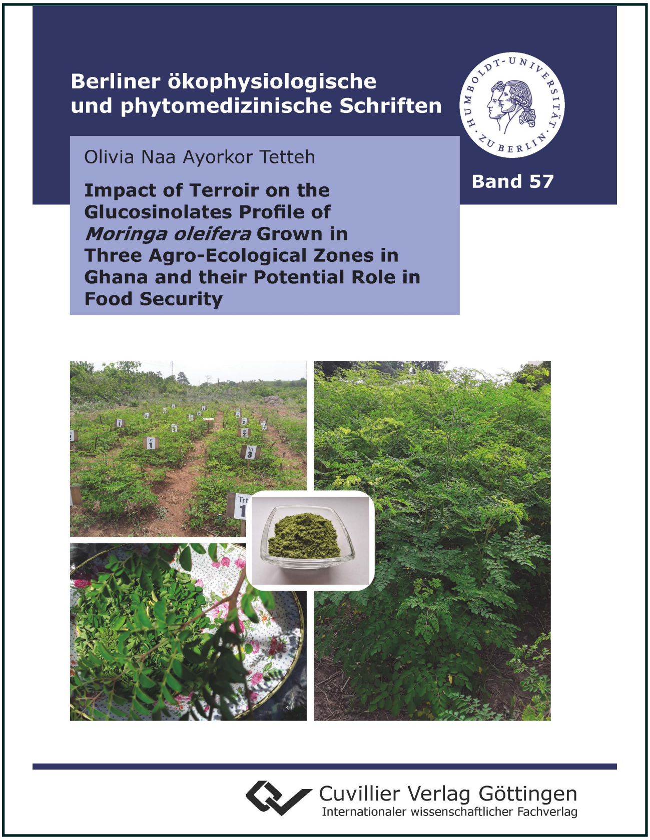
Olivia Naa Ayorkor Tetteh (Autor)

Impact of Terroir on the Glucosinolates Profile of Moringa oleifera Grown in Three Agro-Ecological Zones in Ghana and their Potential Role in Food Security



Berliner ökophysiologische und phytomedizinische Schriften

Olivia Naa Ayorkor Tetteh

Impact of Terroir on the Glucosinolates Profile of *Moringa oleifera* Grown in Three Agro-Ecological Zones in Ghana and their Potential Role in Food Security

Band 57

Cuvillier Verlag Göttingen
Internationaler wissenschaftlicher Fachverlag

https://cuvillier.de/de/shop/publications/9019

1. Introduction

Moringa oleifera Lamarck is a native of the Indian subcontinent and has become popular in tropical and subtropical regions worldwide (Fuglie, 2005). However, it is particularly widely cultivated in dry tropical areas of the Middle East and Africa and, more recently, in other tropical countries such as Nicaragua (Nouman et al., 2014).

The plant's uses for food and medicine due to its high nutritional and medicinal values are well-documented. Its high medicinal value is attributed to the plant's high phytochemical composition, including glucosinolates (GS) (see Chapter 2 “Scientific Background”; Fahey et al., 2018). The GS of *M. oleifera* is of particular interest because one of its breakdown products, isothiocyanate, has health-promoting potential for humans (e.g., Waterman et al., 2014; Fahey, 2017). For example, studies have demonstrated that the compound possesses higher chemoprotective potential than sulforaphane (4-methylsulfinylbutyl isothiocyanate from broccoli sprouts) (see Chapter 2 “Scientific Background”; Fahey et al., 2001; Brunelli et al., 2009; Fahey et al., 2012). Thus, the plant has become phytochemically and pharmacologically unique and highly valuable, particularly with consumers enthused about adopting preventive health care through the consumption of health-promoting produce and plant products. This is because of the high incidence of coronary heart disease, stroke, and cancer (Dauchet et al., 2006; He et al., 2006; Fahey et al., 2012).

Also, recent global food security and nutrition reports indicated that efforts to reduce world hunger and malnutrition by 2030 have become challenging (FAO et al., 2021; World Bank Group, 2021). In West Africa, Ghana is one of the countries most impacted by the food security and malnutrition crises (World Food Program (WFP), 2021). According to the United Nations Committee on World Food Security, food security means that at all times, all people have physical, social, and economic access to adequate, safe, and nutritious food that meets their food preferences and dietary needs for an active and healthy life (FAO, 2006). The FAO, for many decades, has measured food security in terms of an adequate supply of food at the national level as the distribution of calorie requirements on a per-person basis from the daily dietary energy supply per capita (Jones et al., 2013; Ickowitz et al., 2019). Although this is an easy way to measure food security, it gives the notion that in order to improve food security at the national level, more calorie-dense crops should be produced instead of increasing production in different crops with high nutritional and phytochemical values to enhance healthy diets (Ickowitz et al., 2019). Thus, for many years, advocacy to increase agricultural productivity has been on a few staple crops, with food security depending mostly on these crops, including rice, maize, wheat, and potato (Füleky, 2009). Meanwhile, with a high increase in such staple crops (lacking essential micronutrients), the issue of micronutrient deficiency (a form of malnutrition) looming in vulnerable households, particularly in low-income countries like Ghana, could be challenging to resolve. Therefore, there is a need to also increase the cultivation and utilization of under-utilized tropical tree plants such as *M. oleifera*.

Despite numerous studies on the phytochemical attributes of *M. oleifera*, few studies have been conducted on the effect of certain aspects of terroir on the plant's GS profile (e.g., Doerr et al., 2009; Förster et al., 2015a). Terroir is usually associated with wine, particularly in European vineyards (Van Leeuwen et al., 2004), to express the significant effects of the living and non-living components of plants' environment on plant compounds that affect the chemical basis for sensory attributes and health potentials in humans (Radovich, 2010). The physiological aspects of terroir deal with soil, climate, and elevation of the cultivated land (Radovich, 2010). Other factors such as the vine (rootstock and cultivar) and human factors such as viticultural and enological techniques are also included in the term terroir (Van Leeuwen et al., 2004). The literature shows that technically, there exists a scientific relationship between these aspects of terroir and the growth potential and quality attributes of plants produce (e.g., Verkerk et al., 2009; Ahuja et al., 2010; Björkman et al., 2011). These authors reported that biotic (e.g., plant exposure to pests and diseases), abiotic (e.g., season, light, temperature, carbon dioxide concentration), and agronomic factors (e.g., soil type, fertilization with organic or chemical fertilizers, and other cultural practices), determine the growth performance and phytochemical composition of growing plants. For example, Verherk et al. (2009), reported that cultural practices such as site selection, cultivar/accession selection, planting date, irrigation, and plant nutrition influence the GS content in vegetables. Björkman et al. (2011), also reported that abiotic, biotic, and cultural practices influence plants' phytochemical profile, including GS profile.

Problem statement

Many studies conducted on the different GS-containing plants, including the *Brassica* species and the model plant, *Arabidopsis thaliana* (e.g., Rosa & Rodrigues, 1998; Pereira et al., 2002; Klein et al., 2006; Schreiner et al., 2009; Justen et al., 2011; Martínez-Ballesta et al., 2013;

Brandt et al., 2018; Chorol et al., 2018) may potentially provide a comprehensive overview of plants' response to individual aspects (e.g., cultivar or climate or soil) of terroir. However, the response of a fast-growing perennial tree plant such as *M. oleifera* to terroir may be very different from what has been identified in, for instance, non-woody plants such as the *Brassica* species. Moreover, how plants respond to terroir is not well understood, because it is complex and dynamic, especially when the plants under investigation are grown under field conditions with the possibility of the plants experiencing multiple, yet interacting aspects of terroir. Under field conditions, biotic and abiotic factors combined with varying agronomic factors are diverse (Ahuja et al., 2010). Meanwhile, plants' responses are also varied because fundamentally, plants use complex metabolic processes overlaid by and interacting with numerous other factors to maximize these resources for optimal growth and development (Mundim & Pringle, 2018). Moreover, different metabolic processes in individual plants require specific enzymes to alter specific biochemical reactions leading to physiological and morphological changes as the plant grows (Barnabás et al., 2008; Sakata & Higashitani, 2008; Hatfield & Prueger, 2015). In this context, an objective determination of the impact of specific terroir on important secondary plant metabolites such as the GS profile of *M. oleifera* growing, particularly under field conditions, could be challenging. Hence, for the first time, this study determined the impact of terroir on the GS profile of *M. oleifera* by focusing on site selection and cultural practices, including accession selection, fertilization, and harvest time, under field conditions.

With regards to site selection, three agro-ecological zones, i.e., Guinea savannah, Transitional, and Deciduous Forest zones, covering 63%, 28%, and 3% total land area of Ghana, were selected for this study to understand how the growth performance and the GS synthesis in *M. oleifera* are affected by the unique climatic conditions of these zones. By inference, the selected zones cover 94% of the total land area of Ghana (FAO, 2005a; FAO, 2005b); thus, this study's outcome will be impactful for *M. oleifera* farmers in Ghana and other regions of the world with a similar climate like that of Ghana.

One of the cultural practices that can influence growth performance and GS profile in *M. oleifera* and of particular importance in this study is accession selection. A growing number of studies have used different accessions of *M. oleifera* from genebanks to better understand the genetic diversity among accessions for breeding programs (e.g., Muluvi et al., 1999; Rufai et al., 2013; Ganesan et al., 2014). This helps plant breeders monitor, identify, and select the plant's useful diversity to develop new cultivars in a dynamic agricultural environment. Still, an increase in the collection and characterization of unknown accessions of *M. oleifera* from various populations could be useful in selecting well-suited accessions adapted to the different environmental-related stress conditions for optimum yield and GS content. Therefore, further research in this context is still relevant.

Developing the plant's cultivation requires the implementation of more productive cultural practices under favorable environmental conditions that will increase the plant's productivity with enhanced phytochemical composition. Thus, a need to study the impact of terroir on the growth and important secondary plant metabolites such as GS in *M. oleifera* in order to recommend applicable tools and cultivation methods for cultivating healthy *M. oleifera* with an enhanced GS profile.

The applied question behind the entire research was: how does terroir (climate, elevation, soil, and cultural practices) impact the GS profile of *M. oleifera*? Hence, the main focus of this Ph.D. research was to integrate multi-factor field studies to investigate the impact of terroir on the GS profile of both wild-grown (i.e., mature plants growing without human intervention) and cultivated *M. oleifera* accessions under semi-controlled field conditions at three agro-ecological zones in Ghana.

2. Scientific Background

2.1 Utilization of *Moringa oleifera*

Moringa oleifera has been used for many years for many purposes, and the edible parts usually include the leaves, young pods, seeds, and flowers (Thurber & Fahey, 2009; Sauveur & Broin, 2013; Palada, 2019). Different cultures and eating preferences determine the regional use of the plant (Palada, 2019). For example, the young green pods are consumed more than the leaves in India; in the Philippines, the leaves are commonly consumed more than green pods; in Ghana, it is mainly cultivated for the leaves and oil; in Seychelles, mainly for oil, and as an ornamental in Mexico (Sauveur & Broin, 2013; Palada, 2019). However, *Moringa* products, particularly the plant's leaves and seeds are mostly marketed for their nutritional and medicinal reasons. Therefore, this section will highlight the use of *Moringa* leaves and seeds as food in human diets and for medicinal purposes.

2.1.1 Food

The nutritional properties of, especially the leaves, are widely known, and there appears to be no doubt that it provides high nutritional benefits when consumed, particularly in situations where starvation is imminent (Thurber & Fahey, 2009). Indeed, the plant's nutritional attribute is generally linked to the leaves' putatively high protein content, ranging from 23% to over 35.37% on a dry weight basis (Joshi & Mehta, 2010; Compaoré et al., 2011). These values appear to be unreasonably high from a plant physiology perspective, as seeds are usually known to be the main storage organs for proteins. Moreover, the proteins in the leaves have all the essential amino acids in good proportions. The essential amino acid pattern is higher compared to the amino acid pattern of the FAO/WHO reference protein for a 2- to a 5-year-old child and comparable to those in soybeans (Bau et al., 1994; Makkar & Becker, 1997). Thus, the plant's leaves could be an alternative protein-rich food source in areas where children have high protein deficiency (Fuglie, 2005). This notwithstanding, a recent study reported that the plant's protein content and amino acid composition are not exceptional (Lewerenz et al., 2021), as authors (e.g., Oduro et al., 2008; Tetteh et al., 2010; Amaglo et al., 2010) have shown in past years. Lewerenz et al. (2021), suggested that the protein content, as determined according to the Kjeldahl method of analysis for crude protein extracts, is similar to what has been identified in the leaves of other plant species. For example, a previous study revealed that cassava leaves have crude protein content ranging between 29% and 38% (Yeoh & Chew, 1976). Further, a study that investigated 70 different leaf materials from 60 tropical plant species in Ghana reported that ten of the studied species revealed, similarly, protein content higher than 28% in their leaves, and 28 of the species showed protein content higher than 24% (Byers, 1961). Therefore, it was confirmed that the putative high protein content of *M. oleifera* leaves is not exceptional (Lewerenz et al., 2021).

In Africa, particularly in Ghana, the leaves of *M. oleifera* are eaten fresh, cooked, or stored as dried powder for other uses (Popoola & Obembe, 2013; Adu-Dapaah et al., 2017). With a growing need for more sustainable plant-based protein to reduce protein deficiency, particularly among children, *Moringa* has become a useful source of protein in low-income tropical countries including Ghana, where other sources of protein including legumes and meat are limited in the diet of the poor (Fuglie, 2005; Amaglo et al., 2017; Nadathur et al., 2017). *Moringa* leaf powder has also been used as a protein supplement (Waterman et al., 2021). Yet so far, there is no scientific evidence of its proteins' bioavailability and absorption, or determination of changes in the metrics of muscle growth, performance, or exercise recovery in human subjects (Waterman et al., 2021).

The seeds of *M. oleifera*, as previously mentioned, have also been used in human nutrition, besides their use in non-food applications, including water purification, wastewater treatment, biodiesel fuel, cosmetics, machine lubrication, and organic fertilizer for enhanced agricultural productivity (Leone et al., 2016). For this study, the emphasis will be on their use as food.

The seeds are consumed fresh as peas, pounded, roasted, and eaten like nuts (Mbah et al., 2012). The dry seeds contain protein content ranging from 29.36 to 33.30% (Oliveira et al., 1999; Anwar & Rashid, 2007a; Leone et al., 2016). The literature also provides different contents of carbohydrates (16.5 to 19.8 %), fiber (6.8 to 8.0%), ash (4.4 to 6.9%), and moisture (5.7 to 8.9%) for the seeds (Oliveira et al., 1999; Abdulkarim et al., 2005; Anwar & Rashid, 2007a; Leone et al., 2016). These differences are due to differences in environmental factors of the location for cultivation, cultivars, and cultural practices (Leone et al., 2016).

Given the high protein level, the seeds also provide consumers with a high source of protein and can also supplement other protein sources to increase the protein intake of low-income families who cannot afford expensive animal protein sources. Their proteins contain a high level of amino acids, including methionine and cysteine, comparable to milk and eggs (Oliveira et al., 1999). Thus, they can be used to complement legumes that are usually low in these amino acids. Interestingly, the seeds do not contain trypsin inhibitors and urease activity and thus have as high as 93% protein digestibility (Oliveira et al., 1999; Leone et al., 2016).

The seeds of *M. oleifera* are made up of about 36.7% fat (Leone et al., 2016). A three-year-old *M. oleifera* tree can produce 15,000 to 25,000 seeds per annum. One hectare plantation can yield 3000 kg of seeds per annum (Amaglo et al., 2017). This produces 900 kg of oil, translating into a 30% yield of oil compared to soybean, which produces an average of 3000 kg of seeds per hectare, yielding 20% oil (Amaglo et al., 2017). The oil is edible and has a pleasant flavor comparable to peanut oil, using a vapor analyzer (electronic nose) (Abdulkarim et al., 2005).

Fats and oils are predominantly triacylglycerols or triglycerides, accounting for about 92 to 99% of the total content (Narasinga Rao, 2001). For example, a study showed high triacylglycerols content in *M. oleifera* whole- and shelled-seed oil ranging between 95 to 97% (Boukandoul et al., 2017). Apart from the fatty acids forming part of the triacylglycerols, the fats, and oils also contain the nonglycerides, which are fat-soluble phytochemicals in reduced amounts (i.e., 1 to 8%) (Narasinga Rao, 2001). They include sterols, tocopherols (vitamin E), terpene alcohols, hydrocarbons, long-chain alcohols including waxes, carotenoids, and flavor compounds that contain sulfur (S) and nitrogen (N) (Pereira et al., 2016). The most reported of these components of fats and oils for *M. oleifera* are the fatty acids, sterols, and tocopherols because these are mostly extracted with the oils from its seeds and possess distinct functions in human nutrition (Tsaknis et al., 1999; Abdulkarim et al., 2007; Leone et al., 2016; Nadeem & Imran, 2016).

The total monounsaturated fatty acids have been identified to be higher in *M. oleifera* seed oil (78.1%) than those identified in palm olein (46.3%), canola (58.6%), and sunflower oils (25.1%) (Abdulkarim et al., 2007). Its monounsaturated fatty acid chains include two ω-9 monounsaturated acids, [cis-9-octadecenoic (oleic acid) and cis-11-eicosenoic acids], and one ω-7 monounsaturated acid [cis-11-octadecenoic acid (vaccenic acid)] (Vlahov et al., 2002). The seed oils are high in oleic acid (C18:1) content, accounting for more than 70% of the total fatty acids in the oil (Anwar et al., 2005; Amaglo et al., 2010; Leone et al., 2016). Other unsaturated fatty acids present in the oil are 1.86% gadoleic (C20:1), 1.37% palmitoleic (C16:1), and 0.11% erucic (C22:1) acids, while their polyunsaturated fatty acids are very low (1.18%), with linoleic (C18:2), linolenic (C18:3) acids having 0.76% and 0.46%, respectively (Leone et al., 2016). The saturated fatty acids include, palmitic (C16:0), arachidic (C20:0), behenic (C22:0), lignoceric (C24:0), stearic (C18:0), margaric (C17:0) and cerotic acid (C26:0) with mean values of 6.25% 3.23%, 6.02%, 0.36%, 4.97%, 0.07%, 0.92%, respectively (Leone et al., 2016). Reports also indicated that there are traces of lauric n-pentadecanoic (C15:0) and pentadecenoid acids in the oil (Amaglo et al., 2017).

Recent demands from consumers for healthier diets have put the oils from *M. oleifera* in the global vegetable oil industry (Amaglo et al., 2017). This is because the oil from the seeds, which ranges from 34.7 to 40.4% fats (Tsaknis et al., 1999; Abdulkarim et al., 2007; Nadeem & Imran, 2016), has low saturated fatty acid content reported to be between 17 to 27.0% (Amaglo et al., 2010; Pereira et al., 2016), but high content in monounsaturated fatty acids.

Due to the high monounsaturated fatty acid content, particularly oleic acid, *M. oleifera* seed oils are classified among other oils such as olive oils with high health benefits (Abdulkarim et al., 2007; Romano et al., 2021). With a high monounsaturated to saturated fatty acids ratio, it is classified among oils (e.g., olive oil) that have the potential to reduce the risk of cardiovascular diseases (Schwingshackl & Hoffmann, 2014). Indeed, a study reported that oleic acid (cis-9-octadecenoic acid) has the potential to reduce cholesterol (Lokuruka, 2007). Additionally, oils with high oleic acids have good fluidity and low viscosity and are more stable at ambient and high temperatures during cooking (Abdulkarim et al., 2007). Thus, have a low rate of oxidative rancidity, achieving a high degree of oxidative stability in food systems (Abdulkarim et al., 2007). In this context, *M. oleifera* seed oil is also highly stable and has been used like other vegetable oils, including sunflower, canola, and olive oils, for high-heat processing methods such as frying, although the *M. oleifera* oil is more stable than these oils (Abdulkarim et al., 2007; Romano et al., 2021).

M. oleifera seed oil also has different sterol composition from other vegetable oils such as olive oil used in cooking (Tsaknis et al., 1999; Lalas & Tsaknis, 2002). Sterols are essential due to their role in cholesterol metabolism, reducing the level of low-density lipoprotein cholesterol circulating in the blood (Smet et al., 2012).

M. oleifera seed oils contain α-, γ-, δ- and β-tocopherols (Tsaknis et al., 1999; Lalas & Tsaknis, 2002; Anwar et al., 2005; Leone et al., 2016; Boukandoul et al., 2017). The tocopherol profile differs among accessions and differs from other oil such as olive oil (Tsaknis et al., 1999; Lalas & Tsaknis, 2002; Anwar et al., 2005; Leone et al., 2016). For example, a study identified varying amounts of α-, γ-, and δ-tocopherols among different accessions of *M. oleifera* seed oils from Punjab; Sindh; Multan in Pakistan ranging from 114.5 to 140.4 mg/kg, 58.0 to 86.7 mg/kg, and 54.2 to 75.1 mg/kg, respectively (Anwar et al., 2005). Concerning the tocopherols in *M. oleifera* being higher than in other oils, a study reported that, on average, α-, γ- and δ-tocopherols contents are 132.3 mg/kg, 63.9 mg/kg, and 81.2 mg/kg, respectively (Leone et al., 2016). Meanwhile, olive oil, for instance, contains an average of 88.50 mg/kg, 9.90 mg/kg, and 1.60mg/kg contents of α-, γ-, and δ-tocopherols, respectively (Leone et al., 2016; Nadeem & Imran, 2016). Indeed, δ-tocopherol content in *M. oleifera* seed oil (81.2 mg/kg) is higher than those of sunflower (not detected), canola (3 mg/kg), corn (39 mg/kg), soybean (26.7 mg/kg) and olive oils (1.6 mg/kg) (Leone et al., 2016; Nadeem & Imran, 2016). In food systems, the high content of tocopherols, particularly δ-tocopherols, in *M. oleifera* seed oil accounts for their high oxidative stability; thus, preserving the oil during long storage and processing (i.e., cooking and frying) (Tsaknis et al., 1999; Lalas & Tsaknis, 2002; Anwar et al., 2005; Boukandoul et al., 2017). Further, from the standpoint of nutrition, the high α-tocopherol (an active form of vitamin E in humans), with the highest antioxidant activity compound among the four tocopherols, functions as a nonspecific chain-breaking antioxidant, preventing the propagation of free radicals such as peroxyl radicals and singlet oxygen in membranes and plasma lipoproteins (Niki & Kouichi, 2019).

2.1.2 Medicinal and health potential of *Moringa* spp. with particular emphasis on glucosinolates

Glucosinolates in Moringa oleifera

Phytochemicals are categorized into primary and secondary metabolites, depending on their metabolic function in plants (Rabizadeh et al., 2022). Primary metabolites, including carbohydrates, proteins, and lipids are essential for the basic life function of plants, whereas secondary metabolites are the bioactive plant compounds biosynthesized from primary metabolites through various metabolic pathways (Rabizadeh et al., 2022). Secondary metabolites have three forms depending on their biosynthetic pathway and these are; (1) nitrogen-containing compounds, including GS, alkaloids, and cyanogenic glycosides, (2) phenolic compounds, including flavonoids and phenylpropanoids, and (3) terpenes (Jan et al., 2021).

Secondary plant metabolites, including GS, could impact human health and sensory attributes of plant produce, including flavor (i.e., aroma and taste), texture, and color (Jan et al., 2021). *Moringa* contains a range of unique secondary plant metabolites, including GS (Fahey et al., 2001). For *M. oleifera*, the most predominant GS is glucomoringin, 4-(α-L-rhamnopyranosyloxy) benzyl GS, and three monoacetylated isomers of this GS, glucomoringin monoacetyl-isomer I, II, or III (acetyl-4-α-L-rhamnopyranosyloxy-benzyl GS I, II and III) identified, although in lower quantities as compared to glucomoringin in the leaves of

M. oleifera (Bennett et al., 2003) (Fig. 1). Also, two multiple glycosylated GS were identified in *M. oleifera*, which contain a glucopyranosyloxy-benzyl moiety as their R-group (not described previously), as against the rhamnopyranosyloxy-benzyl moiety that has been previously identified in GS from *Moringa* species (Fahey et al., 2018). These GS are 4-(α-L-glucopyranosyloxy) benzyl GS (glucosoonjnain) and 4′-O-acetyl-4-(α-L-glucopyranosyloxy) benzyl GS. Of these two GS, glucosoonjnain and the well-documented glucomoringin (Fig. 2) were the predominant GS in both the wild-type and domestic populations of *M. oleifera* (Chodur et al., 2018; Fahey et al., 2018). The domestic *M. oleifera* contained higher glucomoringin contents than glucosoonjnain content, while the wild-type *M. oleifera* had higher glucosoonjnain content than glucomoringin content (Chodur et al., 2018; Fahey et al., 2018). Other GS such as glucosinalbin (4-hydroxybenzyl GS), glucoiberin (3-methylsulfinylpropyl GS), glucoraphanin (4-methylsulfinylbutyl GS), glucotropaeolin (benzyl GS), 3-hydroxy4-(α-L-rhamnopyranosyloxy)-benzyl GS, 4-(-2′-O-acetyl-αL-rhamnopyranosyloxy)-benzyl GS, 4-(-3′-O-acetyl-α-L-rhamnopyranosyloxy)-benzyl GS, 4-(-4′-O-acetyl-α-L-rhamnopyranosyloxy)-benzyl GS, glucoraphenin, and glucobarbarin have also been identified in different tissues of *M. oleifera* (Maldini et al., 2014).

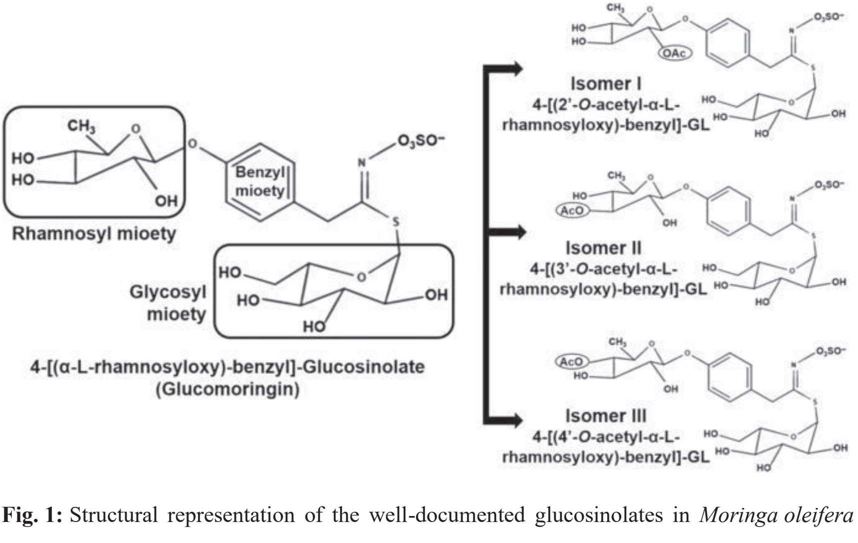


Fig. 1: Structural representation of the well-documented glucosinolates in *Moringa oleifera* leaves. Circled AcO indicates the position of the acetylated group in the isomers (i.e., isomers I, II, and III) (Lopez-Rodriguez et al., 2020)