as a source of gums, oils and pungent principles. Phytochemical studies on its different parts resulted in the isolation of various chemical constituents such as flavonoids, steroids, mustard oils and proteins [5, 7]. *Moringa* seeds of different species also contain an oil, in amounts of 25-50%, which is used traditionally for eating purposes and as an ointment [8, 16]. The kernel from *M. oleifera* yields clear, sweet oil that is applied externally for skin infections [17, 18, 19, 20). The oil consists of a 60% liquid olein fraction and 40% solid fat. The oil is high in oleic acid, stearic acid, behenic acid, myristic acid, palmitic acid and lignoceric acid and is of high market value for cooking and soap manufacture. The presscake remaining after oil extraction contains the active components affecting water coagulation [21, 22, 23].

According to the Indian experience fresh leaves from *M. oleifera* are more nutritious than most other vegetables. The ieaves contain an equal amount of vitamin A as carrot roots and are richer in vitamin C than tomatoes, radishes, carrots and peas. Their protein content is equivalent to peas and Ca and P amounts are higher than in many other vegetables. Further minerals detected in the ash of the ieaves are Fe, Mg, Mn, Na, S and Cl. The seeds are considered a good source of calcium suggesting a possibility of its capacity to absorb fluoride during the treatment. The defluoridation efficiency of *M. oleifera* is 25% in the seed kernel compared to the whole seed. Further, it was found to soften hard water by precipitation, independent of the pH [24, 25, 26).

The vitamin contents of the flowers and seeds of *M. oleifera* were identified and quantified. Significant amounts of thiamin, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, β-carotene and α-tocopheroi were obtained. The flowers were particularly rich in riboflavin, ascorbic acid, nicotinic acid and folic acid, whereas the seeds

contained significantly higher amounts of thiamin, β-carotene and α-tocopherol [27, 28, 29].

In India, *M. oleifera* is cited for the treatment of diseases of the liver and spleen and for articular pains. The tender leaves are used to treat scurvy and catarrh, and given to children suffering from flatulence. Its leaves and young pods are used as vegetables and added to soups and salads to aid digestion and to stimulate appetite [18, 19, 20].

The leaves of *M. oleifera* are rich in amino acids including aspartic acid, glutamic acid, serine, glycine, threonine, α-alanine, valine, leucine, isoleucine, histidine, lysine, cystine, methionine, arginine and tryptophan. The flowers and the fruits also contain amino acids such as α-alanine, arginine, glutamic acid, glycine, serine, threonine, aspartic acid and valine. Lysine was detected only in the flowers [6, 18, 19].

in addition to flocculants and oils, the horseradish-like substance, 4-(α-L-rhamnosyloxy) benzyl isothiocyanate (A), benzyl isothiocyanate (B) and 4-(α-L-rhamnosyloxy) phenyl acetonitrile (C) antibiotics were isolated from the raw seeds of both *M. oleifera* and *M. stenopetala* by hot water extraction. Defatted and shell free seeds of both species contained ~8-10 % of A, but this amount was produced from *M. oleifera* only when ascorbic acid was added during water extraction [7, 8, 18, 19]. *M. stenopetala* seeds released more than twice as much of this antibiotic substance as *M. oleifera* seeds. A and B act on several bacteria and fungi and they were found active against *Bacillus subtilis* but inactive against *Escherichia coli.* C was inactive against both organisms and it was produced from the parent 4-(α-L-rhamnosyloxy)benzyl glucosinolate. When left to stand in an aqueous methanol solution, A decomposes and loses its antibacterial activity. Minimal bactericidal concentration in *vitro* was 40 pmol/L for *Mycobacterium phlei* and 56 pmol/L for *Bacillus subtilis* [26, 27, 28]. 4-(4'-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate has also been proved more effective than the currently used benzyl isothiocyanate and could be a welcome substitute [30, 31]. The precursors of A are found in the highest concentration in dry, matured seeds. if the seed cotyledons are crushed, thioglycosides are hydrolysed in the presence of

thioglycosidase, whereby the strong smelling mustard oil is formed [27, 28].

Most isothiocyanates are unstable and pungent substances easily detected by their biting taste. In higher concentration they possess vesicant and frequently lacrimatory properties. Because of these properties many isothiocyanate-producing plants species have found application as potherbs, condiments and remedies in folk medicine. Some of them are steam volatile oils. Only 4-(a-L-rhamnosyloxy)benzyi isothiocyanate from *M. oleifera* and *M. stenopetala* seeds has so far been assessed in detail as bactericide and fungicide [8, 30, 31, 32].

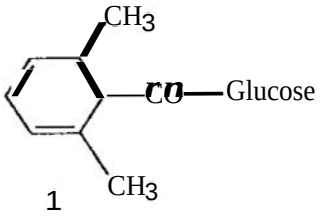
Only the roots of *M. oleifera* contain high concentration the same antimicrobial substances **A** and **B** [30, 31]. Screening tests of the root extracts of *M. oleifera* against larvae of a plant pest feeding on tomato plants and eggs of ascaris have shown very promising nematocidal properties [7, 8]. This effect might also be due to the isothiocyanate. The isothiocyanate in *M. oleifera* seeds might be a repellent and act as a toxic agent to certain lower animals, as ecotoxicological assays have suggested [7, 8, 33, 34].

Mono-palmitic, di-oleic triglyceride with a molecular formula of $C_{55}H_{102}O_6$ has been isolated from the benzene extracts of semi-dried seeds of *Moringa oleifera.* A new glycoside provisionally named as moringyn (1) has been detected from the seeds [29].

In experiments with mammals, isothiocyanates occurring in the genera *Brassica* (cabbage) , *Ruphanus* (radish), *Aimoracia* (horseradish) and *Sinapis* (white mustard) were found to act as goitrogens (cabbage goitre). It is generally accepted that normal consumption of goitrogen containing foods does not cause discernible harm in man, a possible exception being in areas of high incidence of endemic goitre [8, 35]. Compounds having mutagenic activity, 4-hydroxyphenylacetonitrile and 4-hydroxyphenyiacetamide have been isolated from roasted seeds of *M. oleifera* [3, 36]. Fresh flowers of *M. concanensis* were chemically analyzed and quercetin, rutin and kaempferol-3-rutinoside were identified as the main compounds [37]. Rutin was also

detected from acetone extracted leaves of *M. stenopetala* [38]. A new anthocyanin characterized as leucodelphinidin-3-O-β-D-galactosyl (1→4)-glucoside was isolated from *M. oleifera* [39]. Seeds of *M. peregrina*, on treatment with myrosinase, produce 2-propyl, 2-butyl and methyl propyl isothiocyanates in addition to 4-(4'-O-acetyl-α-L-rhamnosyloxy) benzyl isothiocyanate, 4-(α-L-rhamnosyloxy) benzyl isothiocyanate and 5,5-dimethyloxazolidine-2-thione (2) [6].

From stems of *M.oleifera* indian chemists recently succeeded in isolating 4-hydroxymellein, a potential fertility regulating agent which was reported for the first time in a plant species. The stem bark has been shown to contain sterols and terpenes, as well as 4-hydroxymellein (3), vanillin, β-sitosterol, β-sitosterone and octacosanoic acid [8, 40]. The root bark yields the sulfurated amino bases: moringinin, pterygospermin (4) and spirochin, as well as benzylamine (moringin) and glucotropaeolin (5) [30].



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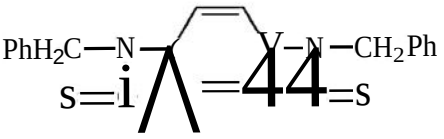

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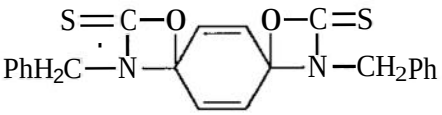

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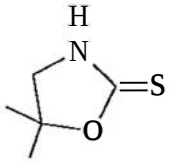

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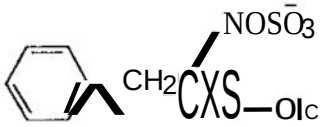

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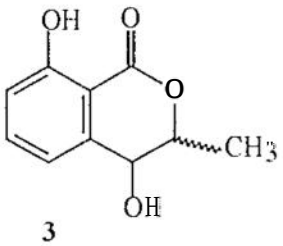


3

Figure 1

Spectroscopic studies of the mucilage designated as drumstick polysaccharide, (DSP), isolated from the pods of *M.oleifera*, revealed the presence of galactose, dextrose, xylose, and Na, K, Mg and Ca salts of glucuronic acid. Chemical analysis of DSP indicated the presence of carbohydrates, gums and mucilages. The chemical studies of DSP indicated the presence of hexopentose linkages through glycosidal bonds with K, Na, Mg and Ca salts of glucuronic acid. It can serve as an alternative for conventional bulk laxative because of its comparable swelling factor with that of agar [41].

Six new and three known glycosides have been isolated from the leaves of *M. oleifera,* employing a bioassay-directed isolation method on the ethanolic extract (Fig 2). Most of the compounds, bearing thiocarbamate, carbamate and nitrile groups, are fully acetylated glycosides, which are very rare in nature [9]. Recently, several novel carbamate and thiocarbamate glycosides possessing hypotensive and antispasmodic activities have been obtained from the leaves. All these glycosides contain rhamnose as the sugar residue, which is either nonacetylated, monoacetylated or triacetylated while no diacetylated rhamnoside was obtained. These triacetylated carbamate/ thiocarbamate glycosides were obtained both in E and Z forms. Acetylation of the non-acetylated or monoacetylated glycoside of the carbamates and thiocarbamates yielded only the Z- isomer of the triacetylated glycosides [10, 42].

Studies on the ethanol extract of the fresh pods of *M. Oliefera* have resulted in the isolation of a novel glycoside niazidin possessing an O-nitrile thiocarbamate group, along with thiocarbamate, carbamate, and isothiocyanate glycosides [43].

Unripe and mature *M. oleifera* seeds are used as foodstuff and spice in many Asian and African countries and are also consumed in drinks prepared in folk medicine [44]. A screening of honey plants has furthermore shown that flowers of *M. oleifera* produce a nectar which should be exploited for beekeeping [7]. In India chemical analysis of the flowers indicated the presence of 0.90% reducing sugars like glucose and 11.8% non-reducing sugars, such as sucrose. Sucrose was also identified from the fruits of *M. oleifera* [8, 45].

Table 1. Medicinal uses of some *Moringa* species

Species	Plant part(s) used	Remedy for	References
M. oleifera	stem, leaves, roots, bark, seeds, flowers	Rheumatism, epilepsy, ascites, paralysis, skin and venereal infection, liver, spleen and articular pains, malaria, hypertension, Antisepetic, nervous disorders, hysteria Spasmolysis, cardiac and circulatory tonic diuretic, analgesic, scurvy, antitubercular, Inflammation, antifertility, fever, diarrhea.	5, 9-11, 18, 19
M. stenopetala	Leaves, seeds, stem, roots	Skin and venereal infection, abortion, Malaria, diarrhea, stomach pain, fever, diabetes, hypertension, inflammation,	4, 7, 8, 38, 65
M. peregrina	seeds, roots, leaves, stem	hypertension, malaria, fever, bacterial infections, inflammation	6, 7, 8

1.4 Secondary metabolites from *Moringa* species.

Various types of secondary metabolites and other compounds have been isolated from different *Moringa* species, especially from *M. oleifera.* Some of them are mustard oil and flavonoid glycosides, steroids, amino acids, fatty acids, vitamins, carbohydrates and minerals [5, 18, 27]. Sugars are often found to occur in organisms in the form of glycosides. Plant glycosides are found especially in leaves, buds, young shoots where metabolism is active and also in the barks and seeds [47]. Thus it has been noticed that glycosides are common compounds in seeds, roots, leaves and stem while proteins and oils are major constituents of leaves and seeds of *Moringa* species, respectively [18].

1.4.1 Glycosides from *Moringa* species

1.4.1.1 Mustard oil glycosides: Glucosinolates are organic substances containing sulfur and nitrogen, mainly nitriles and isothiocyanates (mustard oils), combined with sugar moieties to form glycosides. The most prominent source of isothiocyanate-

producing glycosides is undoubtedly the family of Crucifereae. It can be concluded that the presence of one or more of the thioglycosides is a family characteristic. No Cruciferae species has been rigorously proved to be devoid of these compounds. Equally important is the fact that virtually all investigated species of the related families Moringaceae, Capparidaceae and Resedaceae are sources of the same general types of compounds. The term mustard is believed to be derived from the use of the seeds as a condiment; the sweet *Must* of old wine was mixed with crushed seeds to form a past called "mustum ardens" (hot *must*), hence the mustard. Their compounds are often called mustard oils, since an oil, allyl isothiocyanate, was isolated for the first time from mustard seeds. Glucosinolates are precursors of compounds with goitrogenic (antithyroid) action in mammals including man. They fall into two groups: goitrogenic and mustard oil (isothiocyanate) compounds [34, 35]. The active goitrogenic compounds include thiocyanates, oxazolidine-2-thione, thiourea and thiouracil. If eaten in sufficient quantity over a long period, they have the capacity to prevent the thyroid from accumulating inorganic iodides and so can be responsible for the development of goitre in children and young grazing animals. Seeds always contain much greater amounts than leaves, stems and roots [35].

Mustard oils are formed by enzymatic breakdown of glucosinolates present in the seeds of many plants of the mustard family. Although the glucosinolates themselves are non-irritant, the mustard oils are intensively irritant to animal tissues and if taken in excess can be totally poisonous to livestock or humans [34]. Crystalline salts of K^+, Na^+, etc. are not easily obtained from glucosinolates. This is partly due to difficulties in crystallization and partly due to difficulties in separating mixtures of glucosinolates into individual compounds [35, 47].

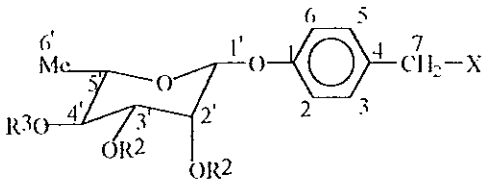


Figure 2

X		R^1	R^2	R^3
—C≡N	1a	-	H	H
	1b	-	H	Ac
	1c	-	Ac	Ac
—N=C=S	2a	-	H	H
	2b	-	H	Ac
–NH–C(=O)–OR'	3a	Me	Ac	Ac
	3b	Et	Ac	Ac
	3c	Me	H	Ac
	3d	Et	H	Ac
—NH–C(=S)–OR'	4a	Me	Ac	Ac
	4b	Et	Ac	Ac
	4c	Et	H	H
	4d	Me	H	Ac
-CHO	5a	H	H	Ac
-NH–C(=S)–OCN	6a	H	H	H

1.4.1.2 Flavonoid glycosides: Flavonoids are common in land plants but not in marine ones. They have a great effect in plant biochemistry and physiology. They are used as pigments, protective agents against UV light or infection by phytopathogenic organisms, antioxidants, etc. Compounds belonging to different classes of flavonoids have been implicated as insect feeding attractants including flavonols, flavones, flavanones and flavanols. The active constituents are usually the O-glycosides rather the aglycones. In most cases flavonoid glycosides rather than aglycones are implicated in feeding stimulation, perhaps because glycosides are more water soluble such that they pass via the blood stream to the site of action. For instance, rutin stimulates feeding of the tobacco hornworm but a mixture of the aglycone quercetin and the sugar

rutinose has no effect. Thus the activity is due to the glycosylated structure rather than to the aglycone and the sugar moieties separately. Naturally occurring flavonoids such as quercetin, rutin, morin and apigenin have also antiviral activity. Quercetin has been reported to inhibit many biochemical events associated with tumour [48]. Rutin has been used as anticoagulant which prevent platelets from aggregation like quercetin. It has also been shown to have therapeutic effect. Isoflavonoids can also be used as antispasmodic, antiarthritic, antiulcer, diuretic and hypotensive agents [35].

Flavonoid glycosides isolated from *Moringa* species are listed in Table 3 and their structures are given in Figure 3.

Table 3. Flavonoids from Moringaceae.

Compound	Species	References.
1. Quercetin	*M. concanensis*	37
2. Rutin	*M. concanensis* and *M. stenopetala*	37, 38
3. Kaempferol-3- rutinoside	*M. concanensis*	37

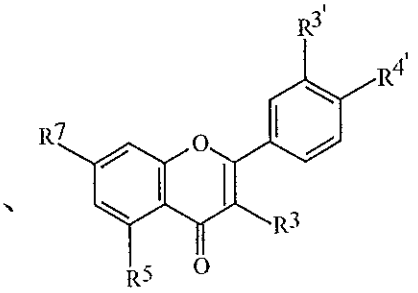


Figure 3

No.	R^3	R^5	R^7	$R^{3'}$	$R^{4'}$
1.	OH	OH	OH	OH	OH
2.	O-glc-rha	OH	OH	OH	OH
3.	O-glc-rha	OH	OH	H	OH

1.4.1.3 Steroids from *Moringa* species

Steroids are a large group of terpenoids including many biologically important compounds like steroid hormones, bile acids, cardiac glycosides, steroid alkaloids and steroid saponins [48]. They have diverse biological activities, such as development and control of the reproductive tract (estradiol, progesterone, testosterone) in man, the moulting of insects (ecdysone), and the induction of sexual reproduction in aquatic fungi (antheridiol). In addition steroids contribute to a wide range of therapeutic application, such as cardiotonics (digitoxin), vitamin D precursors (ergosterol), oral contraceptive agents (semisynthetic estrogens), antiinflammatory agent (contricosteroids) and anabolic agents (androgens) [49].

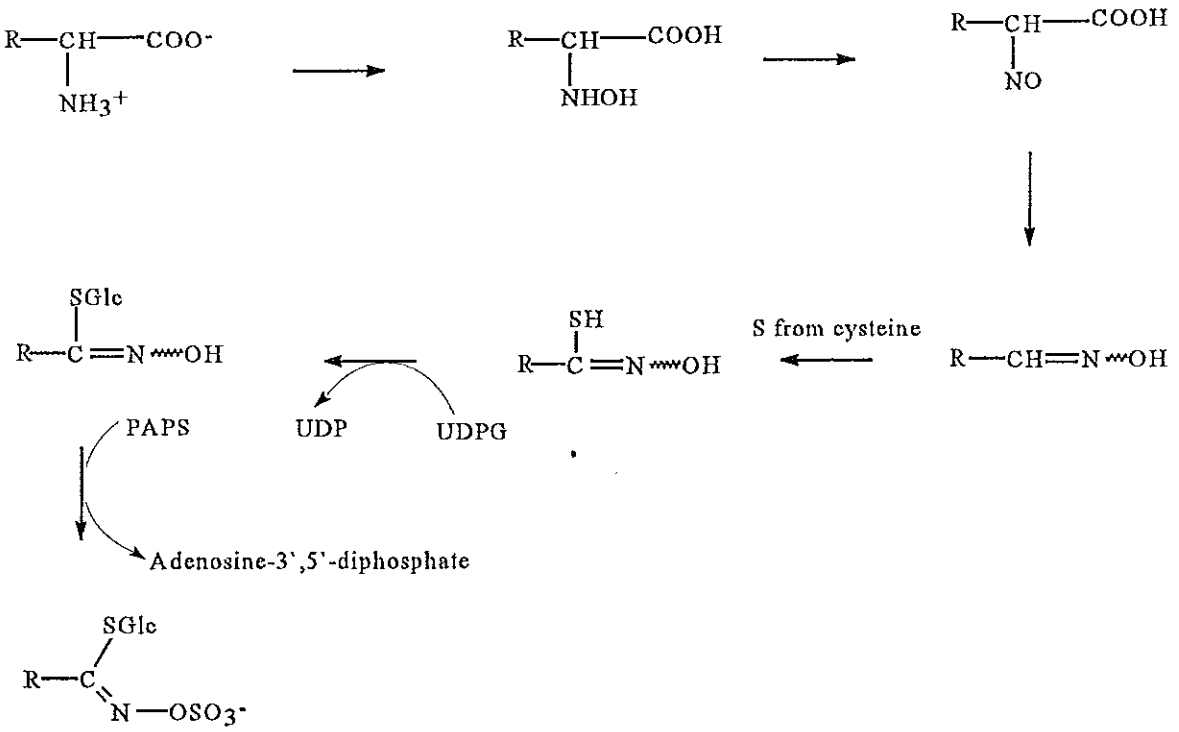
Sterols have a vital role in maintaining the structural integrity of most membranous structures in organisms. They assist in the regulation of the permeability of these membranes to various ions [49]. Steroids isolated upto now from *M. oleifera* are β-sisterol and β-sitosterone [40].

1.5 Biosynthesis of Mustard oil Glycosides.

The similarity between the C-N skeletons of natural L-amino acids and some mustard oil glycosides suggests that amino acids may be the precursors of their aglycone moieties. Feeding experiments with appropriately labeled precursors have shown that the nitrogen of the glycoside is that of precursor amino acids. The necessary precursor amino acids of certain of these glycosides are formed by chain extension of lower homologs [47, 49].

The aglycones of glucosinolates are isothiocyanates, nitriles and thiocyanates. In other words gucosinolates are presumably derived biosynthetically from amino acids in a series of reactions in which the carboxyl group is lost and the α-carbon is transformed into the central carbon in the glucosinolates. The R-group is identical with the substituent on the α-carbon in the amino acid (Scheme 1). The variety of glucosinolates

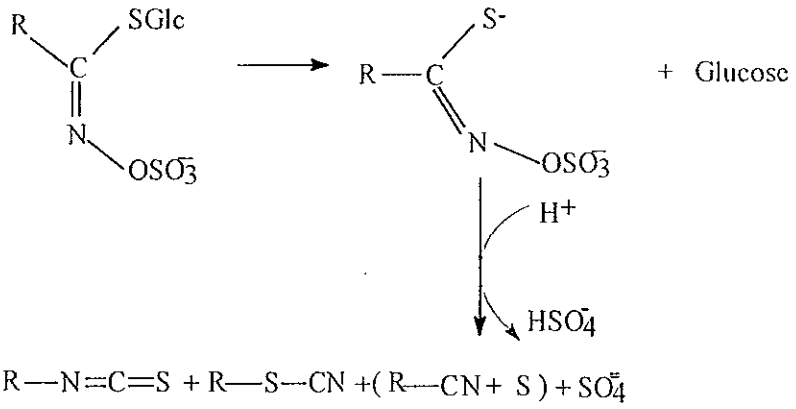
occurs only in the side chain, R, whereas the carbohydrate moiety is always a β-D-glucopyranosyl residue and the configuration around the -C=N bond is always Z. The glycosylation steps are usually at the end of the particular biosynthetic pathway. Because of the low pK value of the sulfonic acid group and the instability of glucosinolates in strong acid, they invariably occur in nature in the anionic form [47, 51].



Scheme I. Biosynthesis of glucosinlates from amino acids

Mustard oils are normally absent in the plant and are first formed when tissue damage permits interaction of glucosinolates and myrosinase. The activation of this enzyme is promoted by its cofactor, ascorbic acid, during tissue damage. Isolation and identification of glucosinolates is difficult since enzymes within the plant frequently decompose the glycosides during extraction [47]. The hydrolysis of the S-glycosidic bond yields the sugar and the aglycone. As a result of molecular rearrangement the aglycone is converted into the sulfates and isothiocyanates depending on their side chains. Smaller amounts of nitriles, thiocyanates and sulfur could also be formed due

to fragmentation. Therefore, during isolation of the native glucosinolates, the enzymes must be denatured [49, 51].



$$R—N{=}C{=}S + R—S—CN + (R—CN + S) + SO_4^{=}$$

Scheme II. Conversion of glucosinolates to their corresponding isothiocyanates by the action of enzymes.

Isothiocyanates with an OH group in the 2- and 3- positions cyclize to give oxazolidine-2-thiones and tetrahydro-1,3-oxazinethiones, respectively. These transformation products are related to toxic properties of glucosinolates and their derivatives. Since isothiocyanates are chemically very reactive, they readily add alcohols and amines to give thiocarbamates (thiourethans) and thioureas, respectively. Some alkyl thiocyanates are useful insecticides. Partial hydrolysis or alcoholysis in the presence of con.H_2SO_4 yields S- alkyl thiocarbamates [47, 52, 53]. These compounds may be present in the leaves of *Moringa* species as their glucosinolates and the myrosinase activity in the leaves is sufficient for the liberation of the thiocarbamates from the corresponding parent compounds [5]. Therefore, the formation of thiocarbamate glycosides, during extraction and isolation, may involve the addition of ethanol or methanol to isothiocyanates. Biogenesis of carbamates may occur through the hydrolysis of thiocarbamates [5, 47].

1.6 Identification

1.6.1 Mustard oil glycosides. Identification of glucosinolates is usually done using

spectroscopic methods and transformation into isothiocyanates [47].

1.6.1.1 Color reaction. Glycosides, due to the presence of hydroxyl functional groups on their basic skeleton, can easily be distinguished by the appearance of a specific color upon spraying aqueous acetone silver nitrate solution on the chromatogram [54, 55].

1.6.1. 2 Chemical transformation

I. *ACETYLATION.* Identification of glycosides using chemical transformation can be done by acetylation. In a few instances, introduction of acetyl groups into the sugar moiety of glycosides and additional acetyl groups on the free hydroxyl groups in the side chain has led to the isolation of crystalline salts of glucosinolates. Acetylation of their mustard oil glycosides yields triacetylated glycosides [8, 47].

II. *ACID HYDROLYSIS.* It cleaves mustard oil glycosides into a sugar and non-carbohydrate portion (aglycone). In our case acid hydrolysis yielded rhamnose as the only sugar component and different mustard oils [47].

1.6. 1. 3 UV-VIS Spectroscopy

The uv-vis absorption spectrum of mustard oil glycosides is considered to originate from the aromatic chromophoric systems that can be modified by either nitrile, isothiocyanate, thiocyanate, carbamate or thiocarbamate functionalities. Thus bands at λ_{max} 184, 204 and 255 nm are associated with aromatic ring and those at about 160-167, 210-220 and 195-210 nm are considered to originate from nitrile, amide and ester functional groups, respectively [56].

1.6.1.4 IR Spectroscopy

IR Spectroscopy is the primary probe for the detection of functional groups and electronic conjugation in a molecule. The IR spectra of all mustard oil glycosides containing aromatic ring shows absorption bands in the region 1500-1650 cm^{-1} along with carbonyl band at 1680-1730 cm^{-1}. The appearance of more than one hydroxyl band

between 3600-3200 cm^{-1} indicates more than one such group attached to the glycoside nucleus. Absorbance between *ca*.930-776 cm^{-1} may indicate either α- or β-glucopyranose. The glycosidic nature of mustard oils is reflected by broad bands at *ca*. 3250 and 1060 cm^{-1}. Carbamates display important bands at 1735 (ester carbonyl) and 1715 cm^{-1} (carbamate carbonyl). Nitriles show weak absorption at about 2250 cm^{-1} and isothiocyanates indicate strong absorption bands at 2160-1990, 649-600, 565-510 and 470-440 cm^{-1} due to the assymetric stretching mode of the cumulative N=C=S double bonds. Furthermore C-O stretching of secondary OH group would absorb between 1040-1020 cm^{-1} [57, 58].

1.6.1.5 ^{1}H and ^{13}C NMR Spectroscopy

^{1}H and ^{13}C NMR have proved invaluable as a guide to structural determination. NMR has been widely used in structural studies of mustard oil glycosides. Chemical shifts, integral values as well as coupling constants provide information about the substitution pattern of mustard oil glycosides [59]. Mustard oil glycosides, which have been isolated and characterized from *Moringa* species, basically contain 4-(α-L- rhamnosyloxy)benzyl skeleton. Furthermore the benzene ring may contain either nitrile, isothiocyanate, carbamate or thiocarbamate functional groups. Therefore the nature of the NMR spectra of these compounds mainly indicates three features: the α-L-rhamnosyl, the benzene ring and the functional group. The structures of glycosides were deduced through extensive ^{1}H and ^{13}C NMR studies, including 2D-NMR and DEPT [60].

In the sugar moiety, the directly bonded C-H group coupling constant of C-1' signals ($^{1}J_{C1'-H1'}$) of hexopyranoses and pentopyranoses are characteristic of the anomeric configuration. $^{1}J_{C1'-H1'}$ was found to depend mainly on the anomeric structure regardless of the variety of aglycones. In other words the anomeric proton signal alone can provide useful information regarding the saccharide moiety. The H-1'/H-2' coupling constant can at times indicate which signal relates to which sugar and more frequently is also indicative of the α- or β-linkage of the glycosidic bonds. For example, α-linked L-rhamnopyranosides, which exhibit H-1'/H-2' coupling constant of 2 Hz, are readily

distinguishable from the β-linked rhamnopyranosides with 1 Hz couplings. Sugar carbon signals other than C-1' were found to be only slightly affected by the structure of aglycones and $^{1}J_{C1'-H1'}$ is consistently 10 Hz smaller when H-1' is axial than when it is equatorial [60].

A chemical shift data relating to the glycosidic H-1' signal can also be of diagnostic value. The anomeric proton signals of α-L-rhamnosides appear always at lower field (5.02-5.92 ppm) than those of the corresponding β-isomers (4.55-4.93 ppm). This is used to differentiate anomeric structures, though occasional deviation by the change of aglycone structures or of the conditions of measurement. This is promising for the determination of the anomeric configuration with the aid of chemical shift differences of C-3' and 5' [61]. The site of the glycosidic linkage with the aromatic ring is inferred from the relatively down field shift of the anomeric proton as compared with its chemical shift when it is linked with saturated carbons [10].

Table 4. ^{1}H NMR chemical shifts and coupling constants for mustard oil glycosides containing carbamate and isothiocyanate functionalities (δ ppm) [9, 11].

H	Carbamates	Isothiocyanates
2,6	7.07-6.97(*d*, 8.7 Hz)	7.09-7.07 (*d*, 8.8 Hz)
3,5	7.27-7.15 (*d*, 8.7 Hz)	7.32-7.25 (*d*, 8.8 Hz)
7	4.10-4.01(*d*, 6.0 Hz)	4.86- 4.64(*s*, 6.0 Hz)
1'	5.62-5.35 (*d*, 1.8 Hz)	5.55-5.45 (*d*, 1.6 Hz)
2'	5.32-3.87(*m*, 3.6, 1.8 Hz)	4.14- 3.88 (*m*, 3.5, 1.6 Hz)
3'	5.27-3.81 (*m*, 9.7, 3.6 Hz)	4.09- 3.81 (*m*, 9.4, 3.5 Hz)
4'	5.14- 3.81 (*t* or *m*, 9.7 Hz)	4.89- 4.85 (*t* or *m*, 9.4 Hz)
5'	3.89- 3.59 (*m*)	3.87- 3.65 (*m*, 9.4, 6.2 Hz)
6'	1.09-0.97(*d*, 6.2 Hz)	1.19(*d*)
OMe	3.95(*s*)	-
NH	7.56- 4.53 (*t*, 5.9 Hz)	-
OCOMe	2.12-1.95 (*s*, 7.1)	2.13-2.04(*s*)

The NH linked with the benzylic methylene carbon (C-7) in carbamates and thiocarbamates was evident from the multiplicities and coupling constants of these protons. In other words the thiocarbamate and carbamate functions could be indicated by the presence of a triplet for NH at about 9.55 and 7.52 ppm, respectively. These vanish on shaking with D_2O while the doublet of the CH_2 group changed to a singlet at about 4.10 ppm. Like wise on shaking with D_2O, the signals of OH in the sugar moiety disappeared and the signals for H-2' and H-3' each collapsed in to a doublet of doublets, and that for H-4' changed into a triplet. The effect of acetylation of the OH group produces small but reproducible changes in the chemical shifts to the OH group. In the ^{13}C NMR spectrum (broad band) shows a peak at 123 ppm attributable to the CN group and 129 ppm to the carbon of N=C=S while OMe and thiocarbonyl carbons were observed at 56.43 and 190.72 ppm, respectively [5 ,9, 11].

1.6.1.6 Mass Spectroscopy

The main applications of MS in the structure analysis of mustard oil glycosides are for the determination of molecular masses of the compounds, establishment of the distribution of substituents and determination of the nature and site of attachement of the sugar moiety. The first objective in the interpretation of glycosides is to identify the unfragmented molecular ion, (M^+), and then to relate other major fragments to it by rationalizing the loss of mass using recognized fragmentation pathway. For glycosides that are too labile thermally to give useful EI, CI or FIMS, the various mild techniques that don`t require heating, such as FAB, laser desorption or electron spray MS will provide better analytical information [62]. FABMS has been used commonly since it usually gives a strong molecular ion peak which indicates clearly the number and type of sugar units present. Therefore the molecular formula of the compounds were established with the aid of FABMS (negative-ion mode). The molecular masses of the glycosides are indicated by the presence of negatively charged molecular-ions, with a reasonable abundance for monoglycosides, a low abundance for diglycosides and a barely detectable (0.01%) abundance for triglycosides [9, 11].

increases the relative resonance contribution of band I and consequently produce considerable bathochromic shift in the band. Methylation and glycosylation cause hypsochromic shift. The orientation of OH-groups in flavonoids skeleton can be determined by using shift reagents [37, 63].

1.6.2.3 IR Spectroscopy

The IR spectra of all the flavonoids and isoflavonoids is characterized by absorption in the region 1500- 1600 cm^{-1} due to aromatic rings, along with carbonyl band at 1620-1670 cm^{-1}. The presence of OH group in flavonoids is evidenced by absorption in the region 3300-3400 cm^{-1}. The presence of gem-dimethyl group is indicated by the appearance of a band at *ca.*1400 and 925 cm^{-1} (methylenedioxy group). The presence of glycosidic linkage to a flavonoid is indicated by broad bands at *ca.* 3250 and 1060 cm^{-1} [37].

1.6.2.4 ^{1}H NMR Spectroscopy

^{1}HNMR is very useful in the structure elucidation of flavonoids. For flavones, flavonols and isoflavones with 5,7-dihydroxy groups, the proton of ring-A located at C-6 and 8 give rise to two doublets (J = 2.5 Hz) in the range 6.0-6.5 ppm due to *meta* coupling. As a result of the *ortho* deshielding effect of the two OH groups, the signal for H-6 is usually observed at higher field (6-6.2 ppm) than H-8 (6.3-6.5 ppm). C- and -O linked sugars (anomeric protons) show signals in the range of 4-5.7 (s or d) ppm. Attachement of sugar residue at C-7 causes a down field shift for H-6 and 8 signals [47, 63, 64].

B-ring protons usually appear in the range 6.7-7.9 ppm. The coupling pattern of these protons is characteristic of the substitution pattern of the ring. For instance, If B-ring is oxygenated at C-4' a typical four peak pattern of two doublets (J = 8.5 Hz) is observed. The doublet for H-3' and 5' always appear upfield (6.65-7.1 ppm) from the H-2' and 6' protons (7.2-7.5 ppm) because they are shielded by the C-4' oxygen substitution [47, 63, 64].

Ring-C proton chemical shift values aid in differentiating flavones and flavonols from isoflavones. For example in flavonols and flavones, C-3 proton appears as a singlet at about 6.3 ppm. In isoflavones, the C-2 proton is at β- position to the C-4 keto and appears in the range 7.6-7.8 ppm. In other words a singlet that appears at *ca* 7.8 ppm representing the C-2 proton of the ring-C is a characteristic feature of an isoflavone. The presence of a methoxy group is readily recognized by the appearance of strong signals at 3.5-4.1 ppm [47, 63, 64].

1.6.2.5 ^{13}C NMR Spectroscopy

^{13}C NMR spectrum of flavonoids can be useful in special cases, although the resonances of the aromatic carbon atoms can't be used to distinguish between the different types of flavonoids. The flavonoids, pterocarpanoids and chalcones possess three aliphatic and 12 aromatic resonances in ^{13}C NMR spectrum. The presence of many signals in the 60-80 ppm region is generally indicative of glycosidic carbons. Carbonyl signals of both flavonoids and isoflavones resonate in the region 174-178 ppm. A carbonyl resonance at 181±2 ppm clearly indicates the presence of a OH moiety at C-5. The chemical shifts of the three carbons of the ring-C are usually quite distinct for the different classes [47, 63, 64].

Table 5. ^{13}C NMR chemical shift of three carbons of the ring-C of flavonoids (ppm) [63]

Flavonoid type	C-2	C-3	C-4
1. Flavonones	75- 80.3 (*d*)	42.8- 44-6 (*t*)	189.5-95.5 (*s*)
2. flavonols	145- 150	136- 139	172- 177
3. Flavones	160.5-165 (*s*)	103-111.8 (*d*)	176.3- 184 (*s*)
4. Isoflavones	149.8- 155.4 (*d*)	122.3- 125.9	174.5-181 (*s*)

1.6.2.6 Mass Spectroscopy

The fragmentation of the M^+ into A and B-ring containing fragments provides useful structural information. The frgments at *m/z* M^+ and M^+-1 are commonly encountered for flavonoids containing OH group at C-3 and C-6 or flavone methyl ethers having OMe at C-3 or C-5 because they give rise to a stable quinonoid ions. M^+ -28 indicates loss of CO or M^+ -29(CHO) from 4-keto functions to form a 5- membered ring, especially with 3-OH flavones and dihydroxyflavonols. An intense M^+ -43 (CH_3CO) peak could also be observed from 6-methoxylated flavonoids and a peak at M^+-55(56) will indicate the presence of prenyl ($-CH_2-CH=C(CH_3)_2$) substituent [47, 63].

1.7 Objective

The main objective of this project is isolation and structure elucidation of secondary metabolites from the leaves of *M. stenopetala.*

1.8 *Moringa stenopetala* of Ethiopia

Ethiopia shows a remarkable wealth in useful plants and some of them are used by the rural population for different purposes. For instance, *M. stenopetala* and *Maerua subcordata* are traditionally used for purification of turbid water [65]. In Ethiopia five species of *Moringa* have been identified and water purification characteristics of three, namely *M. oleifera* (Diredawa), *M. peregerina (*Hararge) and *M. stenopetala* (south Ethiopia) is known [2, 65].

M. stenopetala is a 6 to 10 m tall tree characterized by brittle branches, smooth grey bark and soft wood. It is indigenous to south Ethiopia, north Kenya and east Somalia. It is cultivated traditionally as cabbage tree and planted occasionally as ornamental tree at altitudes between 1,000 and 1800 m with an appropriate temperature range of 20-30°C. It is more drought resistant than *M. oleifera* and stores water in its pulpy roots. In Kenya, drinking water is squeezed out of the roots during drought, yet this practice is

also minorities from the same tribe in the area of north Kenya [4]. It has been planted in Wello, Shoa and Hararge region for demonstration purposes from southern parts of Ethiopia [4].

Very few attempts have been made to isolate and characterize the components from the seeds and leaves of *M. stenopetala* of Ethiopia. Studies of the plant`s chemical components are limited to the crude extracts of the seeds, leaves and roots. In the present work, the fresh leaf extracts of the plant are subjected to a systematic phytochemical isolation procedure and isolated components have been characterized.

-O-acetyl-α-L-rhamnosyloxy)benzaldeyhde. This is the first report of the isolation of the monoacetylated rhamnoside of *p*-disubstituted benzaldehyde from this species; earlier it was isolated from the leaves of *M. oleifera* [10, 11].

Table 7. Comparison of the observed ^{1}H NMR data of **9** with those reported for 4-(4'- O-acetyl-α-L-rhamnosyloxy)benzaldehyde [11].

Observed data (400 MHz, CD_3OD)		Reported data (400 MHz, $(CD_3)_2SO$)
H	ppm	Ppm
3, 5	7.85 (*d*)	7.87 (*d*)
2, 6	7.20 (*d*)	7.25 (*d*)
7	9.95 (*s*)	9.87 (*s*)
1'	5.58 (*d*)	5.61 (*d*)
2'	4.20 (*m*)	3.88 (*m*)
3'	4.10 (*m*)	3.82 (*m*)
4'	4.86 (*t*)	4.86 (*t*)
5'	3.60 (*m*)	3.64 (*m*)
6'	1.20 (*d*)	0.99 (*d*)
$OCOCH_3$	2.16 (*s*)	2.06 (*s*)

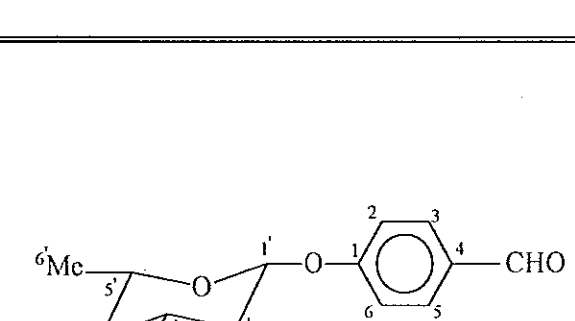


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Figure 5

3. 0 Experimental Section

3.1 Materials and Methods. Melting points were determined on a Bock-Monoscop-NR174 apparatus and are uncorrected. UV and IR data were recorded using Beckman DU-65-spectrophotometer and Perkin-Elmer FTIR 1600 series, respectively. The EI mass spectra were recorded on a VG ZABSPEC instrument and the ^{1}H NMR spectra were taken in CD_3OD and DMSO-d_6 on a Varian VXR-500 Spectrometer operating at 400 MHz at the Chalmers University of Technology, Gothenburg, Sweden. The 300 MHz ^{1}H NMR spectra were recorded in CD_3OD on a Brucker 300 MHz instrument at the University of Botswana, Gaborone, Botswana. The chemical shifts(δ) are in ppm and coupling constant (J) are in Hz.

The purity of compounds was monitored on Si gel GF_{254} plates. Analytical thin layer chromatography were run on silica gel (Merck) coated on aluminium foil, 0.25 mm thickness. The spots were detected by their UV fluorescence and by spraying with aq. $AgNO_3$ and/or 5% methanolic KOH solution. Column chromatography was performed on silica gel 60 (0.040-0.063mesh ASTM-Merck) and Sephadex LH-20 ($CHCl_3$:MeOH, 1:1). All solvents were distilled prior to chromatographic use. The petrol used throughout the study has boiling point (40-60°C). PTLC was run on 0.50 mm thick silica gel $60PF_{254}$ (Merck) and bands were detected by their UV fluorescence.

3.2 Plant material. The leaves of *Moringa stenopetala* were collected from Arbaminch Water Technology Institute in the months of November, 1998 and April, 1999.

3.3 Extraction and Isolation. 1 kg fresh and uncrushed leaves of *M. stenopetala* were extracted six times with 5 L of ethanol each for 48 hrs at room temperature. The first two and the last four extracts were combined separately and freed from the solvent under reduced pressure to give two viscous extracts, A (62 g) and B (27 g), respectively. The residues were defatted with petrol and then each residue was subjected to column chromatography (3 cm x 30 cm). The column was eluted first with petrol then with petrol:$CHCl_3$ and $CHCl_3$:MeOH gradients to pure MeOH. TLC of each fraction was

examined using different solvent systems. Fractions having similar spots were combined to give four major fractions (Fr-I, Fr-II, Fr-III and Fr-IV). Each fraction was further purified by chromatography over Sephadex LH-20 and refractionated using different solvent systems. Purification by PTLC was done for each fraction to give pure compounds. The type of compounds isolated from residues A and B were the same but the masses of the isolated compounds from extract B were much less compared to those from residue A. Extraction and isolation were done repeatedly to collect sufficient amounts for spectroscopic and chemical analysis.

Fr-I (84 mg) mainly contained one spot and it was purified using prep. TLC using petrol. This gave component **1**. Fr-II (242 mg) contained oily substances and chlorophyll. It was subjected to column chromatography over Sephadex LH-20. The presence of oily components made separation difficult. However, with repeated use of column chromatography followed by PTLC ($CHCl_3$) three components (**3**, **4** and **5**) were obtained. Fr-III (122 mg) contained a lot of chlorophyll and it was subjected to column chromatography over Sephadex, PTLC gave compounds **6**, **7** and **8** using $CHCl_3$: MeOH (9:1). Fr-IV (1.2 g) contained gummy polar compounds. The gum was pericipitated out by adding EtOAc:MeOH (6:1). The remaining solution gave compound **12** by direct recrystallization. The rest of the mixture was subjected to column chromatography which gave components **9**, **10**, and **11** which were further purified by PTLC using $CHCl_3$:MeOH (8:2).

Table 9. Solvent systems used for the isolation of compounds.

No.	solvent system	ratio
I	n-BuOH:HOAc:H_2O	4:1:5
II	$CHCl_3$: MeOH	8:2
III	$CHCl_3$: MeOH	9:1
IV	n-BuOH:HOAc:H_2O	4:1:5
V	EtOAc:MeOH	6:4
VI	$CHCl_3$: MeOH	8:2
VII	$CHCl_3$: MeOH	9:1

3.4 Physicochemical data

Rutin (**12**). R_f 0.55 (solv. I), pale yellow crystalline solid, mp 187-190°C (lit.190-195°c [38]), UV (366) fluorescence, deep yellow, UV (MeOH) λ_{max}, 245, 295, 353, 395 nm, IR (KBr)ν_{max} 3421, 2918, 1655, 1601, 1505, 1457, 1363, 1295, 1204, 1064, 806 cm^{-1}, ^{1}H NMR (4000 MHz, DMSO-d_6) δ (ppm), 7.56 (*d*, H-2',H-6'), 6.84 (*d*, H- 5'), 6.38 (*d*, H- 8), 6.18 (*d*, H-6), 5.34 (*d*, glucosyl H-1), 4.38 (*s*, rhamnosyl H-1), 3.50 - 3.06 (*m*, 10H- sugar), 0.95 (*d*, H-6"'), 12.60 (*s*, 5- OH). EIMS *m/z* 302 (M^+- rhamnoglucosyl), 274, 273, 187,153, 121 and 73.

3. 4.1 *Acid hydrolysis of Rutin.* A solution of 1 mg sample and 10ml of 2N HCl:MeOH (1:1) was refluxed on a water bath for 1h, It was then cooled and concentrated to dryness by rotary evaporator. The resulting residue was diluted with water and extracted with ethyl acetate repeatedly. The aqueous layer was further analyzed using paper chromatography (solv IV) and revealed the presence of two compounds. They were identified as L-(+)-rhamnose and D-glucose by comparison with authentic samples. Compound **12** was chromatographed with an authentic sample (rutin) and gave identical chromatogram. The aglycone was also run along with an authentic sample (quercetin) using solvent system V and gave identical R_f.

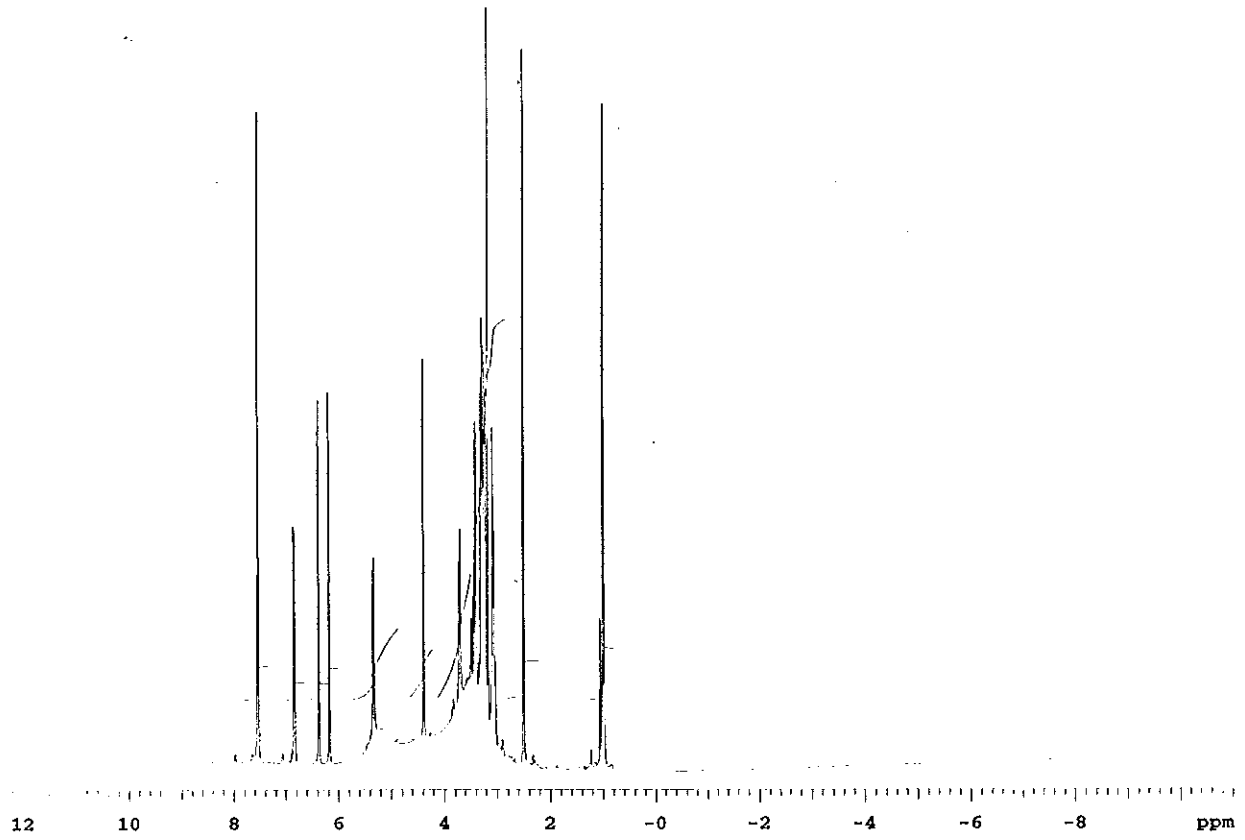
compound **9.** R_f 0.44 (solv.II), light yellow solid, mp 162-165 (d)°C, UV (366) fluorescence, yellow, UV (MeOH)λ_{max}, 240, 260, 280 nm, IR (MeOH) ν_{max} 3400, 2923, 2852, 1734, 1617, 1489, 1458, 1364, 1094, 860, 776, 450 cm^{-1}, ^{1}H NMR (400 MHz, CD_3OD) δ (ppm), 7.85 (*d*, H-3, H-5), 7.20 (*d*, H-2, H-6), 9.95 (*s*, CHO or H-7), 5.58, (*d*, H-1'), 4.2 (*m*, H-2'), 4.10 (*m*, H-3'), 4.86 (*t*, H-4'), 3.6 (*m*, H-5'), 1.20 (*d*, H-6'), and 2.16 (*s*, OAc), EIMS *m/z* 281, 253, 189, 122 (HOC_6H_4CHO), 121 ($HOC_6H_4CO^+$), 105, 93.

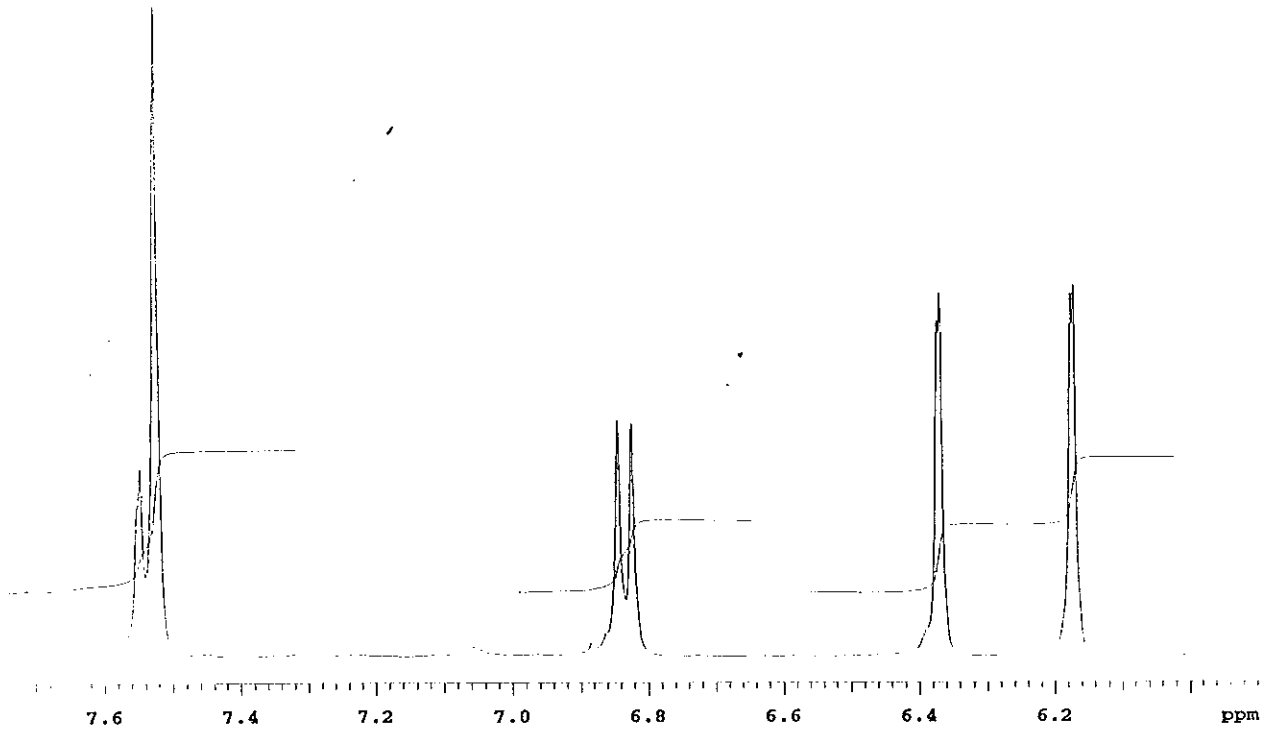
4-(4'-O-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate (**7**). R_f 0.60 (solv. III), light yellow, mp 122-125 (d)°C, UV (366) fluoescence, light brown, UV (MeOH) λ_{max}, 225, 275, 375 nm, IR (KBr) ν_{max} 3383, 2932, 2173, 2091, 1715, 1611, 1510, 1342, 1235, 1063, 1023, 983, 835, 668, 500 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD) δ (ppm), 7.34(*d*, H-3,

H-5), 7.11 (*d*, H-2, H-6), 4.71 (*s*, H-7), 5.49 (*d*, H-1'), 4.05 (*m*, H-2'), 4.03 (*m*, H- 3'), 5.05 (*t*, H-4'), 3.80 (*m*, H-5'), 1.12 (*d*, H-6'), 2.10 (*s*, OAc), EIMS *m/z* 189, 129, 107, 85, 71.

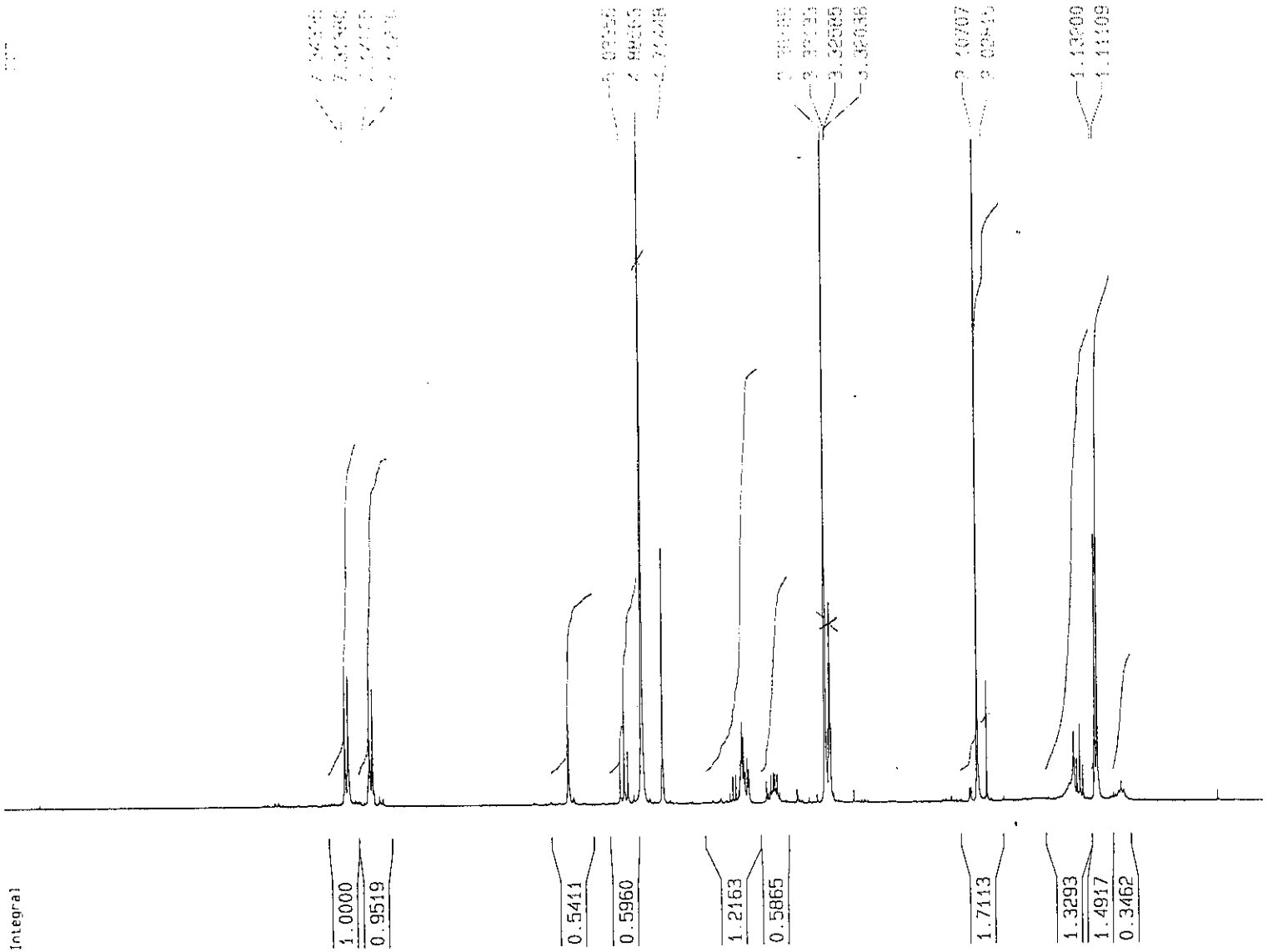
3. 4. 2 Acetylation of compounds **9** and **7.** A mixture 0.5 ml of acetic anhydride, 2.0 mg compound **9** and two drops of pyridine was kept for two days at room temperature. The solution was neutralized with 10 ml of saturated sodiun bicarbonate solution and extracted with 50 ml of chloroform. The organic layer was washed with 0.1N HCl and dried with anhydrous sodium sulfate. The solvent was evaporated to give 1.3 mg of the acetylated product. This product was analyzed by TLC (solv.VI) and gave R_f value greater than the non- acetylated counter part and didn`t change its colour when chromatogram was sprayed with $AgNO_3$ reagent. Acetylation of compound **7** was carried out in a similar manner. The R_f value of the acetylated compound was checked using solvent system VII.

AM-MS-12





AM-MS-7 TG



Current Data Parameters

NAME	AM-MS-7
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	990608
Time	21 03
INSTRUM	spect
PROBHD	5 mm QNP 1H/1
PULPROG	zg30
TD	65536
SOLVENT	MeOH
NS	16
DS	2
SWH	6172.839 Hz
FIDRES	0.094190 Hz
AQ	5.3084660 sec
RG	256
DW	81.000 usec
DE	6.00 usec
TE	300.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	9.70 usec
PL1	0.00 dB
SFO1	300.1316534 MHz

F2 - Processing parameters

SI	32768
SF	300.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	10.189 ppm
F1	3057.99 Hz
F2P	-0.261 ppm
F2	-78.43 Hz
PPMCM	0.52251 ppm/cm
HZCM	156.82103 Hz/cm

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