UNIVERSITY OF NAPLES "FEDERICO II"

DEPARTMENT OF AGRICULTURAL SCIENCES

AND

AN-NAJAH NATIONAL UNIVERSITY

FACULTY OF GRADUATE STUDIES

MASTER DEGREES IN

FOOD SCIENCE AND TECHNOLOGY

AND

NUTRITION AND FOOD TECHNOLOGY

Experimental thesis

METABOlOMICS AND ANTI-MICROBIAL ACTIVITY OF MORINGA OLEIFERA

Tutor:

Dr. Mohammed Sabbah

Tutor:

Prof. Virginia Lanzotti

Co-Tutor:

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Candidate: Mohammed Ammar

N0: 6/00105

Academic year 2020-2021

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Dedication

I dedicate my dissertation work to my family , and my frinds . A special feeling of gratitude to my loving to my parents , Iyad and sabbah whose words of encouragement and push fot tenacity ring in my ears. My brothers and my sister have never left my side and are very special.

I also dedicate this works and give special thanks from my heart to my great and best brother Atitilo Anzano who supproting my at each time and give me a lot of possitive energy to complete my works.

I also dedicate this works and give special thanks to my great and best professors Dr.Mohammed Sabbah, Prof. Virginia Lanzotti and Rosanna Capparelli.

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الأقرار

انا الموقع ادناهو مقدم الرسالة التي تحمل العنوان:

METABOlOMICS AND ANTIMICROBIALACTIVITY OF MORINGA OLEIFERA

اقر بأن ما اشتملت عليه هذه الاطروحه انها هو نتاج جهدي الخاص ، ومشاركه في التحليل من قبل المشرفين . وان هذه الرساله كاملة و او اي جزء منها لم يقد لنيل اي درجه او لقب علمي او بحثي لدى اي مؤسسة تعليمة او بحثية اخرى.

Declaration

The work provide in thesis, unless otherwise referenced, is the researchers own work, and has not been submitted elsewhere for any other degree or qualification.

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Signature: **التوقيع:**

Date: 27/7/2021 **التاريخ:**

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MOS	Moringa oleifera leaves
MOL	Moringa oleifera leaves
As	a polar extract from seeds
AF	a polar extract from leaves
PS	polar extract of Moringa oleifera seeds
PF	polar extract of Moringa oleifera leaves
GABA	gamma-aminobutyric acid
NSAIDs	Non-steroidal anti-inflammatory drugs
D_2O	deuterated water
PPM	parts per million
$CDCl_3$	deuterated chloroform
MHz	megahertz
DSS	sodium trimethylsilylpropanesulfonate
AOAC	Association of official analytical chemists
ALP	alkaline phosphatase
AST	aspartate transaminase
ALT	transaminase
CCl4	Carbon tetrachloride
CCl3.	trichloromethyl
CCl3O2.	trichloromethyl peroxyl
MO	Moringa oleifera

METABOlOMICS AND ANTI-MICROBIAL ACTIVITY OF MORINGA OLEIFERA

By
Mohammed Ammar
Supervisors
Dr. Mohammed Sabbah
Prof. Virginia Lanzotti
Prof. Rosanna Capparelli

Abstract

The World Health Organization's Global Antimicrobial Surveillance System report tracked the occurrence of bacterial infections with antibiotic resistance in half a million people around the world. Moreover, Multi-resistant strains are becoming more common and dispersed, posing a serious threat to public health. As a result of this situation, alternative strategies were investigated, including herbal extracts and plant-derived compounds. Antibiotic resistance is defined as a drug's inability to kill a microbe that it was previously used to inhibit or kill. Antimicrobial resistance is widespread around the world, posing a threat to the ability to manage common infectious diseases, resulting in increased mortality and morbidity. Medicinal plants have been used to treat common infectious diseases since ancient times. Also, it can be considered a safe, and cheap source for microbial treatment. Consequently, the efficacy and efficiency of medicinal plants in the antimicrobial domain that encourages researchers to study their compositions and investigate them as a natural way to control and inhibit bacteria activity. An example of herbs that have antimicrobial activity, which has been studied is Guava (*Psidium guajava*), Sage (*Salvia officinalis*), Rhamnus (*Ziziphusspina Christi*), Mulberry (*Morusalba* L.)

which they show good antimicrobial activity.

Ben oil is an ancient medicine obtained by the seeds of *Moringa oleifera* Lam. Plant. It has been used for a variety of purposes. It has also been the subject of considerable research due to its multiple applications and well-known bactericidal properties. In addition, it contains a substance called pterygospermin, which has antibacterial and fungicidal properties. *M. oleifera* extracts from seeds, leaves, root, and seed oil extracted using various solvents have been shown to inhibit the growth of gram-positive and gram-negative bacteria in general. Furthermore, *M. oleifera* parts have been reported to be a rich source of phytochemicals such as flavonoids, phenolic, and alkaloid compounds, as well as bioactive peptide and polysaccharide compounds which can be used as antimicrobials.

Chapter one
Literature Review

1.1 Introduction

Moringa oleifera Lam., commonly named “Miracle tree” is a member of the Moringa genus, which is the only one of the Moringaceae family. The plant is also known by other names depending on its geographical region. Apart from M. oleifera, the Moringaceae family includes 13 species (M. oleifera being the most popular of the thirteen species in the variety Moringa of family Moringaceae). The species are the following: M. oleifera, M. concanensis, M. drouhardii, M. arborea, M. borziana M. hildebrandtii, M. longituba, M. pygmaea, M. rivae, M. ruspoliana, M. ovalifolia, M. peregrine, and M. stenopetala which are native to Africa, Arabia, India, Southeast Asia, South America, the Pacific Islands, and the Caribbean.

Furthermore, the plant is one of the most popular plants that can be grown in a variety of environments due to its ability to grow in harsh conditions such as high temperatures and limited water availability [6,14]. It can also grow in a variety of soils (semi-dry, desert, or tropical soils) and rainfall conditions. Roots rot in waterlogged soil, whereas they can grow with very little moisture because their roots can store moisture for long periods. The plant tolerates a wide range of soil types and pH levels, ranging from 5.0 to 9.0. It prefers neutral pH and well-drained soils. It thrives in temperatures ranging from 25 to 40 °C, though it can withstand temperature swings of -1

to 3 °C and 38 to 48 °C during the coldest and warmest months, respectively.

M. oleifera is among the food plants richest in nutrients. It has a high content in vitamins, essential amino acids, proteins, minerals, vitamins, polyphenols, and phytochemicals (like flavonoid or isothiocyanates, anthraquinone, alkaloids, essential oils, tannic acid, saponins, steroids, terpenoids, cardiac glycoside, anthocyanin, carotene) [69]. In addition, It is used to treat individuals with extreme malnutrition as well as for its pharmacological (hepatoprotective, antihypertensive, cholesterol-lowering, anti urolithiasis, antifertility, antidiabetic, and antioxidant activity, nutraceutical properties, and antimicrobial (Table 3) [28]. Moreover, Moringa is being used to help to breastfeed mothers improving postpartum milk production [63].

1.2 Plant description

M. oleifera is a short, slender, deciduous, perennial tree that grows to about 10 m tall, slender with drooping branches; branches and stem are brittle, with corky bark.

Leaves are feathery, pale green, compound, tripinnate, (30-60 cm long), with many small leaflets, 1.3-2 cm long, 0.6-0.3 cm wide, lateral ones slightly elliptic, terminal ones obovate, and slightly larger.

Flowers are fragrant, white or creamy-white, 2.5 cm in diameter, and borne in sprays, with five(5) at the flower's top. The stamens are yellow, and the

pods are pendulous, brown, triangular, splitting lengthwise into three parts when dry, and containing about 20 seeds embedded in the pith. The pod has nine (9) ribs on both ends and the seeds are dark brown with three papery wings[71].

1.3 Morphology

M. oleifera consists of different parts, including A) leaves, B) seeds and seed oil, C) flowers (Figure 1).

1.3.1 Leaves

The feathery leaves of the tripinnate complex have green curved leaflets that are 1-4 cm long. Because of its leaves, the tree is frequently mistaken for a leguminous plant. The alternate twice or thrice pinnate leaves appear at the branch tips in most cases. They have a long petiole with 8-10 pairs of pinnae, each bearing two sets of inverse elliptic leaflets and one at the apex, and are 20-70 cm long when young [14,52].

1.3.2 Seeds

The seeds have three papery wings and are oval with a tannish semi-

Figure 1: M. oleifera tree parts

Permeable seed arrangement. Their arrangements are mostly brown to dark brown but can be white if portions are of low viability. It almost within a week, viable seeds sprout. The body has three white wings that run at 130 intervals from start to finish [14, 52].

1.3.3 Flowers

Flowers are prominent, softly fragrant, and are borne on inflorescences 15-25 cm long. They are mostly white to cream in color, 2.5 cm in diameter, and tinged pink in a few varieties. However, the flowers are fragrant and 2.5 cm wide, and they bloom profusely in auxiliary, dropping panicles that are 10-25 cm long. They have a dotted base and are white in color. The direct lanceolate sepals are five-reflexed. The five petals are rumored to be thin. Except for the lowest stamen, they are reflexed and

consist of five stamens and five staminodes [14, 52].

1.4 Traditional uses

People in ancient times used parts of the *M. oleifera* tree for a variety of medical purposes, including the treatment of various diseases, wound healing (Figure2), and nutritional purposes to reduce the risk of malnutrition. These things encourage the researcher and Scientists to investigate and study this plant to learn more about its composition and how to investigate it in various domains.

1.5 Pharmacological Actions

1.5.1 Antidiabetes activity

Diabetes is a disease defined by problems with the hormone insulin production. In healthy individuals, the pancreas produces insulin, which helps the body use and stores fat and sugar obtained from the diet. Insulin levels in diabetics can be harmed in a variety of ways. In addition, the pancreas may not produce any insulin at all in some cases. Other times, the body does not react to insulin properly, which is referred to as "insulin resistance." Finally, diabetes can be caused by an insufficient amount of insulin produced by the pancreas, Interestingly, M. oleifera leaves have a powerful anti-diabetic effect by increasing antioxidant levels and inhibiting pro-inflammatory mediators [4, 8, 71].

1.5.2 Antioxidant activity

Antioxidants are bioactive compounds that inhibit free radicals and substrate oxidation. They play an important role in protecting cells from oxidative damage caused by free radicals by blocking the process of oxidative damage [74]. However, polyphenol-rich natural compounds have strong antioxidant properties and can reduce oxidative damage in tissues by scavenging free radicals. M. oleifera has a high antioxidant activity due to its high content of bioactive polyphenols [39].

1.5.3 Anticancer Activity

The rising global burden of cancer necessitates a new treatment option. Herbal medicine offers a viable alternative to conventional cancer treatment. Furthermore, it has evolved into a very safe, non-toxic, and easily accessible source of cancer-fighting compounds [38]. Several bioactive compounds with antitumor activity have been found in M. oleifera parts (leaves, seeds) that can be effective against several types of human cancer cells [7,20,34,36,59].

1.5.4 Improve radiotherapy

Cancer radiotherapy aims to kill tumor cells effectively, but it also causes irreversible damage to normal tissues, which is undesirable. The success of radiotherapy is because normal tissues are spared from radiation damage. Natural phytochemicals can protect normal cells while also increasing tumor cell susceptibility to radiotherapy by modulating cellular molecular

targets. M. oleifera contains bioactive compounds such as polyphenols (alkaloids and flavonoids), which are naturally occurring phytochemicals with potent antioxidant activity and assistance in free radical scavenging. It can also be used for radioprotection and radiosensitization. They were also found to suppress the cytokines tumor necrosis factor-alpha and inducible nitric oxide production [67].

1.5.5 Anti-Inflammatory Activity

Inflammation is a natural defense mechanism that helps the body fight infection and heals tissue damage. Chronic inflammation, on the other hand, can lead to chronic inflammation-related diseases and disorders such as diabetes, cancer, autoimmune diseases, cardiovascular diseases, sepsis, colitis, and arthritis [8, 11]. Inflammatory cytokines such as interleukin-1 beta (IL-1) and tumor necrosis factor-alpha (TNF-) can stimulate or enhance the activity of inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), and microsomal PGE synthase-1 (mPGES-1) in target cells by upregulating the production of nitric oxide (NO) and prostaglandin E2 (PGE-2) [40]. M. oleifera has been shown to reduce the production of TNF-, IL-6, and IL-8 in response to both lipopolysaccharide (LPS) and lipopolysaccharide (LPS). In addition, in the inflammation stage, inhibit the expression of RelA, a gene involved in nuclear factor-kappa B (NF-B) p65 signaling [51]. Besides that, in lipopolysaccharide (LPS)-induced RAW264.7 cells, M. oleifera can inhibit the production of iNOS and COX-2, as well as the secretion of NO and other inflammatory markers like

PGE-2, TNF-, IL-6, and IL-1[55]. Meanwhile, it can contribute to the suppression of the NF-B signaling pathway by inducing the production of IL-10 in LPS-stimulated macrophages in a dose-dependent manner [39].

1.5.6 Anti-ulcer Activity

A gastric ulcer is caused by a break in the stomach's tissue lining. The term peptic ulcer refers to ulcers that develop in the stomach or the duodenum, the first part of the small intestine that leads out of the stomach. However, a variety of factors can cause damage to the gastroduodenal mucosa, including systemic events like stress or local administration of irritants like aspirin and Non-steroidal anti-inflammatory drugs (NSAIDs), which are common mucosal barrier breakers [46]. Furthermore, Cerebellar nodulation, which is considered to be responsible for the pathogenesis of gastroduodenal damage by reducing mucous secretion, can cause it.

The neurotransmitter 5-HT, also known as serotonin, is found in the EC cells of the gastric mucosa and acts through 5-HT3 receptors in the gastrointestinal tract. In general, EC cells are found throughout the alimentary canal, primarily in the gastric mucosa, and there is a strong correlation between EC cell count and 5-HT content. According to a study using the 5-HT3 receptor antagonist ondansetron, M. oleifera may trigger the healing of gastric damage by releasing 5-HT from EC cells of the gastric mucosa via 5-HT3 receptors [19]. The use of this antagonist reduced the amount of 5-HT without affecting the number of EC cells in the stomach, causing gastric tissue damage and reducing the protective

effect of MO. The formation of EC cells in the gastric mucosa may also be stimulated by MO. As a result of the current study, it can be concluded that MO protects against gastric ulcers by modulating 5-HT and EC cells [19].

1.5.7 Cardioprotective

Cardioprotective drugs are important in the treatment of patients who are at risk for cardiovascular disease or who already have it (CVD). M. oleifera parts(leaves and seeds) can be used in the treatment of cardiovascular diseases, interestingly, Moringa oleifera leaves(MOL) contain bioactive polyphenols like niazinin A, niazinin B, niazimicin, and niazinin AB which help to reduce blood pressure [58]. Besides that, the polyphenolic fraction of M. oleifera leaf extract reduced oxidative stress and restored increased levels of creatine kinase, serum troponin-I, lactate dehydrogenase, and heart tissue malondialdehyde content to normal levels, resulting in reduced myocardial damage and oxidative stress [41,58]. Additionally, β-sitosterol derived from Moringa leaves has a cholesterol-lowering effect, and Flavonoids-Quercetin has hypolipidemic activity. The inflection of glutathione, superoxide dismutase, catalase, creatine kinase-MB, lactate dehydrogenase, and peroxidase enzymatic parameters are thought to be the mechanism of M. oleifera's cardioprotective effect [11], Otherwise, the chemical constituents of the seeds are nitrile, mustard oil glycosides, and thiocarbamate glycosides, which are thought to be responsible for cholesterol-lowering, antiulcer, hepatoprotective, and cardiovascular-protective properties [32].

1.5.8 Hepatoprotective

The liver is involved in a variety of physiological processes in our bodies, including metabolic, secretory, and storing functions. It also plays a role in the detoxification of a wide range of drugs and xenobiotics. In this case, the liver is vulnerable to these agents' toxicity because the metabolic products of detoxification reactions can be harmful to the liver [30].

Carbon tetrachloride (CCl4) is one of the most widely used hepatotoxins in research on liver injury caused by oxidative stress and free radicals. Reductive dehalogenation products such as trichloromethyl (CCl3.) and trichloromethyl peroxyl (CCl3O2.) radicals are responsible for CCl4's hepatotoxicity. These radicals can bind to proteins and lipids, or remove a hydrogen atom from unsaturated fatty acids, causing lipid peroxidation and liver injury [22]. However, Moringa oleifera leaves extract could improve the hepatomegaly induced by chronic CCl4 administration in rats. The reduction in transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities as a result of M. oleifera leaves extract suggests an early improvement in hepatic cell cellular membrane integrity, which is a clear manifestation of its anti-hepatotoxic effect[17,22].

1.5.9 Anti-allergic

In an allergic reaction, histamine is a well-known mediator that causes bronchoconstriction and vasodilation. After allergen sensitization, the early phase of an allergic reaction occurs, releasing histamine and other

mediators. After 2 to 6 hours of sensitization, cytokines such as IL-13, IL-4, IL-9, IL-5, IFN-, and TNF- are released, indicating the late phase of the allergic reaction. The most pivotal cytokines in an allergic reaction are IL-4 and TNF-α. Moringa oleifera leaves (MOL) are high in flavonoids (quercetin), which have been shown to inhibit histamine and IL-4 production [1, 37]. Furthermore, MOL Aqueous extract, which helps in the suppression of mast cell activation and/or the improvement of the Th1/Th2 balance to Th1 dominance [29]. As well the release of beta-hexosaminidase, histamine, and TNF- was inhibited more effectively by M. oleifera seeds extract than by ketotifen fumarate. It could be due to the presence of glucomoringin, an isolated compound [1, 64].

1.5.10 Hyperthyroidism activities

Thyroid hormone has a significant impact on cardiac function and structure, and hyperthyroidism is one of the most common thyroid gland disorders that is increasing day by day globally, especially in developing countries. Thyroid hormone overproduction affects cardiovascular hemodynamics, resulting in high-output heart failure and, later, dilated cardiomyopathy [66]. the thyroid gland produces two hormones, T4 (is synthesized primarily in the thyroid gland and is directly secreted into the blood, where it is converted to T3 by the activity of type-I 5′-iodothyronine monodeiodinase (5′ -DI)) and T3 (is the major thyroid hormone, responsible for most of the metabolic effects of an organism including calorigenesis, oxygen consumption and maintenance of the basal metabolic

rate) These hormones control the metabolism and function of many organs. MOL extract reduces type-I 5′-iodothyronine monodeiodinase (5′ -DI) activity and hepatic lipid peroxidation LPO [70]. In addition, there a significant effect of moringa oleifera extract on decreasing of TSH hormone and reduced total cholesterol concentration and low-density lipoproteins cholesterol (LDL).

1.5.11 Antimicrobial

Antibiotic resistance is on the rise, and with it comes a drop in antimicrobial discovery. This has prompted researchers to look into alternative antimicrobial therapies. Humans have relied on plants for effective antimicrobial agents for centuries. Plants, on the other hand, appear to have the ability to enhance the activity of other antimicrobials, according to research. As a result, the goal of this study is to see how effective M. oleifera (leaves, seeds, and seed oil) extracts are against bacterial triggers of autoimmune diseases on their own. The study will also look at the M. oleifera extracts' qualitative phytochemical to know more about the chemical and biological properties of a plant that is widely used in traditional medicine around the world. However, M. oleifera parts (leaves and seed) were extracted using solvents of varying polarity and found to have promising antibacterial properties, with strong inhibitory effects on Gram-positive species (Staphylococcus aureus and Enterococcus faecalis) and weak inhibitory effects on Gram-negative species (Escherichia coli, Salmonella, Pseudomonas aeruginosa, Vibrio

parahaemolyticus, and Aeromonas caviae) [2, 16, 21, 24, 26, 53, 56, 57, 72].

1.6 Nutritional value

M. oleifera trees have been used in ancient times to combat malnutrition, especially among infants and nursing mothers. It one of a natural plant with high nutritional value. However, *Moringa* leaves, pods, and seeds contain a variety of essential phytochemicals, making it more nutrient-dense plants. *Moringa* is said to have 7 times the vitamin C of oranges, 10 times the vitamin A of carrots, 17 times the calcium of milk, 9 times the protein of yogurt, 15 times the potassium of bananas, and 25 times the iron of spinach. *M. oleifera* leaves are considered a rich source of certain macro and micronutrients, interestingly, moringa leaf contains high quantities of macronutrient (fat, carbohydrate), and micronutrients vitamins (folates, niacin, riboflavin, thiamin, vitamin A and vitamin C), minerals (calcium, iron, magnesium, phosphorus, selenium, and zinc) and electrolyte (mainly potassium and sodium) [69].

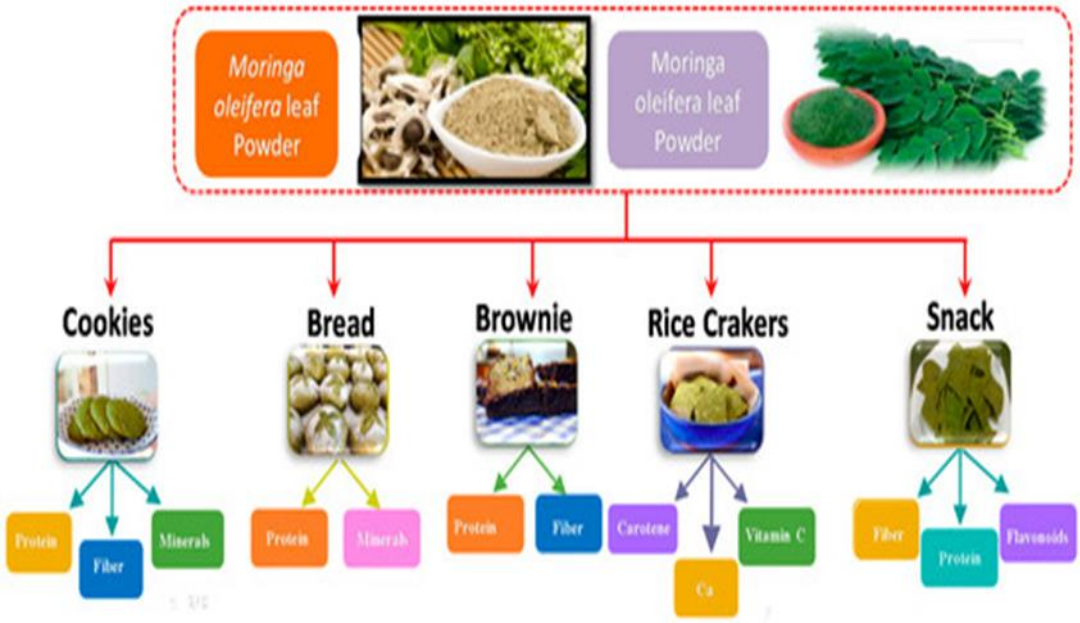

Figure 2.M. oleifera bakery industries application[50]

This encourages industrial factories to incorporate moringa in the manufactured product as a raw ingredient in manufacturing products, for example, bakery (cookies, bread, brownie, rice crackers, and snacks) (Figure 2) [55].

1.7 Chemical composition

Table 1. Chemical composition of *Moringa oleifera*

Moringa part	Component	References
Leaf	Alkaloids Tannin Saponin Flavonoids **Minerals** **Macro-elements** Sodium Potassium Calcium Magnesium Phosphorus Sulfur **Micro-elements** Zinc Iron Manganese Copper	[11,22,53]

	Selenium Lead **Nutrients** Carbohydrate Protein Fat Fiber	
Seed and leaves*	Vitamin B1 Vitamin B2 Vitamin B3 Vitamin C Vitamin E **Amino acids** **Essential amino acids** Threonine Valine Leucine Lysine Phenylalanine Methionine Cysteine Tryptophan Histidine **Non-essential amino acids** Asparagine Proline Arginine Alanine Glycine Serine	[11,42]
Seed	Saponins Tannins Alkaloids Flavonoids Cardiac glycosides	[42]

*** Richer in leaves**

Table 2: Main organic compound isolated from M. oleifera plant.

Compound	Molecular formula	Plant organ		Quantity		Method	Referances
Fatty acid name				**leave**	**seed**		
Caproic acid		Leave	-	0.1%	-	GC-MS	
Capric acid	$C_6H_{12}O_2$	Leave	-	0.1%	-	GC-MS	
Lauric acid	$CH_3(CH_2)_8COOH$	Leave	-	0.72%	-	GC-MS	
Myristic acid	$C_{12}H_{24}O_2$	Leave	-	1.13%	-	GC-MS	
Palmitic acid	$CH_3(CH_2)_{12}COOH$	Leave	Seed	23.65%	8%	GC-MS	
Palmitoleic acid	$C_{16}H_{32}O_2$	-	Seed	-	1.5%	GC-MS	
Heptadecanoic acid	$C_{16}H_{30}O_2$	Leave	Seed	0.46%	0.32%	GC-MS	[43]
Stearic acid	$CH_3(CH_2)_{15}CO_2H.$	Leave	Seed	4%	7%	GC-MS	
Oleic acid	$C_{18}H_{36}O_2$	Leave	Seed	6%	74.5%	GC-MS	
Linoleic acid	$C_{18}H_{34}O_2$	Leave	Seed	6.84%	0.62%	GC-MS	
Arachidic acid	$C_{18}H_{32}O_2$	Leave	Seed	0.72%	4.28%	GC-MS	
Cis-11-Eicosenoic acid	$C_{20}H_{40}O_2$	Leave	-	54.44%	-	GC-MS	
Linolenic acid	$C_{20}H_{38}O_2$	-	Seed	-	2.5%	GC-MS	
Cis-11, 14-Eicosadienoic acid	$C_{18}H_{30}O_2$ $C_{20}H_{36}O_2$	-	Seed	-	0.48	GC-MS	
Cis-5, 8, 11, 14, 17-Eicosapentaenoic acid.	$C_{21}H_{32}O_2$	Leave	Seed	2%		GC-MS	
P-Methoxyphenyl acetic acid butyl ester (MIMO1).	$C_{13}H_{18}O_3$	Leave	-	222.3 g/mol.	0.60% -	NMR	[32]
P-Hydroxyphenyl acetic acid butyl ester (MIMO2).	$C_{12}H_{16}O_3$	Leave	-	208.3 g/mol		NMR	
Amino Acid					-		
Lysine	$C_6H_{14}N_2O_2$	Leave	-	69.13±0.13mg.100g-^{1}	-	HPLC	
Histidine	$C_6H_9N_3O_2$	Leave	-	29.56±0.21mg.100g-^{1}	-	HPLC	

Valine	$C_5H_{11}NO_2$	Leave	-	62.34±0.19 mg.100g-^{1}	-	HPLC	
Leucine	$C_6H_{13}NO_2$	Leave	-	94.36±0.31 mg.100g-^{1}	-	HPLC	
Isoleucine	$C_6H_{13}NO_2$	Leave	-	46.98±0.15 mg.100g-^{1}	-	HPLC	[15,67]
Threonine	$C_4H_9NO_3$	Leave	-	48.35±0.26 mg.100g-^{1}	-	HPLC	
Alanine	$C_3H_7NO_2$	Leave	-	4.93±0.12 mg.100g-^{1}	-	HPLC	
Aspartic acid	$C_4H_7NO_4$	Leave	-	13.76±0.15 mg.100g-^{1}	-	HPLC	
Serine	$C_3H_7NO_3$	Leave	-	3.13±0.15 mg.100g-^{1}	-	HPLC	
Proline	$C_5H_9NO_2$	Leave	-	1.86±0.13 mg.100g-^{1}	-	HPLC	
Glutamic acid	$C_5H_9NO_4$	Leave	-	18.03±0.09 mg.100g-^{1}	-	HPLC	
Glycine	C2H5NO2	Leave	-	2.31±0.21 mg.100g-^{1}	-	HPLC	
Arginine	$C_6H_{14}N_4O_2$	Leave	-	7.65±0.10 mg.100g-^{1}	-	HPLC	
Cysteine	$C_3H_7NO_2S$	Leave	-	2.15±0.11 mg.100g-^{1}	-	HPLC	
Tyrosine	$C_9H_{11}NO_3$	Leave	-	2.03±0.13 mg.100g-^{1}		HPLC	
Methionine	$C_5H_{11}NO_2S$	Leave		0.43±0.14 mg.100g-^{1}	-	HPLC	
Phenylalanine	$C_9H_{11}NO_2$	Leave	-	3.42 mg.100g-^{1}	-	HPLC	
Bioactive compound			-				
Epicatechin		Leave		5.68 mg/g		HPLC	
Isorhamnetin	$C_{15}H_{14}O_6$	Leave		0.03-0.18 mg/g	-	HPLC-DAD-electrospray mass	
	$C_{16}H_{12}O_7$		-				
Kaempferol		Leave		0.8-3.5 mg/g		spectrometry	[15]
	$C_{15}H_{10}O_6$				-	.	
					-	HPLC-DAD-electrospray	
					-		
1, 30-triacontane diol					-	mass	
Octacosane	$C_{30}H_{62}O_2$	Leave	-	14.98%		spectrometry	
Z-14-nonacosane	$C_{28}H_{58}$	Leave	-	8.57%	-	, LC/MS.	
2,2-dimethyl-1-oxa-2-	$C_{29}H_{58}$	Leave	-	8.3%	-		
silacyclo hexane	$C_6H_{14}OSi$	Leave	-	8.28%	-	GC-MS	
Nonacosane			-		-		
Gamma.-Sitosterol	$C_{29}H_{60}$	Leave	-	15.55%	-	GC-MS	
Pyridine-3- carboxamide	$C_{29}H_{50}O$	Leave	-	9.56%	-	GC-MS	
2-myristynoyl-	$C_6H_6N_2O$	Leave	-	0.87-5%	-	GC-MS	

glycinamide	$C_{16}H_{28}N_2O_2$	Leave	-	0.49-1.33%	-	GC-MS	
9 ,12, 15-	$C_{19}H_{32}O_2$	Leave	-	0.44%	-	GC-MS	
octadecatrienoic acid	$C_{10}H_{18}$	Leave	-	0.83%	-	GC-MS	
Ethylidene cyclooctane	$C_{20}H_{40}O$	Leave	-	0.94%	-	GC-MS	
phytol	$C_{11}H_{20}O$	Leave	-	2%	-		
Cyclopropaneoctanal.	$C_{18}H_{34}O_2$	Leave		0.94%	-	GC-MS	
9-octadecenoic acid	$C_{16}H_{34}$	Leave	-	1%		GC-MS	
Hexadecane	C_9H_8	Leave	-	0.58%	-	GC-MS	
1H-indene	C_4H_7NO	Leave	-	0.52-1.87%	-	GC-MS	
Cyclopropane	$C_{18}H_{32}O_2$	Leave		1.16%	-	GC-MS	
carboxamide			-			GC-MS	
Z, Z- 8,10 hexadecadien	$C_{20}H_{42}$	Leave		0.12-3.66%	-	GC-MS	
1ol acetate	$C_{22}H_{43}NO$	Leave	-	7.15%		GC-MS	
Eicosane	$C_8H_5N_3O_4$	Leave	-	6.51%	-	GC-MS	
13-docosenamide (z)-.			-		-	GC-MS	
5-Acetamido-4,7-dioxo-	$C_{28}H_{58}$	Leave	-	8.57%	-	GC-MS	
4,7-dihydrobenzofurazan						GC-MS	
Octacosane	$C_{14}H_{22}O_2$	Leave	-	5.79%		GC-MS	
1 2-benzenediol 3 5-	$C_{26}H_{50}$	Leave	-	1.86%	-	GC-MS	
bis(1 1-dimethylethyl)-.	$C_{28}H_{48}O$	Leave	-	5%	-		
13-hexacosyne	$C_{16}H_{15}N_3S$	Leave	-	1.26%	-	GC-MS	
Campesterol					-	GC-MS	
5-(p-aminophenyl)-4-(p-	$C_{27}H_{56}$	Leave	-	5.12%		GC-MS	
tolyl)-thiazolamine.	$C_7H_{12}O_2$	Leave	-	6%	-		[20,15,45,73]
Heptacosane	$C_{21}H_{27}NO_2$	Leave	-	3%	-	GC-MS	
cyclohexanecarboxylic	$C_6H_{13}NS$	Leave		0.07%	-		
acid			-			GC-MS	
Methadone N-oxide	$C_{12}H_{18}O$	Leave	-	0.13%	-	GC-MS	
N-Methyl-à-	$C_{16}H_{32}O_2$	Leave	-	22%	-	GC-MS	
diMethylThioPropylami	$C_{18}H_{36}O_2$	Leave	-	0.40%	-	GC/MS	
de.					-		
6-Dodecanone	$C_{18}H_{30}O$	Leave	-	0.16%		GC-MS	
Pentadecanoic acid,	$C_{23}H_{48}$	Leave	-	0.20%	-	GC-MS	

methyl ester	$C_{36}H_{74}$	Leave	-	1.61%	-	GC-MS	
Hexadecanoic acid, 15-methyl, methyl ester.	$C_{24}H_{38}O_4$	Leave	-	0.68%	-	GC/MS	
(E, E)-farnesylacetone	$C_{10}H_{16}O$	Leave	-	0.29%	-	GC-MS	
Heptadecane, 9-hexyl-.	$C_{20}H_{34}O$	Leave	-	0.31%	-	GC-MS	
Hexatriacontane	$C_{14}H_{30}O$	Leave		0.19%	-	GC-MS	
1,2-Benzenedicarboxylic acid, Dioctyl ester	$C_{32}H_{66}$ $C_7H_6O_5$	Leave	-	0.34%		GC/MS	
Limonene oxide	C_7H_6O	Leave	-	0.04 –4.98		GC-MS	
Trans-Geranylgeraniol		Leave		0.06 –4.99 mg/mL	-	GC-MS	
1-Tetradecanol		Leave		0.28-7.57 mg/mL		GC-MS	
Docosane, 11-decyl	$C_{14}H_{19}NO_{10}S_2$	Leave	-	5.47-15 mg/mL			
Flavonoids	$C_{16}H_{19}NO_6S$		-		-	GC/MS	
Isoquercetin		Leave	-	0.39 mg/mL	-	HPLC	
Astragalin			-		-	HPLC	
Myricetin			-		-	HPLC and	
Quercetin	$C_{16}H_{19}NO_6S$	Leave	-	0.06 – 4.98mg/mL	-	MS/MS	
		Leave	-	0.409 mg/g	-		
Rutin		Leave		0.018 -0.489mg/g	-	HPLC-DAD-	
		Leave		0.009- 0.189 mg/g		electrospray	
	$C_{16}H_{19}NO_6S$	Leave	-	0.078 - 0.128 mg/g		mass	
Phenolics		Leave	-	1.034 mg/g	-	spectrometry	
Cryptochlorogenic acid	$C_{21}H_{20}O_{12}$	Leave		ND		.	
Caffeic acid	$C_{21}H_{20}O_{11}$					HPLC	
Chlorogenic acid	$C_{15}H_{10}O_8$						
Ellagic acid	$C_{15}H_{10}O_7$	Leave	-	0.59 mg/g	-	HPLC	
Ferulic acid		Leave		0.69 -5 mg/g			
Gallic acid	$C_{27}H_{30}O_{16}$						
Gentistic acid						HPLC	
Glucosinolates			-		-	HPLC and	
		Leave		0.45-1.5 mg/g		MS/MS	[44,49]
4-hydroxybenzyl (sinalbin)	$C_{16}H_{18}O_9$					HPLC and MS/MS	

4-O-(α-L-acetyl rhamnopyranosyl oxy)-benzyl Glucosinolate isomer 1.	$C_9H_8O_4$ $C_{16}H_{18}O_9$ $C_{14}H_6O_8$ $C_{10}H_{10}O_4$	Leave	-	5.04 -50.2 mg/g	-	HPLC and MS/MS	
4-O-(α-L-acetylrhamnopyranosyloxy)- benzyl Glucosinolate isomer 2	$C_7H_6O_5$ C_7H_6O	Leave Leave	-	21.84–22.56 mg/g 33.79 µmol/g	-	HPLC HPLC-DAD-electrospray mass spectrometry	
4-O-(α-L-acetylrhamnopyranosyloxy)- benzyl Glucosinolate isomer 3	$C_{14}H_{19}NO_{10}S_2$ $C_{16}H_{19}NO_6S$	Leave	- - -	5-20.6 g/kg	- -		[44]
Glucomoringin Glucosoonjnain		Leave	- -	2-50 gDE/kg	- -	HPLC-DAD-electrospray mass spectrometry	
Total tannins Tannins	$C_{16}H_{19}NO_6S$	Leave Leave	-	430-500 mg/100 g 21-31 g/kg	-	, LC/MS	
Total saponins Saponin	$C_{16}H_{19}NO_6S$	Leave Leave	- -	6.63-40 mg/100 g 6.94,102 mg/100 g	- -	HPLC-DAD-electrospray mass spectrometry	[25]
Oxalates and phytates Oxalates Phytates	$C_{21}H_{31}NO_{14}S_2$	Leave Leave	-	26.45% 1.63%	-	, LC/MS.	
Carotenoids β-carotene Lutein	$C_{21}H_{31}NO_{15}S_2$	Leave	-	0.33% 8.66% 1%		HPLC HLCP	
Other phytochemical constituents *cis*-Vaccenic acid 1,2,3-Cyclopentanetriol Diethyl phthalate	$C_{76}H5_2O_{46}$	Leave Leave	-	5-20.6 g/kg 2-50 gDE/kg			

(Z)-Hexyl oleate							
Mannitol,1,4-di-O-methyl-, tetraacetate	$C_{58}H_{94}O_{27}$	Leave	-	430-500 mg/100 g 21-31 g/kg		Folin-Ciocalteu modified	
		Leave	-	6.63-40 mg/100 g			
	$C_2O_4(_2^-)$	Leave		6.94,102 mg/100 g			
	$C_6H_{18}O_{24}P_6$	Leave	-	26.45%		Spectrophotometric method	
	$C_{40}H_{56}$	Leave		1.63%			
	$C_{40}H_{56}O_2$	Leave		0.33% 8.66%		AOAC 2004 Colorimetric method	
	$C_{18}O_{34}O_2$	Leave	-				
		Leave	-			AOAC 2004, HLCP	
	$C_5H_{10}O_3$	Leave	-				
	$C_{12}H_{14}O_4$	Leave	-			HLCP	
	$C_{24}H_{46}O_2$						
	$C_{16}H_{26}O_{10}$					HS-SPME-GC–MS	
						HS-SPME-GC–MS	
						HS-SPME-GC–MS	
						HS-SPME-GC–MS	
						HS-SPME-GC–MS	

***Association of official analytical chemists (AOAC)**

Table 3: Bioactive compounds with antimicrobial and antioxidant activity

Plant part	Compound	Bioactive	Type of pathogen bacteria	References	Cited by
Seed Extract	4-(α-L-rhamnopyranosyloxy)benzyl isothiocyanate, methyl N-4-(α-L-rhamnopyranosyloxy)benzyl carbamate, and 4-(β-D-glucopyranosyl-1→4-α-L-rhamnopyranosyloxy)- benzyl thiocarboxamide	Antimicrobial Activity	*Shigella dysenteriae, Bacillus cereus, E. coli*, and *Salmonella typhi.*	[34,54]	(by 62; 13)
	Saponins		chloroform extract (*E. coli* and *Salmonella typhimurium*).	[13]	by 280
	Water-soluble lectin from Moringa oleifera seeds (WSMoL).	Bacteriostatic and bactericidal activities	*Enterococcus faecalis, Klebsiella pneumonia*, and *Serratia sp.*, *Micrococcus luteus.*	[16]	by 3
	4-(α-L-rhamnosyloxy)benzyl glucosinolate (glucomoringin; GMG)	Antimicrobial Activity	Myrosinase-catalyzed hydrolysis (*Staphylococcus aureus, Enterococcus casseliflavus*, and on the yeast *Candida albicans*).	[26]	by 54
	4-(α-L-Rhamnosyloxy)benzyl isothiocyanate, and 4-(4'-O-acetyl-α-L-rhamnosyloxy)- benzyl isothiocyanate	Antimicrobial Activity	Dichloromethane (good antimicrobial against *S. aureus*, moderate activity against B. subtilis	[57]	by 46
	4 (ά– L – rhamnosyloxy) benzyl-isothiocyanate, Alkaloids, Flavonoids.		Chloroform extract(*E. coli, S. Typhi, P. aeruginosa*) Aqueous Extract (*E. coli, S.* typhi).	[21]	by 14

Leaf Extracts	Alkaloids, tannins, and Saponins	Antimicrobial Activity	Ethanol extract (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter aerogenes susceptible*).	[13]	by 280
	Myricetin, quercetin, and hydroxybenzoic acid derivatives like gallic acid, protocatechuic acid, syringic acid, and gentisic acid	Antimicrobial and antioxidant activity	methanol, ethanol, and ethyl acetate extracts (*Pseudomonas aeruginosa, Erwinia carotovora*)	[60]	by 32
	alkaloids, flavonoids, glycosides, saponins, and tannins.	Antimicrobial and antioxidant activity	Ethanol extract (*Pseudomonas aeroginosa, Proteus vulgaris, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus mutans)*	[27]	by 14
	Alkaloids, glycosides, volatile oils, or tannins	Antimicrobial	Petroleum Ether (*P. aeruginosa , P. vulgaris, B. subtilis)* chloroform extract (*Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, B. subtilis, S. typhimurium*).	[61]	by 22
	Methanol extract(Alkaloids,saponins,anthraquinones tannins,flavonoids,and phenol). Aqueous extract(Alkaloids, anthraquinones, tannins, flavonoids, and phenol).	Antimicrobial	Methanol extract (more effect on *E. coli* less than *S. aureus* and *S. pneumonia*). Aqueous extract (less effect on *E. coli* less, *S. aureus*, and *S. pneumonia*.	[3]	by 14
Seeds and leaves	unsaturated fatty acids (palmitoleic acid, oleic acid, linoleic acid, linolenic acid, cis-11-eicosenoic acid, and cis-11,14-eicosadienoic acid)	Antibiofilm	*Staphylococcus aureus*	[43]	by 23

1.8 Objective

The purposes of this study were stated as below:

1. To obtain an organic extract from *M. oleifera* leaves and seeds using different extraction methods

2. To determine the qualitative phytochemical profile of *M. oleifera* leaves and seeds extracts.

3. To evaluate antimicrobial and anti-biofilm activities of *M. oleifera* leaves and seeds extracts.

Chapter Two
Material and Methods

2.1 Materials

M. oleifera leaves and seeds were purchased in Palestine from a local market. The leaves and seeds were air-dried at room temperature, shielded from dust and sunlight, and ground using a mortar and pestle to reach a fine texture. After that, all samples of leaves and seeds were mixed separately, to create a homogeneous sample, which was then stored at 4 ℃ in a plastic storage box.

Bacterial strains used in this work are *E.coli*, *Salmonella* spp, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*. Culture broths were subjected to vigorous agitation (180 rpm), while biofilm formation was assessed in static conditions. Bacterial strains grown in nutrient broth were kept at -80◦C with glycerol at a final concentration of 15%.

2.2 Plant extraction

M. oleifera seeds and leaves were extracted by using different solvents (hexane, methanol-water 1:1 mixture). Non-polar extracts from *M. oleifera* leaves and seeds were obtained by mixing 4 g of crushed *M. oleifera* leaves and seed powder with 40 ml hexane, stirring for 1 hour on a magnetic stirrer under the fume-hood, and then filtering. The supernatants and crude extract of the leaves and seeds were dried at room temperature before starting the polar extraction of the dried crude extract. Polar extract

samples (4g) were extracted with 25 mL of water/methanol mixture at a 1:1 ratio, mixed for 1 hour under the fume-hood with a magnetic stirrer, and then filtered. The mixture was centrifuged at 5000g for 5 min, after which the aqueous and organic fractions were accurately separated. To obtain dry extracts, the fractions were dried under vacuum (Rotavapor R-114, Büchi) at 30 °C. The dried samples from both extractions (polar, and non-polar) were stored at 4℃ until analysis. Validation of the extraction protocol has been obtained by using a standardized sample preparation protocol for leave and seed extraction [18].

2.1.1 Preparation sample for Nuclear magnetic resonance spectroscopy (NMR).

Dried aqueous fractions were diluted in 600 L of 99.8% deuterium oxide (D_2O) and transferred to a 5 mm NMR tube as an aliquot. The internal standard, sodium trimethylsilylpropanesulfonate (DSS), was added to the NMR tube at a concentration of 0.2 mg/m. On a Varian Unity Inova spectrometer operating at 700 MHz, the NMR spectra were recorded at 298 K. All of the data, as well as the time and angle required for measurement, were adjusted. All spectra were phased and baseline corrected using the iNMR program (www.inmr.net). Signal integration was used to quantify the data in relation to the internal standard, DSS. The solvent peaks region was left out of the analysis. Spectral peak assignments of the detected compounds were obtained based on pure standards [48] and two-dimensional NMR spectra.

2.1.2 Minimal Inhibitory Concentration

Each extract's antimicrobial activity was tested. According to the Clinical & Laboratory Standards Institute (CLSI) and the National Committee for Clinical Laboratory Standards (NCCLS), the minimal inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that results in a 99.9% reduction (i.e. three log-units) in bacterial growth when compared to untreated bacteria. MIC was determined in duplicate by twofold serial dilution (CLSI, 2017).

The wells of a sterile 96-well flat-bottomed polystyrene plate were filled with 200 µl of 1/100 diluted overnight bacterial cultures grown in nutrient broth. Each extract was tested starting from a concentration of 4 mg/ml, as reported in the literature [23, 75]. After overnight incubation at 37◦C, the antimicrobial activity was optically evaluated comparing treated and untreated samples.

Chapter Three
Result and Discussion

The NMR spectra of a polar fraction of seeds and leaves were run in deuterated chloroform ($CDCl_3$) at 400 MHz.

The ^1H NMR spectra of the oil obtained from the plant seeds (Figure 3) showed as the main component of the extract signals of monounsaturated fatty acids. In particular, the terminal methyl of the fatty acid chain resonated as a triplet at δ 0.86 while the methylene signals of the alkyl chain resonated at the classical value of δ 1.26. Further signals of the alkyl chain were: a triplet at δ 2.25 (alfa-carbon, fatty acid), a multiplet a δ 1.56 (beta-carbon, fatty acid). The unsaturation on the chain was indicated by the signal at δ 5.30 (double bond protons) and δ 1.95 (allylic protons). Signals for glycerol resonated at δ 5.22, 4.25, and 4.10.

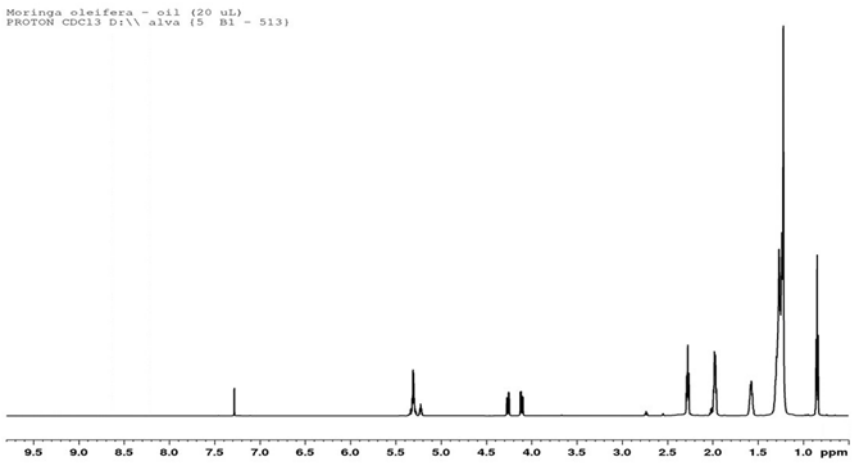

Figure 3. ^1H NMR of M. oleifera oil (20uL) in CDCl3

Our data agree with those reported in the literature reporting the monounsaturated oleic acid as the main component of the fatty acid mixture (around 78%), followed by saturated fatty acid (20%) and by polyunsaturated fatty acids (2%). Among the monounsaturated fatty acid, it is of interest the finding of behenic acid that is present in *M. oleifera* and it is responsible for the common name given "Ben-oil".

The ^{1}H NMR spectra of the seeds (Figure 4) were almost superimposable to that of the oil thus indicating the same chemical composition and the monounsaturated oleic acid as the main component of the a polar extract.

The ^{1}H NMR spectra of the seeds thus contained saturated and polyunsaturated fatty acids as minor components of a polar fractions.

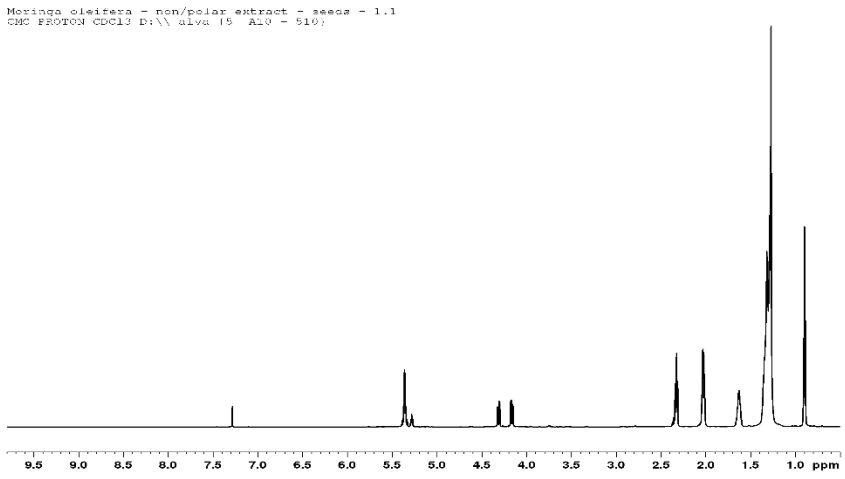

Figure 4*:* ^{1}H NMR of M. oleifera seeds apolar extract in $CDCl_3$

The ^{1}H NMR spectra of the leaves showed a very different profile (Figure 5). Besides the signals of the fatty acid at δ 0.86 and 1.26 that accounted for 40% of the total extract, the spectra showed signals due to aliphatic

alcohols resonating at δ 1.50, 1.60, 2.0, and 5.05, accounting for 60% of the total extract.

Interestingly, in the plant leaves are reported the long-chain alcohols: phytol (C20 $H_{40}O$) and 1-hexadecanol ($C_{18}H_{38}O$). These compounds are components of the waxes that are present on the leaves' surface. They act decreasing surface wetting and moisture loss, protecting by ultraviolet light, assisting in the formation of an ultra-hydrophobic and self-cleaning surface, and acting as an anti-climb surface [76].

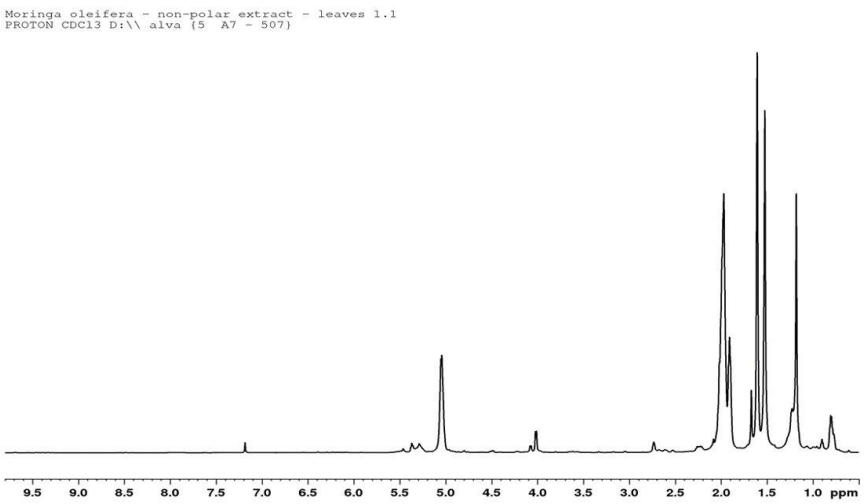

Figure 5. ^{1}H NMR of M. oleifera leaves apolar extract in $CDCl_3$

The ^{1}H NMR spectra of the polar extract (Figure 6) of the leaves appeared quite complicated and with some signals partially overlapped. The spectra run in deuterated water (D_2O) could be divided into three sub-region:1) the aliphatic region from 0.5 to 3.0 ppm; 2) the carbohydrate region from 3.1 to 5.7 ppm, 3) the aromatic and phenolic region from 5.8 to 9.5 ppm.

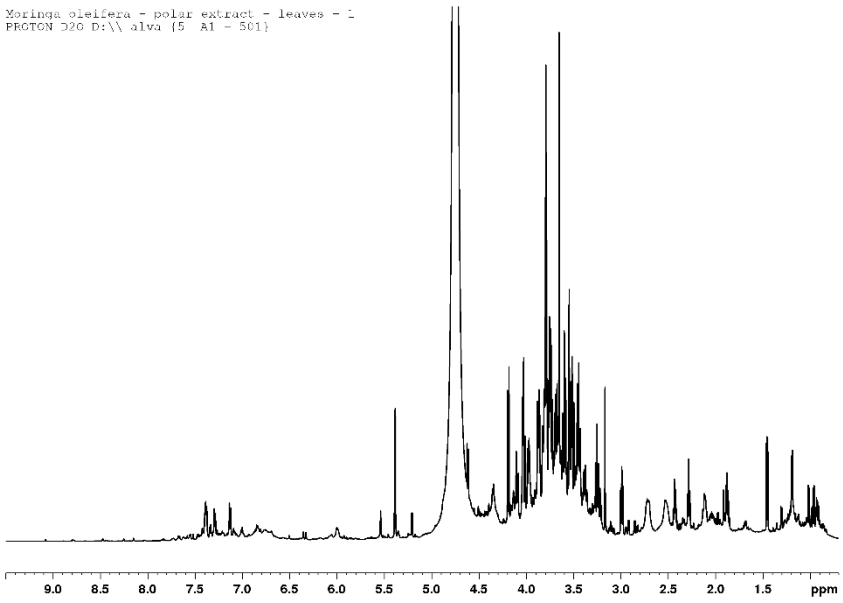

Figure 6: ^{1}H NMR of M. oleifera leaves polar extract in D2O

Analysis of the spectra was performed by comparison of the chemical shifts and coupling constants of the signals in comparison with data in the literature for standard metabolites and with the aid of 2D NMR spectra.

The spectra appeared quite similar to those of the seeds reported in Figure 7.

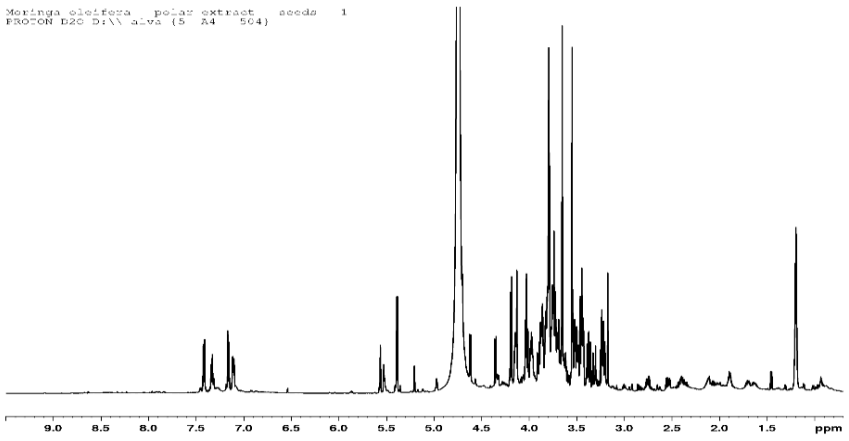

Figure 7: ^{1}H NMR of M. oleifera seeds polar extract in D_2O

Table 4 lists the metabolites identified in both polar extract extracts. The compounds belong to different classes of organic compounds including amino acids, organic acids, carbohydrates, nucleotides.

Among amino acids, valine, leucine, isoleucine, threonine, and asparagine have been detected. In addition, signals for lactic acid, malic acid, citric acid, succinic acid, and acetic acid were also identified along with gamma-aminobutyric acid (GABA).

Concerning carbohydrates. The monosaccharides glucose and fructose were major metabolites along with the disaccharides, saccharose, and maltose.

Myo-inositol, adenosine, and trigonelline were also identified from their characteristic signals in the spectra.

Table 4: Compounds identified in the ^{1}H NMR spectra of polar extracts of *M. oleifera* leaves and seeds.

PEAK NUMBER	IDENTIFIED COMPOUND	^{1}H (PPM)	Multiplicity [J (Hz)]	Leaves	Seeds
1	Valine	0.97 1.01	d, 7 d, 7	X	X
2	Leucine	0.94	d, 7	X	X
3	Isoleucine	0.92 0.99	t, 7 d, 7	X	X
4	Threonine	1.31	d, 7	X	X
5	Lactate	1.31	d, 7	X	X
6	Alanine	1.46	d, 7	X	X
7	4-Aminobutyrate (GABA)	1.88 2.28 3.00	m t, 7 t, 7	X	X
8	Acetate	1.92	s	X	X
9	Malate	2.43 2.70* 4.34*	dd, 15.5, 9.0	X	X
10	Succinate	2.44	s	X	X
11	Citrate	2.53	m	X	X
12	Asparagine	2.88	dds	X	X
13	Ethanolamine	3.13	t	X	X

14	Myo-inositol	3.27 3.61	t t	X	X
15a 15b	α-Glucose (GLC) β-Glucose (GLC)	5.22 4.63	d, 4 d, 8	X	X
16	Fructose	4.01 3.69 3.58	m m m	X	X
17a 17b	Sucrose	5.39 4.19	d, 4 d, 8	X	X
18	Maltose	5.22 5.40	d, 4 d, 8	X	X
19	Adenosine	6.07 8.25	d, 4 s	X	X
20	Trigonelline	4.43 8.07 8.82 9.08	s t, 8 d, 8 bs	X	X

*Signal overlapped

A total of 20 metabolites, present as main components, were unequivocally identified both from leaves and seeds of *M. oleifera*. Concerning the phenolics, the spectra overlapping did not allow the determination of unequivocally single metabolites although signals for the flavonoids kaempferol and quercetin and the flavonoid glycosides rutin were recognized in the NMR spectra of the polar extract.

3.1 Antimicrobial test for the polar extract of *M.oleifera* seeds and leaves.

Microbial cultures were incubated in the absence (NT) and in presence of different concentrations (4,2,1 mg/ml) of polar extract from leaves (PF), polar extract from seeds (PS), a polar extract from leaves (AF), and apolar extract from seeds (AS) at 37°C. After 24 h bacterial viability was assessed by measuring the optical density at 600 nm (OD_{600nm}) (Figure 8).

The results showed that *M.oleifera* seeds nonpolar extracts at a concentration of 4 mg/ml have antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis*, no effect on *Pseudomonas aeruginosa and Salmonella spp* at the same concentration. There is an adverse effect for the growth of *Staphylococcus epidermidis* at the different concentrations on *M.oleifera* polar extracts (4, 2 mg/ml). In

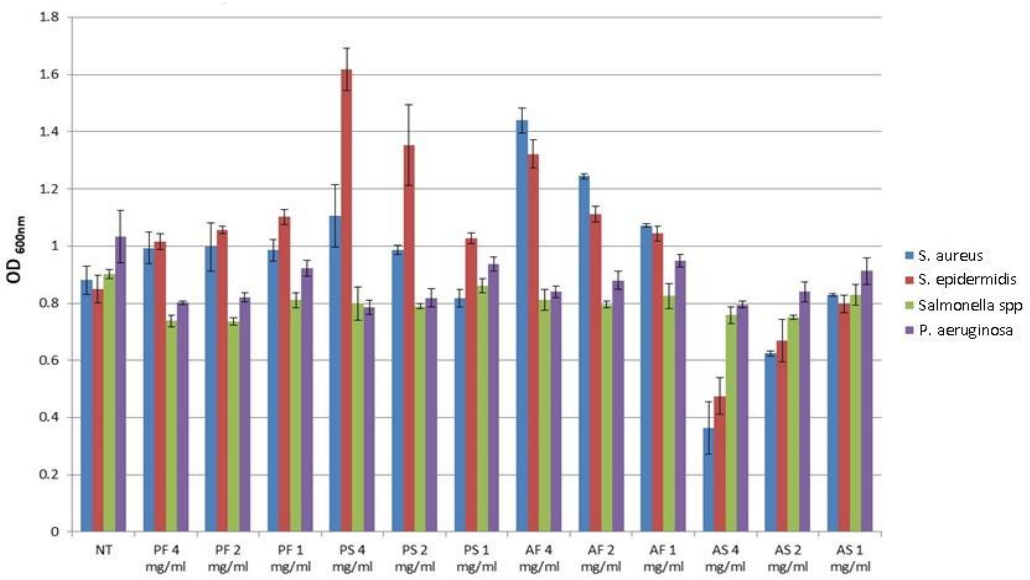

Figure 8: Antimicrobial activity of polar extract of *Moringa oleifera* leaves (PF) and seeds (PS).

Addition at 4 mg/ml, there is an adverse effect for *M.oleifera* leaves polar extract on growth on *Staphylococcus aureus.*

Table 5: Weight of polar extracts from M.oleifera leaves and seeds.

Leaves(L)	**Seeds(S)**
1L=0.598g	**1S**=9,166
2L=0.633g	**2S**=0.507g
3L=0.793g	**3S**=0.426g

Table 6: Weight of apolar extracts from M.oleifera leaves and seeds.

Leaves(L)	**Seeds(S)**
1.1L=0.119g	**1.1S**=127g
1.2L=0.137g	**1.2S**=0.192g
2.1L=0.108g	**2.1S**=0.203g
2.2L=0.116g	**2.2S**=0.074g
3L=0.170g	**3.1S**=0.155g
-	**3.2S**=0.169

Because of the rise in multidrug-resistant pathogens and deadly infectious diseases, the need for affordable and sustainable health care has never been greater. Despite the fact that a growing number of scientific studies on plant antimicrobial properties are being conducted, the vast majority of medicinal plants remain untapped globally [74]. Even fewer studies have looked into the antimicrobial interactions between traditional plants and antibiotics, highlighting the need for more scientific research in this field. The goal of our research was to determine the antimicrobial activity of *M. oleifera* extracts polar and a polar against a variety of microbial autoimmune disease triggers. Furthermore, preliminary phytochemical analysis of the polar and a polar *Moringa oleifera* extracts was a major goal of this research.

The antimicrobial test (Figure 8) resulted in a positive effect for a polar extract of *Moringa oleifera* seeds against *Staphylococcus aureus* and *Staphylococcus epidermidis* at a concentration of 4 mg/ml compared to 1, 2 mg/ml concentrations that showed no effect on all types of bacteria by comparing with control. By contrast, the polar extracts of *Moringa seeds* showed negative effects against *Staphylococcus epidermidis*, which we

noticed their growth rate increased at 2, 4 mg/ml.

The result of a polar extract of *M.oleifera* leaves showed a negative effect against S*taphylococcus aureus and Staphylococcus epidermidis*, to increase their growth rate at 2, 4 mg/ml concentration. The growth rate of these bacteria was increased by increase the concentration of the extract. By contrast, the polar extracts of *Moringa leaves were* showed no effects against *all types of bacteria* for all extracts concentrations.

M.oleifera plant considers a rich source of phytochemicals and fatty acids, which help to enhance their antimicrobial activities. However, the *Moringa oleifera* seed part composition shows an abundant amount of fatty acid mainly oleic acid and other bioactive components [5]. A polar extract of *M.oleifera* seed shows the highest antimicrobial activity against bacteria especially S*taphylococcus aureus and Staphylococcus epidermidis* which fit with previous research [54, 60].

There is a need for further investigation of this plant in order to identify and isolate the active antimicrobial compound and understand the mechanism of their action on bacteria.

Chapter Four
Conclusion

4.1 Conclusion

Moringa oleifera is a large plant that can grow in a variety of climates, allowing it to adapt to harsh environments. *Moringa oleifera* is considered one of the richest plants due to its high nutritional value and biological health benefits. Recently, there has been a surge in research interest in using this plant in various aspects of the food industry. Inaddtion it has an antimicrobial effect.

Using an NMR approach, we performed an experiment to test antimicrobial activity and characterize *Moringa oleifera* composition. The findings revealed that *Moringa* seeds have antimicrobial activity against *Staphylococcus aureus and Staphylococcus epidermidis*, which we believe is due to the fatty acid composition and other bioactive constituents. Furthermore, we obtained NMR spectra of a polar fraction of seeds, which confirmed the high fatty acid content. This could back up our theory that a polar extracts from *Moringa* seeds have antimicrobial properties.

More research is needed to look into these properties on a larger scale, particularly to figure out how fatty acids exert these effects on microbes. This could be crucial for new applications in food science and technology, particularly for the use of food life extenders.

References

- Abd Rani, N. Z., Kumolosasi, E., Jasamai, M., Jamal, J. A., Lam, K. W., & Husain, K. (2019). In vitro anti-allergic activity of *Moringa oleifera* Lam. extracts and their isolated compounds. *BMC Complementary and Alternative Medicine*, *19*(1), 1–16. https://doi.org/10.1186/s12906-019-2776-1

- Abdulkadir, A. R., Hasan, M. M., & Jahan, M. S. (2018). Antimalarial, antioxidant, antimicrobial properties of *Moringa oleifera* Lam: A review. *Australian Journal of Crop Science*, *12*(6), 905–908. https://doi.org/10.21475/ajcs.18.12.06.PNE920

- Abubakar, I., & Usman, A. (2016). Phytochemical and antibacterial investigations of moringa (*Moringa oleifera*) leaf extract on selected bacterial pathogens. *Journal of Microbiology and Antimicrobials*, *8*(5), 28–33. https://doi.org/10.5897/jma2016.0361

- Akinyeye, A. J., Solanke, E. O., & Adebiyi, I. O. (2014). Phytochemical and antimicrobial evaluation of leaf and seed of *Moringa oleifera* extracts. *International Journal of Research In Medical and Health Sciences*, *4*(6), 1–10.

- Aldakheel, R. K., Rehman, S., Almessiere, M. A., Khan, F. A., Gondal, M. A., Mostafa, A., & Baykal, A. (2020). Bactericidal and in vitro cytotoxicity of moringa oleifera seed extract and its elemental analysis

using laser-induced breakdown spectroscopy. Pharmaceuticals, 13(8), 1–18. https://doi.org/10.3390/ph13080193

- Alli, E., And, R., & Arumugam, T. (2017). Moringa oleifera (Lam)-A nutritional powerhouse. *J. Crop and Weed*, *13*(2), 238–246.

- Alphonsine, R.-T., Moumouni, K., Nabere, O., Marius, L., & Innocent, P. G. (2019). A comparative study of phytochemical profile and antioxidant activity of Sahelian plants used in the treatment of infectious diseases in northern part of Burkina Faso: Acacia seyal Delile and Acacia tortilis (Forssk.) Hayne subsp. raddiana (Savi). *Journal of Pharmacognosy and Phytotherapy*, *11*(3), 74–79. https://doi.org/10.5897/jpp2019.0555.

- Anwer, T., Safhi, M. M., Makeen, H. A., Alshahrani, S., Siddiqui, R., Sivakumar, S. M., Shaheen, E. S., & Alam, M. F. (2021). Antidiabetic potential of *Moringa oleifera* Lam. leaf extract in type 2 diabetic rats, and its mechanism of action. *Tropical Journal of Pharmaceutical Research*, *20*(2), 97–104. https://doi.org/10.4314/tjpr.v20i1.15

- Ariel, A., & Serhan, C. N. (2007). Resolvins and protectins in the termination program of acute inflammation. *Trends in Immunology*, *28*(4), 176–183. https://doi.org/10.1016/j.it.2007.02.007

- Bakare, S. B., & Ghareeb, M. A. (2019). *Chemical profile , antimicrobial and antioxidant activities of Moringa oleifera Lam leaves grown in Saudi Arabia*. *14*, 45–52.

- Bamagous, G. A., Al Ghamdi, S. S., Ibrahim, I. A. A., Mahfoz, A. M., Afify, M. A., Alsugoor, M. H. M., Shammah, A. A., Arulselvan, P., & Rengarajan, T. (2018). Antidiabetic and antioxidant activity of ethyl acetate extract fraction of Moringa oleifera leaves in streptozotocin-induced diabetes rats via inhibition of inflammatory mediators. *Asian Pacific Journal of Tropical Biomedicine*, *8*(6), 320–327. https://doi.org/10.4103/2221-1691.235327

- Bhatelia, K., Singh, K., & Singh, R. (2014). TLRs: Linking inflammation and breast cancer. *Cellular Signalling*, *26*(11), 2350–2357. https://doi.org/10.1016/j.cellsig.2014.07.035

- Bukar, A., Uba, A., & Oyeyi, T. (2010). Antimicrobial profile of moringa oleifera lam. Extracts against some foodborne microorganisms. *Bayero Journal of Pure and Applied Sciences*, *3*(1), 43–48. https://doi.org/10.4314/bajopas.v3i1.58706

- Chaudhary, K., & Chaurasia, S. (2017). Nutraceutical Properties of Moringa oleifera : A Review. *European Journal of Pharmaceutical and Medical Research*, *April*, 646–655.

- Chelliah, R., Ramakrishnan, S., & Antony, U. (2017). Nutritional quality of *Moringa oleifera* for its bioactivity and antibacterial properties. *International Food Research Journal*, *24*(2), 825–833.

- Coriolano, M. C., Brito, J. S., Ferreira, G. R. S., Moura, M. C., Melo, C. M. L., Soares, A. K. A., Lorena, V. M. B., Figueiredo, R. C. B. Q., Paiva, P. M. G., Napoleão, T. H., & Coelho, L. C. B. B. (2020). Antibacterial lectin from Moringa oleifera seeds (WSMoL) has differential action on growth, membrane permeability, and protease secretory ability of Gram-positive and Gram-negative pathogens. *South African Journal of Botany*, *129*, 198–205. https://doi.org/10.1016/j.sajb.2019.06.014

- Das, N., Sikder, K., Ghosh, S., Fromenty, B., & Dey, S. (2012). Moringa oleifera lam. leaf extract prevents early liver injury and restores antioxidant status in mice fed with a high-fat diet. *Indian Journal of Experimental Biology*, *50*(6), 404–412.

- De Falco, B., Grauso, L., Fiore, A., Bochicchio, R., Amato, M., & Lanzotti, V. (2021). Metabolomic analysis and antioxidant activity of wild type and mutant chia (*Salvia hispanica* L.) stem and flower grown under different irrigation regimes. *Journal of the Science of Food and Agriculture*, *April*. https://doi.org/10.1002/jsfa.11256

- Debnath, S., & Guha, D. (2007). Role of Moringa oleifera on enterochromaffin cell count and serotonin content of experimental ulcer model. *Indian Journal of Experimental Biology*, *45*(8), 726–731.

- Dubey, D. K., Dora, J., Kumar, A., & Gulsan, R. K. (2013). A Multipurpose Tree- *Moringa oleifera*. *International Journal of Pharmaceutical and Chemical Sciences*, *2*(1), 415–423.

- E. Abalaka, M., Y. Daniyan, S., B. Oyeleke, S., & O. Adeyemo, S. (2012). The Antibacterial Evaluation of *Moringa oleifera* Leaf Extracts on Selected Bacterial Pathogens. *Journal of Microbiology Research*, *2*(2), 1–4. https://doi.org/10.5923/j.microbiology.20120202.01

- El-bakry, K., Toson, E., Serag, M., & Aboser, M. (2016). Hepatoprotective Effect of *Moringa oleifera* Leaves Extract Against Carbon Tetrachloride- Induced Liver. *World Journal of Pharmacy and Pharmaceutical Sciences*, *5*(5), 76–89. https://doi.org/10.20959/wjpps20165-6638

- Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, *64*(8), 711–713. https://doi.org/10.1055/s-2006-957563

- El-Sayed, O., El-Taweel, H., El-Shibiny, A., & Kamal, M. (2016). Using moringa leaves powder in the production of probiotic yogurt. *Sinai Journal of Applied Sciences*, 5(2), 197–208. https://doi.org/10.21608/sinjas.2016.78645

- Falowo, A. B., Mukumbo, F. E., Idamokoro, E. M., Lorenzo, J. M., Afolayan, A. J., & Muchenje, V. (2018). Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products: A review. *Food Research International*, *106*(January), 317–334. https://doi.org/10.1016/j.foodres.2017.12.079

- Galuppo, M., De Nicola, G. R., Iori, R., Dell'Utri, P., Bramanti, P., & Mazzon, E. (2013). Antibacterial activity of glucomoringin bioactivated with myrosinase against two important pathogens affecting the health of long-term patients in hospitals. *Molecules*, *18*(11), 14340–14348. https://doi.org/10.3390/molecules181114340

- Gebregiorgis Amabye, T., & Mekonen Tadesse, F. (2016). Phytochemical and Antibacterial Activity of *Moringa oleifera* Available in the Market of Mekelle. *Journal of Analytical & Pharmaceutical Research*, *2*(1), 2–5. https://doi.org/10.15406/japlr.2016.02.00011

- Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*, *5*(2), 49–56. https://doi.org/10.1016/j.fshw.2016.04.001

- Hagiwara, A., Hidaka, M., Takeda, S., Yoshida, H., Kai, H., Sugita, C., Watanabe, W., & Kurokawa, M. (2016). Anti-Allergic Action of Aqueous Extract of *Moringa oleifera* Lam. Leaves in Mice. *European Journal of Medicinal Plants*, *16*(3), 1–10. https://doi.org/10.9734/ejmp/2016/28566

- Hassan, T., D, V. , Naseer, I., & N, A. (2019). Hepatoprotective Activity of Some Medicinal Plants: a Review. *International Research Journal of Pharmacy*, *10*(5), 9–16. https://doi.org/10.7897/2230-8407.1005154

- Hemeg, H. A., Moussa, I. M., Ibrahim, S., Dawoud, T. M., Alhaji, J. H., Mubarak, A. S., Kabli, S. A., Alsubki, R. A., Tawfik, A. M., & Marouf, S. A. (2020). Antimicrobial effect of different herbal plant extracts against different microbial populations. *Saudi Journal of Biological Sciences*, *27*(12), 3221–3227. https://doi.org/10.1016/j.sjbs.2020.08.015

- Igado, O. O., Glaser, J., Ramos-Tirado, M., Bankoğlu, E. E., Atiba, F. A., Holzgrabe, U., Stopper, H., & Olopade, J. O. (2018). Isolation of a novel compound (MIMO2) from the methanolic extract of *Moringa oleifera* leaves: protective effects against vanadium-induced cytotoxicity. *Drug and Chemical Toxicology*, *41*(3), 249–258. https://doi.org/10.1080/01480545.2017.1366504

- Jang, M. H., Piao, X. L., Kim, J. M., Kwon, S. W., & Park, J. H. (2008). Inhibition of cholinesterase and amyloid-&bgr; aggregation by resveratrol oligomers from Vitis amurensis. *Phytotherapy Research*, *22*(4), 544–549. https://doi.org/10.1002/ptr

- Jeon, S. R., Lee, K. H., Shin, D. H., Kwon, S. S., & Hwang, J. S. (2015). Synergistic antimicrobial efficacy of mesoporous ZnO loaded with 4-(α-l-rhamnosyloxy)-benzyl isothiocyanate isolated from the *Moringa oleifera* seed. *Journal of General and Applied Microbiology*, *60*(6), 251–255. https://doi.org/10.2323/jgam.60.251

- Jung, I. L., Lee, J. H., & Kang, S. C. (2015). A potential oral anticancer drug candidate, *Moringa oleifera* leaf extract, induces the apoptosis of human hepatocellular carcinoma cells. *Oncology Letters*, *10*(3), 1597–1604. https://doi.org/10.3892/ol.2015.3482

- Kaur, H. S. (2015). Anticancer activity of a constituent from Moringa oleifera leaves. *Journal of Chemical and Pharmaceutical Research*, *7*(1), 701–705.

- Kawai, M., Hirano, T., Higa, S., Arimitsu, J., Maruta, M., Kuwahara, Y., Ohkawara, T., Hagihara, K., Yamadori, T., Shima, Y., Ogata, A., Kawase, I., & Tanaka, T. (2007). Flavonoids and related compounds as anti-allergic substances. *Allergology International*, *56*(2), 113–123. https://doi.org/10.2332/allergolint.R-06-135

- Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S. A., Afridi, S., & Shinwari, Z. K. (2020). Anticancer plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects. *Biomolecules*, *10*(1). https://doi.org/10.3390/biom10010047

- Kou, X., Li, B., Olayanju, J. B., Drake, J. M., & Chen, N. (2018). Nutraceutical or pharmacological potential of *Moringa oleifera* Lam. *Nutrients*, *10*(3). https://doi.org/10.3390/nu10030343

- Kou, X., Qi, S., Dai, W., Luo, L., & Yin, Z. (2011). Arctigenin inhibits lipopolysaccharide-induced iNOS expression in RAW264.7 cells through suppressing the JAK-STAT signal pathway. *International*

Immunopharmacology, *11*(8), 1095–1102. https://doi.org/10.1016/j.intimp.2011.03.005

- Καραγεώργου, I. (2018). *Extraction of antioxidant polyphenols from Moringa oleifera leaves using a biomelecule based low transition temperature mixture.*

- Lakshmana Prabu, S., Umamaheswari, A., & Puratchikody, A. (2019). Phytopharmacological potential of the natural gift Moringa oleifera Lam and its therapeutic application: An overview. *Asian Pacific Journal of Tropical Medicine*, *12*(11), 485–498. https://doi.org/10.4103/1995-7645.271288

- Lee, J. H., Kim, Y. G., Park, J. G., & Lee, J. (2017). Supercritical fluid extracts of *Moringa oleifera* and their unsaturated fatty acid components inhibit biofilm formation by *Staphylococcus aureus*. *Food Control*, *80*, 74–82. https://doi.org/10.1016/j.foodcont.2017.04.035

- Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: An overview. *International Journal of Molecular Sciences*, *16*(6), 12791–12835. https://doi.org/10.3390/ijms160612791

- Lopez-Rodriguez, N. A., Gaytán-Martínez, M., de la Luz Reyes-Vega, M., & Loarca-Piña, G. (2020). Glucosinolates and Isothiocyanates from *Moringa oleifera*: Chemical and Biological Approaches. *Plant Foods*

for Human Nutrition, *75*(4), 447–457. https://doi.org/10.1007/s11130-020-00851-x

- Lu, W. Y., Rhoney, D. H., Boling, W. B., Johnson, J. D., & Smith, T. C. (1997). A Review of Stress Ulcer Prophylaxis in the neurosurgical intensive care unit. *Neurosurgery*, *41*(2), 416–426. https://doi.org/10.1097/00006123-199708000-00017

- Madhuri, S., Mandloi, A. K., Govind, P., & Sahni, Y. P. (2012). Antimicrobial activity of some medicinal plants against fish pathogens. *International Research Journal of Pharmacy*, *3*(4), 28–30.

- Mahmud, I., Chowdhury, K., & Boroujerdi, A. (2014). Tissue-specific metabolic profile study of *Moringa oleifera* L. using nuclear magnetic resonance spectroscopy. *Plant Tissue Culture and Biotechnology*, *24*(1), 77–86. https://doi.org/10.3329/ptcb.v24i1.19214

- Mekonnen, Y., & Dräger, B. (2003). Glucosinolates in *Moringa stenopetala*. *Planta Medica*, *69*(4), 380–382. https://doi.org/10.1055/s-2003-38881

- Milla, P. G., Peñalver, R., & Nieto, G. (2021). Health benefits of uses and applications of *Moringa oleifera* in bakery products. Plants, 10(2), 1–17. https://doi.org/10.3390/plants10020318

- Minaiyan, M., Sajjadi, S. E., Naderi, N., & Taheri, D. (2014). Anti-inflammatory effect of *Kelussia odoratissima* mozaff. Hydroalcoholicextract on acetic acid- induced acute colitis in rats.

Journal of Reports in Pharmaceutical Sciences, *3*(1), 28–35. https://doi.org/10.22110/jrps.v3i1.1479

- Mohanty, M., Mohanty, S., Bhuyan, S. K., & Bhuyan, R. (2021). Phytoperspective of *Moringa oleifera* for oral health care: An innovative ethnomedicinal approach. *Phytotherapy Research*, *35*(3), 1345–1357. https://doi.org/10.1002/ptr.6896

- Oluduro, A. O. (2012). Evaluation of Antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-Western Nigeria. *Malaysian Journal of Microbiology*, *8*(2), 59–67. https://doi.org/10.21161/mjm.02912

- Oluduro, O. A., Aderiye, B. I., Connolly, J. D., Akintayo, E. T., & Famurewa, O. (2010). Characterization and Antimicrobial Activity of 4-(β-d-Glucopyranosyl-1→4-α-L-rhamnopyranosyloxy)-benzyl thiocarboxamide; a Novel Bioactive Compound from *Moringa oleifera* Seed Extract. *Folia Microbiologica*, *55*(5), 422–426. https://doi.org/10.1007/s12223-010-0071-0

- Ott, L. W., Resing, K. A., Sizemore, A. W., Heyen, J. W., Cocklin, R. R., Pedrick, N. M., Woods, H. C., Chen, J. Y., Goebl, M. G., Witzmann, F. A., & Harrington, M. A. (2007). Tumor necrosis factor-α- and interleukin-1-induced cellular responses: Coupling proteomic and genomic information. *Journal of Proteome Research*, *6*(6), 2176–2185. https://doi.org/10.1021/pr060665l

- Özcan, M. M. (2020). Moringa spp: Composition and bioactive properties. *South African Journal of Botany*, *129*, 25–31. https://doi.org/10.1016/j.sajb.2018.11.017

- Padla, E. P., Solis, L. T., Levida, R. M., Shen, C.-C., & Ragasa, C. Y. (2012). Antimicrobial Isothiocyanates from the Seeds of *Moringa oleifera* Lam. *Zeitschrift Für Naturforschung C*, *67*, 0557. https://doi.org/10.5560/znc.2012.67c0557

- Pagadala, P., & Shankar, V. (2020). *Moringa olifera*: Constituents and protective effects on organ systems. *Physiology and Pharmacology (Iran)*, *24*(2), 82–88. https://doi.org/10.32598/ppj.24.2.40

- Pandey, A. (2012). *Moringa oleifera* Lam. (Sahijan) - A Plant with a Plethora of Diverse Therapeutic Benefits: An Updated Retrospection. *Medicinal & Aromatic Plants*, *01*(01). https://doi.org/10.4172/2167-0412.1000101

- Prabakaran, M., Kim, S. H., Sasireka, A., Chandrasekaran, M., & Chung, I. M. (2018). Polyphenol composition and antimicrobial activity of various solvent extracts from different plant parts of *Moringa oleifera*. *Food Bioscience*, *26* (February), 23–29. https://doi.org/10.1016/j.fbio.2018.09.003

- Priadarshini, A., Pankaj, P. P., Varma, M. C., & Kumar, K. (2013). Evaluation of the antibacterial potential of *Moringa oleifera* and Azadirachta indica against some pathogenic microbes: A comparative

study. *International Journal of Drug Development and Research*, *5*(1), 214–218.

- Ríos, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, *100*(1–2), 80–84. https://doi.org/10.1016/j.jep.2005.04.025

- Renityas, N. N. (2018). The effectiveness of moringa leaves extract and cancunpoint massage towards breast milk volume on breastfeeding mothers. *Jurnal Ners Dan Kebidanan (Journal of Ners and Midwifery)*, *5*(2), 150–153. https://doi.org/10.26699/jnk.v5i2.art.p150-153

- Sánchez-Machado, D. I., Núñez-Gastélum, J. A., Reyes-Moreno, C., Ramírez-Wong, B., & López-Cervantes, J. (2010). Nutritional quality of edible parts of *Moringa oleifera*. *Food Analytical Methods*, *3*(3), 175–180. https://doi.org/10.1007/s12161-009-9106-z

- Seleshe, S., & Kang, S. N. (2019). In vitro antimicrobial activity of different solvent extracts from *Moringa stenopetala* leaves. *Preventive Nutrition and Food Science*, *24*(1), 70–74. https://doi.org/10.3746/pnf.2019.24.1.70

- Shrestha, D. S. (2021). Review on Hypothyroidism as per Ayurveda. *The Healer*, *2*(1), 74–79. https://doi.org/10.51649/healer.46

- Singh, A., Dayal, R., Ojha, R. P., & Mishra, K. P. (2015). Promising Role of *Moringa* Radiotherapy : An Overview In Improving. *Journal of Innovations in Pharmaceuticals and Biological Sciences*, *2*(2), 182–192.

- Sohaimy, S. A. El, Hamad, G. M., Mohamed, S. E., Amar, M. H., & Al-hindi, R. R. (2015). *Biochemical and functional properties of Moringa oleifera leaves and their potential as a functional food*. *4*(4), 188–199.

- Taher, M. A., Nyeem, M. A. Bin, Ahammed, M. M., Hossain, M. M., & Islam, M. N. (2017). *Moringa oleifera* (Shajna): the wonderful indigenous medicinal plant. *Asian Journal of Medical and Biological Research*, *3*(1), 20–30. https://doi.org/10.3329/ajmbr.v3i1.32032

- Tahiliani, P., & Kar, A. (2000). Role of Moringa oleifera leaf extract in the regulation of thyroid hormone status in adult male and female rats. *Pharmacological Research*, *41*(3), 319–323. https://doi.org/10.1006/phrs.1999.0587

- Technology, R., & Road, F. (2017). *International Journal of Education and Science Research*. *1*(3), 21–29.

- Vieira, G. H. F., Mourão, J. A., Ângelo, Â. M., Costa, R. A., & Vieira, R. H. S. dos F. (2010). Antibacterial effect (in vitro) of *Moringa oleifera* and *Annona muricata* against gram positive and gram negative bacteria. *Revista Do Instituto de Medicina Tropical de Sao Paulo*, *52*(3), 129–132. https://doi.org/10.1590/S0036-46652010000300003

- Vongsak, B., Sithisarn, P., & Gritsanapan, W. (2014). Simultaneous HPLC quantitative analysis of active compounds in leaves of *Moringa oleifera* Lam. *Journal of Chromatographic Science*, *52*(7), 641–645. https://doi.org/10.1093/chromsci/bmt093

- Wahyuni, R., Wignyanto, W., Wijana, S., & Sucipto, S. (2020). Optimization of protein and tannin extraction in *Moringa oleifera* leaf as antioxidant source. *Food Research*, *4*(6), 2224–2232. https://doi.org/10.26656/fr.2017.4(6).293

- Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, *3*(2), 163–175. https://doi.org/10.1038/nprot.2007.521

- Holloway, P.J.; Jeffree, C.E. (2005). "Epicuticular waxes". Encyclopedia of Applied Plant Sciences. 3: 1190–1204

الأيض والنشاط المضاد للميكروبات من المورينجا أوليفيرا

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روزانا كاباريلي

الملخص

تتبع تقرير نظام المراقبة العالمية لمضادات الميكروبات التابع لمنظمة الصحة العالمية حدوث عدوى بكتيرية ذات مقاومة للمضادات الحيوية في نصف مليون شخص حول العالم. علاوة على ذلك، أصبحت السلالات متعددة المقاومة أكثر شيوعًا وتشتتًا، مما يشكل تهديدًا خطيرًا للصحة العامة. نتيجة لهذا الموقف، تم التحقيق في استراتيجيات بديلة، بما في ذلك المستخلصات العشبية والمركبات المشتقة من النباتات. تُعرَّف مقاومة المضادات الحيوية بأنها عدم قدرة الدواء على قتل ميكروب كان يستخدم سابقًا للتثبيط أو القتل. تنتشر مقاومة مضادات الميكروبات على نطاق واسع في جميع أنحاء العالم، مما يشكل تهديدًا للقدرة على إدارة الأمراض المعدية الشائعة، مما يؤدي إلى زيادة معدل الوفيات والمراضة. تستخدم النباتات الطبية لعلاج الأمراض المعدية الشائعة منذ العصور القديمة. أيضًا، يمكن اعتباره مصدرًا آمنًا ورخيصًا للعلاج الميكروبي. وبالتالي، فإن فعالية وكفاءة النباتات الطبية في مجال مضادات الميكروبات تشجع الباحثين على دراسة تركيباتها والتحقيق فيها كطريقة طبيعية للتحكم في نشاط البكتيريا وتثبيطه. من الأمثلة على الأعشاب التي لها نشاط مضاد للميكروبات، والتي تمت دراستها الجوافة (بسيديوم الجوافا)، والمريمية (سالفيا أوفيسيناليس)، ورامنوس (زيزيفوسبينا كريستي)، والتوت (موروسالبا إل) التي تظهر نشاطًا جيدًا كمضاد للميكروبات. زيت بن هو دواء قديم حصلت عليه بذور المورينجا أوليفيرا لام. مصنع. لقد تم استخدامه لمجموعة متنوعة من الأغراض. لقد كان أيضًا موضوعًا لبحث كبير نظرًا لتطبيقاته المتعددة وخصائصه المعروفة للجراثيم. بالإضافة إلى ذلك، فهو يحتوي على مادة تسمى الجفنة،

والتي لها خصائص مضادة للجراثيم ومبيدات الفطريات. لقد ثبت أن مستخلصات المورينجا اوليفيرا من البذور والأوراق والجذور وزيت البذور المستخرجة باستخدام مذيبات مختلفة تمنع نمو البكتيريا موجبة الجرام وسالبة الجرام بشكل عام. علاوة على ذلك، تم الإبلاغ عن أن أجزاء المورينجا اوليفيرا مصدر غني للمواد الكيميائية النباتية مثل مركبات الفلافونويد والفينول والقلويد، وكذلك الببتيد النشط بيولوجيًا ومركبات السكاريد التي يمكن استخدامها كمضادات للميكروبات.