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Valorization of the fixed oils of the *Moringa oleifera*

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Dedication

To our parents

To our sisters and brothers

To our friends

Acknowledgment

Our praise and thanks to Almighty Allah the most Gracious who gave to us the strength to finish this work.

We would like to express our gratitude to our supervisor professor **SEGNI LADJEL** for this careful supervision, valuable advice and kindness.

Thanks are also due to all those who helped us and encourage us to do this work.

ملخص:

هدفت هذه الدراسة إلى تقييم الزيت الثابت لبذور نبات *المورينجا أوليفيرا* الذي تم زراعته في الجزائر،منطقة تمنراست. من خلال استخلاص وإجراء التحليلات الفيزيائية والكيميائية والتركيب، تم استخلاص الزيت الثابت *للمورينغا أوليفيرا* من البذور باستخدام تقنيتين مختلفتين للاستخلاص، التقنية الكيميائية (استخلاص صلب - سائل) والتقنية الفيزيائية (الضغط على البارد). وإجراء مقارنة بينهما، كانت النسبة المئوية لمردود للزيت 24.43 / و71 / على التوالي، وأظهرت نتائج التحاليل الكيميائية أن الكثافة ومعامل الانكسار كانت 2004 و1.4550 على التوالي، ومع الضغط البارد كانت التخليق التوالي، ومع المنعط البارد كانت النسبة المئوية لمردود للزيت 1.452 / و17 / على التوالي، وأظهرت نتائج التحاليل الكيميائية أن الكثافة ومعامل الانكسار كانت 2004 و1.4550 على التوالي، ومع الضغط البارد كانت العارية التحاليل الكيميائية أن الكثافة ومعامل الانكسار كانت 2004 و 0.9045 على التوالي، ومع الضبط البارد كانت التائج التحاليل الكيميائية أن الكثافة ومعامل الانكسار كانت 2004 و 0.9045 على التوالي، ومع الضبط البارد كانت الغازية لقياس الطيف الكتلي (MS / 30) وتم تحديد أكثر من 40 مركبًا منها 7 مركبات أساسية، نسبة حمض الأوليك شكلت الحصة الأكبر بينهم بمعدل 61.30/، زيت البذور من مورينجا اوليفير/ أظهر خصائص فيزيائية وكيميائية جيدة يمكن استخدامها بنجاح في التطبيقات الصناعية.

الكلمات المفتاحية: الاستخلاص، المورينجاأوليفيرا، الضغط البارد، الزيت الثابت، GC / MS ، زيت المورينجا اوليفيرا

ABSTRACT

This study aimed to evaluate the fixed oil of the seeds of the *Moringa oleifera* plant that was cultivated in Algeria, Tamanrasset. By extracting and conducting physical and chemical analyzes and composition, the fixed oil of *Moringa oleifera* was extracted from seeds by using two different extraction techniques, the chemical technique (solid-liquid «Soxhlet" extraction) and the physical technique (cold press). And a comparison between them, the percentage yield of the oil was 24.43% and17% respectively, the results of the chemical analyzes showed that the density and refractive index were 0.9042 & 1.4550 respectively, and with the cold press was 0.9143 and 1.4651 respectively. The oil extracted by chemical technique was also analyzed by gas chromatography-mass spectrometry (GC/MS) and more than 40 compounds were identified Including 7 basic compounds, and the proportion of oleic acid made up the largest share, at a rate of 61.30 %, The seed oil of *Moringa Oleifera* showed good physicochemical properties and could be utilized successfully in industrial applications.

Keywords: Extraction, Moringa oleifera L, cold press, fixed oil, GC/MS, Moringa oil

Résumé:Cette étude vise à évaluer l'huile fixe des graines de la plante *Moringa oleifera* qui a été cultivée en Algérie, région de Tamanrasset.Par extraction et effectuer des analyses physiques et chimiques et composition, de l'huile fixe de *Moringa oleifera* a été extraite des graines en utilisant deux techniques d'extraction différentes, la technique (extraction solideliquide « Soxhlet ») et la technique physique (presse à froid). Et une comparaison entre eux, le rendement en pourcentage de l'huile était de 24,43% et 17% respectivement, les résultats des analyses chimiques ont montré que la densité et l'indice de réfraction étaient respectivement de 0,9042 et 1,4550, et avec la presse à froid de 0,9143 et 1,4651 respectivement.L'huile extraite par technique chimique a également été analysée par chromatographie en phase gazeuse-spectrométrie de masse (GC/MS) et plus de 40 composés ont été identifiés dont 7 composés basiques, et la proportion d'acide oléique constituait la plus grande part, à un taux de 61,30%, L'huile de graines de *Moringa Oleifera* a montré de bonnes propriétés physico-chimiques et pourrait être utilisé avec succès dans des applications industrielles.

Mots clés : Extraction, Moringa oleifera L, pressage à froid, huile fixe, GC/MS, huile de Moringa

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LIST OF ABBREVIATIONS

FAO: Food and Agriculture Organization

- WHO: World Health Organization
- EPA: Environmental Protection Agency
- APG: Angiosperm Phylogeny Group
- NIST: National Institute of Standards and Technology

Introduction general

Applications of vegetable oils expand continuously, providing a major boost for the market. Several vegetable oils are currently in high demand in world markets, for both food and non-food applications.

Among the types of vegetable oils, recently, the seed oil of the *Moringa oleifera* tree has attracted great scientific interest.*Moringa oleifera (Moringaceae)* is a fast-growing tree indigenous to sub-Himalayan tracts of Northern India. Nowadays, *M. oleifera* is mainly found in the Middle East and in African and Asian countries, but, due to its adaptability, it is spreading to other areas, especially tropical and subtropical lands affected by drought, M. oleifera It is one of 13 species within the same genus that gets the most attention in Worldwide, and is among the most economically important tree crops, especially in developing countries [1] as almost all parts of the tree are used [2], The WHO has recommended *Moringa* as an alternative to imported food supplies for the treatment of malnutrition [3] Today, *Moringa oleifera* cultivation has reached in several provinces of the country. especially in the southern provinces

Our interest in this topic is due to the seed kernel of *M. oleifera* contains a large amount of oil. (up to 40%) with a high-quality fatty acid composition (oleic acid > 70%) and, after refining, a notable resistance to oxidative degradation [4]. The oil is known commercially as "Ben oil" or "Behen oil". Its properties make it suitable for both human consumption and commercial purposes, like biodiesel, cosmetics, and perfumes, and as a lubricant for fine machinery with a great potential to become a promising commercial source of edible oil for the food industry. Moreover, after oil extraction, the seed cake can be used in wastewater treatment as a natural coagulant [5] or as an organic fertilizer to improve agricultural productivity [6]

Despite the great economic returns and the availability of all the ingredients for the production of *Moringa* oil grown in Algeria, this oil was not given much attention

This study aimed to evaluate the fixed oil of the seeds of the *Moringa oleifera* plant that was cultivated in Algeria, (Tamanrasset)

4 The plan that was followed in this work:

- Extracting Moringa oleifera seed oil using two methods: (solid-liquid) soxhlet and cold pressing
- Determination of physical and chemical properties
- ➤ -Analysis of extracted oil by GC-MS.

4 This dissertation is organized as follows:

Two parts (theoretical and practical) with a general introduction and aconclusion

The theoretical part is composed of three chapters.

- ➤ -The first chapter is devoted to green chemistry
- The second chapter is devoted to the study of the Moringa oleifera plant.
- > -The third chapter is devoted to general information about fixed oils
- The practical part contains materials and methods, results and discussions.

CHAPTER I: Green Chemistry

1. Introduction

Since the 1940s, environmental issues began to emerge in relation to the growth of industrial activities. In the face of environmental problems and concerns, companies have changed their position on conventional production and product development habits through conferences, political agreements and advances in chemical research and ecological engineering adopting sustainable processes to the present.

In the 1990s, Paul Anastas and John Warner postulated the12 principles of Green Chemistry, still in use today, that rely on the minimization or non-use oftoxic solvents in chemical processes and analyzes, as well as the non-generation of wastes from these processes. These principles propose environmentally favorable actions from the planning of the product to its synthesis, processing, analysis and its destination after use, The main objective is to minimize the environmental and occupational hazards inherent inindustrial activities .

Later, Paul Anastasdiscussed the importance of using these 12 principles in the development of new methods and analytical techniques, with the purpose of reducing their environmental impacts. Thus, one of the most active areas of Research and Development in Green Chemistry is the development of analytical methodologies. New methods and techniques that are able to reduce the use and generation of hazardous substances in all stages of chemical analysis are the maingoals of the so-called Green Analytical Chemistry. In this context, Galuszka, Migaszewski and Namienski, in year 2013, adapted the 12 principles of Green Chemistry, to better fit the Green AnalyticalChemistry.

The impacts of green chemistry are multidimensional. Each analytical choice hasconsequences both in the final product and in everything that surrounds it, from theenvironment, population, analyst and even the company.[7]

2 History

The green chemistry movement started over two decades ago. Initial motivation for redesigning chemicals and chemical process came from the pollution prevention legislation in the early 1990s authored by (EPA). This legislation clearly articulated a shift toward inherently safer and sustainable chemicals as being the best pollution prevention strategy

Key early support of green chemistry came from the U.S. Presidential Green Chemistry Challenge Awards established in 1995, the Green Chemistry Institute founded in 1997, and the publication of the inaugural issue of the Royal Society of Chemistry journal, Green Chemistry, in 1999. The publication of Green Chemistry: Theory and Practice in 1998 clearly explained the 12 principles of green chemistry and helped provide a coherent vision for the emerging green chemistry movement. Although seemingly intuitive, the formulation of these principles helped chemists and chemical engineer nderstand how principles of sustainability could be applied to their research [54]

3 Green chemistry

The main concept of Green Chemistry is the use of chemical skills and knowledge to reduce or eliminate the use or generation of hazardous substances during the planning, manufacturing, and application of chemicals in order to minimize threats to the health of operators and the environment. Thus, the concern to eliminate or minimize the generation of toxic waste has become greater than treating the waste already generated. In 1998, Paul Anastas and John Warner published the first manual of Green Chemistry, in which they proposed 12 principles for the theme, which have been described in the next title and Fig. 1. In summary, the 12 principles of Green Chemistry are based on the minimization or non-use of toxic solvents in the chemical processes and analyzes, as well as on the non-generation of residues resulting from these processes. For this, the atomic and energy economies occupy prominent places, as well as the use of renewable and innocuous raw materials. In addition, the acceleration of chemical reactions through catalysis can help, for example, in energy savings and less waste generation. One of the principles is also concerned with the conscious development of chemicals so that after their useful life they must decompose and become degradation products harmless to the environment, also avoiding bioaccumulation. Thus, it is observed that these principles are concerned with the planning of the product, through its synthesis, processing, analysis, and its destination after the use. The main objective is to minimize the environmental and occupational risks inherent in industrial activities. At the same time, it is possible to predict some of the economic benefits generated by the adoption of Green Chemistry in industrial chemical processes, such as the lesser need for investments in effluent storage and treatment, as well as the payment of indemnities for environmental damages. This is an important aspect since it is clear that if Green Chemistry does not bring economic benefits to the market, it will not be viable. However, if the market ignores the needs of the environment, it will not prosper. [7]

4 The 12 Principles of Green Chemistry

[7]

> *Prevention* It is better to prevent waste than to treat or clean up waste after it has been created.

Atom Economy Synthetic methods should be designed to maximize the incorporation of all

materials used in the process into the final product

Less Hazardous Chemical Syntheses Wherever practicable, synthetic methods should be

designed to use and generate substances that possess little or no toxicity to human health and

the environment.

Designing Safer Chemicals Chemical products should be designed to effect their desired

function while minimizing toxicity

Safer Solvents and Auxiliaries The use of auxiliary substances (e.g. solvents, separation

agents, etc.) should be made unnecessary wherever possible and innocuous when used.

- Design for Energy Efficiency Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, syntheticmethods should be conducted at ambient temperature and pressure.
- Use of Renewable Feedstocks A raw material or feedstock should be renewable rather than

depleting whenever technically and economically practicable.

Reduce Derivatives Unnecessary derivatization (use of blocking groups,

protection/deprotection, temporary modification of physical/chemical processes) should be

minimized or avoided if possible, because such steps require additional reagents and can

generate waste.

Catalysis Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

Design for Degradation Chemical products should be designed so that at the end of their

function they break down into innocuous degradation products and do not persist in theenvironment.

Real-time analysis for Pollution Prevention Analytical methodologies need to be further

developed to allow for real-time, in-process monitoring and control prior to the formation ofhazardous substances.

Inherently Safer Chemistry for Accident Prevention Substances and the form of a substanceused in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.



Figure 1. 12 Principles of Green Chemistry proposed by Anastas and Warner

5 Examples of Implementation of Green Chemistry Principles IntoPractise

Table	1.	Exam	oles	of im	plementation	of g	reen ch	emistrv	princi	ples	into	practise.	221
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Nr	Principle	Examples
1	Prevention	Use of solvent-less sample preparation techniques
2	Atom Economy	Hydrogenation of carboxylic acid to aldehydes using solid catalysts
3	Less Hazardous Chemical	Adipic acid synthesis by oxidation of cyclohexene using hydrogen peroxide
	Syntheses	
4	Designing Safer Chemicals	New, less hazardous pesticide (e.g., Spinosad)
5	Safer Solvents and Auxiliaries	Supercritical fluid extraction, and synthesis in ionic liquids
6	Design for Energy Efficiency	Polyolefins-polymer alternative to PWC (polymerization may be carried with
		lower energyconsumption)
7	Use of Renewable Feedstocks	Production of surfactants
8	Reduce Derivatives	On-fiber derivatization vs derivatization in solution in sample preparation
9	Catalysis	Efficient Au (III)-catalyzed synthesis of b-enaminones from 1,3-dicarbonyl
		compounds. and amines
10	Design for Degradation	Synthesis of biodegradable polymers
11	Real-time analysis forPollution	Use of in-line analyzers for wastewater monitoring
	Prevention	
12	Inherently Safer Chemistry	Inherently Safer Chemistry for Di-Me carbonate (DMC) is an environmentally
	forAccident Prevention	friendly substitute for di-Me sulfate and Mehalides in methylation reactions.

6 FUTURE OF GREEN CHEMISTRY

Green chemistry continues to grow, affecting scientists and engineers around the world. The growing international community is now leading educational and/or research initiatives in more than 25 countries. New technical journals, several international conferences, and emerging social networking sites for green chemistry have helped practitioners collaborate. Many of these collaborations are built around educating chemists about the potential benefits of green chemistry. In order for green chemistry to influence the way materials are produced, concepts of sustainability must be integrated throughout the educational process. [54]

7 Conclusion

Green chemistry is concerned with designing products and processes and minimizing the use and production of hazardous materials. New technology considers green chemistry an essential element in everyday life. The purpose of this chapter is to understand the concept of green chemistry, the principles of green chemistry, and the future prospects for green Chemistry

CHAPTER II: Moringa Oleifera

1. Intoroduction

The Moringaoleifera Lam. belongs to the Moringaceae family, which includes about 13 different species of trees [51], Moringa oleiferaLam.(SynonymMoringapterygospermaGaertn) Is a tropical multipurpose tree originally growing in India, Pakistan, Asia Minor, Africa, and Arabia that has been later distributed to Central America, North, and South America ,and the Caribbean Islands [52], The trees of Moringa grow mainly in semi-arid zones, but it develops better in dry sandy soils. The growth is fast and resistant to drought [53], Among its 13 species, Moringa oleifera Lam. is the one receiving more attention worldwide, being among the most economically important tree crops, especially in developing countries. Several factors contribute to this widespread interest, including its easy cultivation in a variable range of climatic and geographical conditions, its high production yields, the multipurpose uses of all its vegetative structures (leaves, flowers, immature pods, seeds, etc.), with nutritional relevance for humans and animals, traditional use for medicinal purposes, in agroforestry systems, and for water purification. Its seeds are rich in oil, used for cosmetic and perfume production since ancient times, as lubricants in machinery, and, more recently, in biodiesel production. *M. oleifera* oil has oleic acid as its principal fatty acid, similarly to olive oil, with a great potential to become a promising commercial source of edible oil for the food industry [1]

2 History

Antiquity; The history of the *M. oleifera* tree dates back to 150 B.C. Historical evidence reveals that ancient kings and queens used *M. oleifera* leaves and fruits in their diet to maintain a state of mental alertness and healthy skin. Ancient Mauryan warriors in India drank *M. oleifera* leaf extract on the war front and this drink was believed to be a kind of elixir that gave them extra energy and relieved them of the stress and pain suffered during the war. Eventually, these brave soldiers were the ones who defeated Alexander the Great, *Moringa* is considered to have its origin in Agra and Oudh, in the northwest region of India, south of the Himalaya Mountains. Although the name "Shigon" for *M. oleifera* is mentioned in the "Shushruta Sanhita" which was written at the beginning of the first century A.D., there is evidence that the cultivation of this tree in India dates back many thousands of years. Indians have been using it as a regular component of conventional eatables for nearly 5000 years.[8],

Modern times; In the 1990s, the cultivation of *Moringa* became popular because of the recognition that *Moringa* was a useful plant. It is a multi-purpose tree because all parts of *Moringa* can be used for different purposes. Since then, *Moringa* has been known as one of the most economically valued crops, particularly in developing countries for food, industrial, agricultural, and medicinal uses. [9]

Traditional Medicine; Moringa oleifera has been used as a medicine in India since the 18th century BC. Traditional healers used different parts of the plant as traditional medicines. The medicinal uses are numerous and have long been recognized as an Ayurvedic and Unani system of medicine. Almost all parts of the plant: root, bark, gum, leaf, fruit (pods), flowers, seeds, and seed oil, have been used to treat various diseases, like skin infections, swelling, anemia, asthma, bronchitis, diarrhea, headache, joint pain, rheumatism, gout, diarrhea heart problems, fevers, digestive disorders, wounds, diabetes, conjunctivitis, hemorrhoids, goiter, earache, measles, and smallpox in the indigenous system of medicine.[10]

3 Botanical Description of the Crop:

M. oleifera can be taxonomically identified according to the most up-to-date classification of APG IV [21], which is based on phylogenetic criteria. The taxonomic classification would be as follows:

Class Eudicotyledoneae

Subclass Magnoliidae

CladoMalvidae

Order Brassicales

Family Moringaceae

Genus Moringa

Species Moringa oleifera Lam. [12]

Moringa oleifera Ranging in height from 5 to 12m with an open, umbrella-shaped crown, straight trunk and corky, whitish bark, the tree, produces a tuberous tap root. The evergreen or deciduous foliage (depending on climate) has leaflets 1 to 2 cm in diameter; the flowers are white or cream coloured. The fruits (pods) are initially light green, slim and tender, eventually

becoming dark green, firm and up to 120cm long, depending on the variety. Fully mature, dried seeds are round or triangular, the kernel being surrounded by a lightly wooded shell with three papery wings.[11]

4 The species and their distributions

•

The *Moringa* genus comprises 13 species distributed through southwest Asia, southwest Africa, northeast Africa, and Madagascar. The species and their distributions are listed in Table.

Species	Country	Trivial name
M. arboreaVerdcourt	Kenya, Somalia	_
M. borziana Mattei	Kenya, Somalia	_
M. concanensisNimmo	India	-
M. drouhardii Jumelle	Southern Madagascar	—
M. hildebrandtiiEngler	Southwest Madagascar	Hildebrandt's Moringa
M. longitubaEngler	Kenya,	Moringa tubiflora
	SoutheastEthiopia,	
	Somalia	
<i>M. oleifera</i> Lam	India	Horseradish, Ben-oil
		Drumstick, Kelor
M. ovalifoliaDinter ex Berger	Namibia, Southwest	Phantom Tree, Ghost Tree,
	Angola	African Moringo
M. peregrinaForssk. Ex Fiori	Red Sea, Arabia,	Ben tree, wispy-needled
	Northeast Africa	Yasar tree, Wild drumstick
		tree, Yusor, Al Yassar, Al
		Ban
M. pygmaeaVerdcourt	North Somalia	-
M. rivaeChiovenda	Kenya, Ethiopia	Swanjehro
M. ruspolianaEngler	Kenya, Ethiopia,	-
	Somalia	
M. stenopetala (Baker f.) Cufodontis	Kenya, Southwest	Cabbage tree, Haleko,
	Ethiopia, Somalia	Shelagda, Shiferaw

Table 2. List of *Moringa* species throughout the world [22]

Source: All listed species were validated taxonomically from The Plant List (www.theplantlist.org, V1.1, 2013).

5 Parts of the *M. oleifera* and Their Composition



Figure. 2. Parts of the plant moringa oleifera

In the case of leaves, they contain the greatest amount of nutrients compared to other parts of M. oleifera, especially in terms of protein content (19–29%). In addition, they are excellent as a source of vitamin E, vitamin A (four times more than the content of a carrot), vitamin C (in fresh leaves, the amount is seven times higher than in an orange), and vitamin B. They are also one of the best vegetable sources of minerals since their calcium content is very high for a plant (more than four times the amount of milk) and the iron content is very interesting; it becomes very useful against anemia. It also has high amounts of potassium-three times the amount of a banana. Except for vitamin C, the nutritional value of M. oleifera leaf powder is higher than that of fresh leaves. This can be interesting, as dried leaves can be stored so their use is guaranteed throughout the year. In many cultures of poor countries, they are often the only source of additional proteins, minerals, and vitamins. In addition, its content of fats, carbohydrates, and phosphorus is very low, which makes it one of the best plant foods .M. *oleifera* flowers also serve as a good source of a wide variety of nutrients, including proteins, potassium, calcium antioxidants (α and γ tocopherol), and polyunsaturated fatty acids, leading them to be ready food or tea and dietary supplement after processed. Fried M. oleifera flowers taste like mushroom. High content of nutrition in pods and seeds of M. oleifera have been reported in many studies. There is about 9.98-51.80 g crude protein, 17.26-20.00 g crude fiber, 3.36-18.00 g carbohydrate, 38.67-43.60 g fat, and 3.60-5.00 g ash per 100 g *M*. *oleifera* seeds .Pods contain abundance of dietary fiber, low content of lipid, and a reasonable amount of unsaturated and essential fatty acids, especially oleic acid [12].

6 Uses of the Parts of the M. oleifera

6.1 In nutrition

M. oleifera is an essential source of essential nutrients; rich in protein, essential amino acids, minerals, and vitamins, with a relatively low amount of antinutrients [13].

Seed and pods

The seed of this plant contains oil that can be used for cooking [12], and young fruits are eaten as a vegetable. can be also used in Bakery Products (Bread and Cookies)[10], The consumption of pods can be both human and animal. Immature pods can be eaten raw or prepared as peas or green beans and are reported to taste like asparagus; while ripe pods are usually fried and taste like peanuts

Leaves

The leaves can be eaten fresh in salads, or as a spice, They can also be cooked in soups, Its protein content (22–24%) and This makes it one of the best sources of vegetable protein, can be used in Bakery Products (Bread, Cookies, Brownie (cake))[10], Using as a tea

Flawers

The dried flowers are infused for tea and have been proved to be rich in potassium and calcium [12].

6.2 In medicinal

Seed

Anti-inflammatory, Anti-fibrotic, Antidiuretic, antitumor, genitourinary, antituberculous, antibacterial and hepatoprotective, anti-asthmatic [15] [14]

Leaves

Antimicrobial antiulcer, Anticatarrhal, antidiabetic, antiscab, antihypertensive, antiproliferative, antioxidant, anxiolytic, diuretic, pharyngitis, cholesterol-lowering effect, hemorrhoids, glandular swellings, anti-inflammatory and anti-hyperthyroidism [12].

Flawers

Anti-inflammatory, antipsychotic and anti-tumor.[12]

Root and Bark

Analgesic, anti-inflammatory, antitumor, antidiabetic, snake bite, antiulcer, antispasmodic, cholesterol-lowering effect, antibacterial, antiurolytic, antifungal, antidiuretic and antihypertensive.[12]

6.3 In industrial

Seed

The seed of this plant contains oil that can be used for the cosmetics industry, extraction of *Moringa* oil generates a waste (65%–75% of seeds weight) which contains awater soluble protein able to be used either in drinking water clarification or wastewatertreatment, the waste of *Moringa oleifera* extraction was used as coagulant toremove five reactive dyes from synthetic textile effluents, This waste constitutes a natural coagulant which was demonstrated to be effective for the treatment of industrial reactive [16], Other important uses of Moranga oleifera seed waste include: Animal feed fortification[17] [18], Plant growth enhancer [17]

Leaves

The leaves can also be used in animal feed [17]

7 Cultivation and Climatic Requirements

M. oleifera is the unique species for which cultivation practices have been developed and reported in the literature . Cultivation and propagation information on the other *Moringa* species are very restricted. Therefore, in the absence of cultivation practices of other species, and with the growing demands by local populations, wild-harvest and over-browsing is decimating natural tree resources *.Moringa* is widely known as the "Never Die" plant because of its large-scale adaptability to climate, soil and other environmental variations *.M. oleifera*

grows in almost all types of soils, except stiff, heavy clays, and it does not tolerate stagnant water or frequent flooding [19]. It grows well in sandy or loamy soils with a slightly acidic to highly alkaline pH, a rainfall range of 250-3000 mm and a temperature range of 25-35 °C [56]. Soil compositions was found to have no significant influence on the growth character of M. oleifera including the number of the leaves, stem diameter and plant height, although it affects seed weight. Direct seeding is the most adopted method because of its high germination rate and formation of deep, stout taproot with a wide-spreading system of thick, tuberous lateral roots. The percentage of M. oleifera seeds' germination is generally high, ranging from 60% to 100%. Steinitz et al., while examining the possibility of initiating micro-cloning in vitro of seedlings, found comparable germination rate for M. oleifera, M. stenopetala, and M. peregrine, of 77%, 72% and 69%, respectively. Cultivation by cuttings and transplantation are also used, but the plants do not develop taproots, making them more sensitive to drought and wind. Still, propagation by cuttings seems to be preferred by some farmers, since it promises a quick flowering and fruiting rates, and gives the best quality fruits . Still, transplanting young plants requires utmost care as the tap roots are tender . Cultivation methods should be also adapted to the major crop purpose, for seed or leaf production. Seeds production requires a low density plantation (2.5 \times 2.5 m or 3 \times 3 m), while for leaf production it can vary from intensive (spacing from 10×10 cm to 20×20 cm), semiintensive (spacing 50×50 cm), or integrated into an agroforestry system (spacing distance of 2-4 m). Intercropping with other staple food crops, like cassava, maize and sorghum, is very frequent in south Ethiopia and Kenya: Moringa leaves shed on the soil serve as green manure to increase soil fertility and to maximize crop yield . Pruning increases branching and vegetative growth, being adequate to maximize leaf production, but the number of pods per plant decreases, despite showing no significant effect on seed weight .Moringa seeds are usually sown during the rainy season and can germinate and grow without any irrigation. Still, for commercial purposes, Moringa seedlings are usually watered twice a day during three months, and irrigation through a drip system is recommended, in order to maintain leaf and seed production during the dry season . Also, watering regimes had a significant effect on some growth parameters; the plants watered twice a day gave higher number of leaves and increased seeds weight and oil yield. Ferreira da Costa et al. investigated the cultivation of M. oleifera under low temperature conditions and observed a slow initial growth rate (in autumn/winter season), with high mortality. Accordingly, extreme frost or freeze conditions are not recommended for Moringa cultivation [20], [21].

8 Pests and Diseases

Moringa is generally resistant to the most severe pests and pathogens in its country of origin or new forms. Without suffering much from insect attacks or illnesses. However, Some exceptions may occur, Such as new climate changes or certain other practices, Insect lesions associated with M. oleifera can be classified as devolutaries, sap nutrients, and bark. Pod and seeds, with some non - insect lesions. Insect Larva, They feed mostly on leaves, causing them to feed. Low biomass production of damaged leaves and leaves is not suitable for human consumption. Seeds, When the caterpillar punctures it, it becomes unfit for seedlings and "M. oleifera" other uses. Plants growing on (for example, Mistletoe Phoradendronquadrangulare (Viscaceae) in Mexico, Farmers also listed dendrophotoevalcata as a problem. while all M. peregrina trees remained unaffected[20].

9 BENEFITS OF MORINGA

9.1 Nutritional Uses/ Benefit;

A large number of reports on the nutritional qualities of Moringa now exist in both the scientific and the popular literature. Moringa has been in use since centuries for nutritional as well medicinal purposes. These include vitamin C, which fights a host of illnesses including colds and flu; vitamin A, which acts as a shield against eye disease, skin disease, heart ailments, diarrhea, and many other diseases; Calcium, which builds strong bones and teeth and helps prevent osteoporosis; Potassium, which is essential for the functioning of the brain and nerves, and Proteins, the basic building blocks of all our body cells. Another important point is that Moringa leaves contain all of the essential amino acids, which are the building blocks of proteins. It is very rare for a vegetable to contain all of these amino acids. And Moringa contains these amino acids in a good proportion, so that they are very useful to our bodies. These leaves could be a great boon topeople who do not get protein from meat. Moringa even contains argenine and histidine two amino acids especially important forinfants. Argenine and histidine, are especially important for infants who are unable to make enough protein for their growth requirements. Experts tell us that 30% of children in subSaharan Africa are protein deficient. Moringa could be an extremely valuable food source[18].

9.2 Therapeutic uses/ benefit

Phytochemicals refers to only those chemicals which may have an impact on health, or on flavor, texture, smell, or color of the plants, but are not required by humans as essential

nutrients. Moringa contains a range of fairly unique phytochemicals. containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates. Six such phytochemicals have been reported to have hypotensive, anticancer. and antibacterial activity include benzyl isothiocyanate, niazimicin ,pterygospermin, benzyl isothiocyanate, and 4-{a-Lrhamnopyranosyloxy}benzyl glucosinolate Numerous studies now point to the elevation of a variety of detoxication and antioxidant enzymes and biomarkers as a result of treatment with Moringa or with phytochemicals isolated from Moringa have shown, antiulcer, effect on immune response, spasmolytic activities, hypocholesterolemic effects, antibacterial activity. sympatholytic activity and antiviral activity against herpes simplex virus type-1. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. The data obtained in suggests that the extracts of Moringa oleifera both mature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules, and afford significant protection against oxidative damage.[18].

CHAPTER III: Fixed oil

1. INTRODUCTION

The use of fats and fixed oils by man dates back to antiquity. Their chemical composition and specific properties have allowed them to find use as foods, fuels and lubricants. Their sources are numerous, encompassing vegetable, animal, and marine sources. As it is with all matter, their usefulness to man is determined by their chemical nature; and all fats and oils have certain characteristics in common.[23] Fixed oil and fatsare veritable source of energy, helps in growth and development, provides essential fatty acids and fat-soluble vitamins for the system, boost the immune system and improves food palatability.[24]

This chapter presents an overview of the fixed oils and their properties and types, uses, we will also explain the difference between fixed oils and essential oils, then we will learn about the methods of extracting the fixed oils.

2 Definition of fixed oils:

Fixed oils are oily substances that are mainly obtained from vegetable sources, a carrier oil and does not evaporate, non-volatile.

They are produced in various organs of plants.Chemically, fixed oils are glycerol esters of various fatty acids.Different fixed oils differ from each other in the types of the fatty acids that form the oil, it is Chemicallysimilar to fats but differ in their physical state of occurrence. Fixed oils are usually oily liquids and the fats are generally solid or semi-solid substances. Fixed oils are non-volatile in nature and leave permanent translucent greasy spot on paper. Fixed oils are freely soluble in ether, chloroform and light petroleum. They are insoluble in and immiscible with water. Their specific gravity is less than 1.0

Many fixed oils are in use in pharmacy both as pharmaceutic necessities (excipients) and as solvents or carriers of a number of medicinal substances.

The general chemical structure of fixed oils shown in the figure (figure 3)



Figure 3: The general chemical structure of fixed oils.

3 CHEMISTRY OF FIXED OILS:

Chemically fixed oils are glycerides of fatty acids. [26]

General formula:

- Triglyceride CH2-O-CO -R CH -O-CO-R' CH2-O-CO-R''
- Mono-glyceride CH2-O-CO-R CH -OH CH2-OH
- Di-glyceride
- CH2-O-CO-R
- ¢H-O-CO-R'
- CH2-OH

- 1. If R, R', R'' are same = simple glycerides results.
- 2. If R, R', R'' are different = a mixed glyceride result.
- 3. The glycerides of unsaturated fatty acids are liquid.
- 4. The glycerides of saturated fatty acids are solid.

4 Physiochemical properties of fixed oils:

4.1 Chemical's properties of fixed oils:

- On hydrolysis we get sodium salts of fatty acids (soap) and glycerol, this process is known as saponification.
- Soluble in benzene. Chloroform and ether.
- Fixed oils can be easily hydrolyzed with aqueous sodium hydroxide.
- They are insoluble in water. [27]
- Liquid oils that contain unsaturated fatty acids can be transformed into solid oils that contain saturated fatty acids by the hydrogenation process. [28]

4.2 Physical properties:

- Fixed oils are thick, viscous, yellow colored liquids with characteristic odor.
- They are non-volatile and cannot be distilled.
- They turn rancid on storage due to free acidity as a result of the formation of aldehydic compounds or ketones or alcoholic.
- Its melting point is relatively low, so it is usually liquid or semi-solid at room temperature.
- Lighter than water, its density is about 0.8 g/cm³.[28]

5 The difference between fixed oils and essential oils:

The Table (3) illustrates difference between volatile oil and fixed oils. [29]

Table 3: Points of differentiation exist between essential oils	and fixed oils:
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Essential oils	Fixedoils
Oxygenated or dehydrogenated or hydrogenated derivatives of hydrocarbons.	Consist of glycerol esters of fatty acids.
Evaporate at room temperature.	Do not evaporate at room temperature or ordinary temperature.
Possess high refractive index.	Possesslowrefractive index.
Do not leave a permanent grease spot on paper.	Leave a permanent grease spot on paper.
Cannotbesaponified.	Can besaponified.
Their primary source is leaves, roots, in petals and bark.	Their major source is seeds of the plant.
They can be extracted easily by the distillation process.	They require some specific technique for extraction.
Do not become rancid upon long storage. Instead on exposure to light and air, theybecomeoxidized.	Become rancid upon long storage.

6 Types of fixed oils:

Fixed oils are classified into three types:

a) Dry oils are a group of oils characterized by their ability to absorb oxygen from the air

To form a solid layer used in the manufacture of paints such as linseed oil.

- b) Semi-dry oils are a group of oils characterized by partial absorption of oxygen and containing double bonds like olive oil.
- c) Non-drying oils are oils that do not absorb oxygen like peanut oil. [28]

7 Extraction methods of fixed oils:

Fixed oils and fats of vegetable origin are obtained by:

Extraction by pressing:

Fixed oils are obtained by pressing in hydraulic presses, extraction by cold pressing (the simplest) gives a directly consumable product. Coupled with physical or mechanical steps (crushing, shelling, mixing), it is suitable for artisanal practice. Nevertheless, it is also a method commonly used in industry to produce continuously, either with a screw press in the state of palm pulp and oil-rich seeds (palm kernel, copra, groundnut, sunflower), or by mixing-decanting-centrifugation in the state of olive. [30]

Chemical or solvent extraction

Solvent extraction is the process of removal of a solute component from the solid by using a liquid solvent; it is called leaching or solid-liquid extraction. According to this method, n-hexane yields a higher amount of oil compared to other solvents . There are various factors such as particle size, solvent type, and temperature that were found to affect the extraction of oil . The small particle size is preferred as it allows for a large interfacial area between the solid and liquid. The solubility of the material increases with an increase in temperature. Agitation of the solvent increases the eddy diffusion and therefore increases the transfer of materials from the surface to the particles, There are three methods of extraction of this type: Hot water extraction,Soxhlet extraction. Ultrasonication technique [31]

Accelerated solvent extraction

ASE is also called pressurized solvent extraction (PSE), this is one of the modern extraction processes. According to this method, the oil from seeds is extracted by using organic or aqueous solvents at elevated temperatures and pressures. It was observed that elevated pressure prevents boiling at temperatures above the normal boiling point of solvent but high temperature accelerates the extraction rate of oil. This method has reduced time as well as solvent consumption when compared to the other solvent extraction techniques [31]
Enzyme Assisted Extraction (EAE)

Plant cell walls are made up of lignocellulosic, and other polymers intertwined with each other, which provide a barrier to the extraction of its components. Enzymes are used for the digestion of these cellular materials and disruption of pores as a pre-extraction step which eases the diffusion of the oil into the extraction medium. Some of the most effective enzymes used are from the fungus Trichoderma. The choice of enzyme used depends on the structure of oilseed, enzyme composition, type of enzyme, experimental conditions, etc. Cellulases, hemicellulases, and pectinases are commonly used during EAE to hydrolyze and degrade the cell wall thus improving the release of intracellular content [32]

Supercritical fluid extraction

The extraction of oil from seeds using various solvent extraction techniques has been found to be more time-consuming and a problem in solvent disposal. Therefore, the problem of solvent use as well as time consumption, often in large quantities and toxicity, SFE technique has been developed. The method has been in practice since the 1980s to avoid the use of organic solvents as well as to increase the speed of extraction. Solvents used in this method include compressed gases such as ethane, propane, ethylene, dinitrogen oxide, and carbon dioxide [25], SFE using CO2 has been found to have advantages over solvent extraction. Carbon dioxide with properties of 10–50 MPa pressures and 35–80 °C temperatures[36], However, the main constraint of the SFE is its high cost at the production scale. [31]

Microwave-assisted extraction

Microwave-assisted extraction (MAE) is one of the most recent and attractive alternative oil extraction methods [33], It is a new extraction technique that is a combination of microwave and traditional solvent extraction. The MAE process has many advantages over other extraction processes, such as low cost of production with the shorter time period of processing, less solvent requirement, and a higher extraction rate with reduced energy consumption and CO2 emission [31]

8 Analytical Parameters of Fixed Oils:

Following are the parameters used to analysis the fats and oils.

1) Iodine value: The iodine value is the mass of iodine in grams that is consumed by 100 g of fats or oil. An iodine solution is violet in color and any chemical group in the substance that

reacts with iodine will make the color disappear at a precise concentration. The amount of iodine solution thus required to keep the solution violet is a measure of the amount of iodine sensitive reactive groups. It is a measure of the extent of unsaturation and higher the iodine value, the more chance for rancidity.

2) Saponification value: The saponification value is the number of milligrams of potassium hydroxide required to saponify 1 g of fat under the conditions specified. It is a measure of the average molecular weight of all the fatty acids present.

3) Hydroxyl value: The hydroxyl value is the number of mg of potassium hydroxide (KOH) required to neutralize acetic acid combined to hydroxyl groups, when 1 g of a sample is acetylated.

4) Ester value: The ester value is the number of mg of potassium hydroxide (KOH) required to saponify the ester contained in 1 g of a sample.

5) Unsaponifiable matter: The principle is the saponification of the fat or oil by boiling under reflux with an ethanolic potassium hydroxide solution. Unsaponifiable matter is then extracted from the soap solution by diethyl ether. The solvent is evaporated and then the residue is dried and weighed.

6) Acid value: It is the amount of free acid present in fat as measured by the milligrams of potassium hydroxide needed to neutralize it. As the glycerides in fat slowly-decompose the acid value increases.

7) Peroxide value: One of the most widely used tests for oxidative rancidity; peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Milliequivalents of peroxide per kg of fat are measured by titration with iodide ion. Peroxide values are not static and care must be taken in handling and testing samples. It is difficult to provide a specific guideline relating peroxide value to rancidity. High peroxide values are a definite indication of a rancid fat, but moderate values may be the result of depletion of peroxides after reaching high concentrations.

9 Fixed oil uses and benefits :

Fixed oils and fats are important products used pharmaceutically, industrially and nutritionally.

- ➤ Industrially :
 - Manufacturing of detergents, soaps, paints, and varnishes and as lubricant.
 - It is used in many natural cosmetic, and hair, skin care. It is also used in medicinal oil formulations
- > Pharmaceutically :
 - As composite substance = peanut oil, sesame oil is used as solvent in the preparation of certain I/ M injection.
 - As stimulant, cathartic, purgative, for example castor oil.
 - As emollient for ointments, liniment creams and other preparations, for example almond oil, coconut oil.
- > Nutritionally :
 - Unsaturated fatty acids have an anti-inflammatory effect, reduce blood pressure, improve the work of the heart, are important for growth and development, and in the regulation of the nervous system, and have an effect in inhibiting cancer cells, it also maintains the fluidity of living cell membranes and thus improves body functions.

10 Toxic Fixed Oils and Fats:

Though fixed oil and fats serve as one of the main components of humandiets, however some of them portend danger to man as a result of theirtoxicity. Toxic fixed oil and fats include ones containing cyclopropenes, long-chain monoene, trans-unsaturated fatty acids and lipid peroxides. [34]

11 Conclusion :

Fixed oils are used in a wide variety of consumer goods such as detergents, soaps, cosmetics, pharmaceuticals ..., Production technology is an essential element to improve the overall yield and quality of fixed oils, the traditional technologies pertaining to fixed oils processing are of great significance and are still being used in many parts of the globe.

The purpose of this chapter was to understand the concept of the fixed oils, and to have an idea of its composition and properties, and we have mentioned the main methods of extraction and Analytical Parameters of Fixed Oils.

CHAPTER IV: MATERIAL AND METHODS

1. Introduction

The study of *M. oleifera* seeds is important because it is a vegetal specie with high oil content (30 - 45 %). This oil has several well-known worldwide uses; among these it is possible to mention its potential as biofuel, specifically to produce biodiesel⁵ for lubricating oil and cosmetics applications. Oil has a high content of monounsaturated or polyunsaturated fatty acids, *M. oleifera* oil, also known as oil of Behen, contains an important percentage ofoleic acid (around 70 %). Oleic acid is a monounsaturated fatty acid that confers great stability to this oil in front of oxidation. For such a motive, moringa's oil is more stable than the canola oil, soja bean oil, and palm tree oil.[53]

2 Framework for Study

This work was carried out within the laboratory of the Scientific Research Center of Research Process Engineering, Faculty of Applied Sciences, KasdiMerbah University of Ouargla. The latter that deals with the extraction and characterization of vegetable oils, as well as antibacterial and antioxidant activities.

This work is based on the following experimental protocol:

✓ Extracting the vegetable oil of the studied species using two methods, the chemical method and the physical method

Analyzes carried out on the extract:

- ✓ Physical and chemical analyses.
- ✓ Determination of ingredients and their formula by GC/MS

3 General ExperimentalWork Plan



Figure 4 : The experimental work plan.

4 Study Area Overview (TAMANRASSET)

Tamanrasset is known as an oasis city in Ahaggar Mountains located in Southern Algeria an altitude of 1,320 m with very high temperatures of over 47°C with an interesting development of various plants just after rain.[**35**] The climate of Tamanrasset: a desert climate the soil characteristics in the Tamanrasset area (rocky/gravel and clay-like desert soil),[**37**]



Figure 5 : Presentation of the *Moringa* seed collection region.

5 Materials

- Soxhlet extraction system A 250 ml "solid-liquid "
- Condenser
- Extraction cartridges
- Tow neck round bottom flask 500ml
- Beaker 150 ml
- Heating mantle 250 ml (balloon heater)
- ➤ Funnel
- Vacuum pump (Model RS-0.5)
- Pestle and mortar
- Pasteur pipette
- Porous cellulose thimble
- ➢ Filter paper

6 Apparatus

- ➢ GCMS-TQ8040 NX
- Abbe refractometer
- ➢ Thermometer 200°C
- ➢ Analytical balance 10^{−4} g
- Rotary evaporator
- Cold press machine

7 Extraction processes

We extracted Morinaga oleiferaoil by using soxhlet and cold press, soxhlet was used by using an n-hexane as a solvent.

7.1 Soxhlet extraction

7.1.1 Solvent applied in the solid liquid extraction

Hexane (boiling point: 68.7 °C, specific gravity: 0.659, refractive index: 1.375 and purity > 98 %).

7.1.2 Pre-treatment procedure

7.1.2.1 Seed collection

Moringa Oleifera seed pods were obtained from a local farm in Tamanrasset"Abalessa"

(Figure n°11)

7.1.2.2 Drying

The fruits (pods) were dried by leaving them in a tree exposed to the sun after their harvest time (May) for a while until the peel opened.

7.1.2.3 Seeds preparation

The seeds were prepared from the pods by separating the cotyledons (exerting manually pressure on them).

7.1.2.4 Grinding

The seeds were placed in a mortar plant to be ground to reduce their size (to increase the contact area between the seeds and the solvent during the oil extraction process).



Figure 6: Sample preparation

7.1.3 Principle:

The principle of extraction work is the passage of solvent through the crushed seeds, in a circular format in several cycles and all of this is done by a Soxhlet system Supplied with condenser and a round bottom flask, The extraction was run for eight hours on four copies. The oil is separated and the solvent is recovered by rotary evaporator.

7.1.4 Operating mode

The grounded *moringa* seed samples were placed in a porous cellulose thimble. The thimble is then placed in an extraction chamber which is being suspended above a flask containing the solvent and below a condenser. Heat is being applied to the flask and the solvent evaporates and moves to the condenser where it is converted into liquid that trickles into the extraction chamber containing the sample. The extraction chamber is made in such a way that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. The flask containing solvent and lipid is removed at the end of the extraction process, and In order to separate the oil and recover the solvent, rotary evaporator is used, and to speed up the process, therotary evaporator is connected to the vacuum pump .



Figure 7 : Moringa Oil Extraction Protocol by Soxhlet

7.2 Cold press extraction

7.2.1 Principle:

The principle of the machine is to combine friction and constant pressure by means of a screw motor to move and compress the seeds at a temperature of less than 60 °C. The oil seeps through small openings that do not allow seed fiber solids to pass

7.2.2 Operating mode:

M. oleifera seeds are left to ripen naturally on the tree before harvest. The dried seeds are separated from the husks to obtain the kernels. The grains are dried again for a week at room temperature, 100g of seeds were weighed and placed in the funnel of the device, the cold pressing machine was turned on to start working at 60 $^{\circ}$ C, and then for almost a minute, the oil started to drip, Put the oil in a beaker and allow it to settle for a few minutes and use the sedimentation method to remove the Moringa cake, Filter the oil to remove the remaining solid particles in the oil

Note: In cold pressing oil plants are not heated before pressing. They are kept at low temperatures before being fed into the oil press. Cold pressing is generally at a temperature of less than 60°C [55]



Figure 8 : Moringa Oil Extraction Protocol by cold press

8 Determination of extraction efficiency

8.1 Percentage (%) Yield

Moringa oil yield is the ratio between the weight of the extracted oil and the weight of the seeds to be processed.

The extraction yield is calculated by the following equation:



9 Determination of physico-chemicalparameters

We have worked on getting some of the following important characteristics.

9.1 Density

9.1.1 Principle:

The principle is based on the measurement of the mass, at temperature ambient, of a volume of fatty substances contained in syringe (A plastic medical syringe, fitted with a detachable

stainless-steelneedle, volume 5 ml), In order to calculate the density of oil at 20 °C, we follow the following equation:

9.1.2 Expression results:

 $\mathbf{d^{20}} = \mathbf{d^{t}} + \mathbf{0.00068} \ \mathbf{(T-20^{\circ}C)} \dots \dots \dots \dots \dots (2)$

d²⁰:density at 20°C;

d^t: density at ambient or measurement temperature

0.00068: correction factor (the variation in density when the temperature varies by 1°C).

T: ambient or measurement temperature



Figure 9: Density determination

9.2 Refractive Index

9.2.1 Principle:

The measuring principle of an Abbe refractometer is based on the principle of total reflection, The light from a radiation source is reflected by a mirror and hits a double prism. A few sample drops are placed between this so-called Abbe double prism.

The incident light beams pass through the double prism and sample only if their angles of incidence at the interface are less than the critical angle of total reflection. A microscope and a mirror with a suitable mechanism are used to determine the light / dark boundary line (shadow line).

$\eta^{20} = \eta^{t} + 0,0003 \text{ (T-20°C)} \dots (3)$

η²⁰: index at 20°C,

n^t: index at ambient or measurement temperature

0.0003: the variation in refractive index when the temperature varies by 1°C.

T: ambient or measurement temperature.

9.2.2 Operating mode:

- \checkmark Clean the surface of the prism with alcohol using cotton and allow it to dry.
- ✓ Using a dropper put 2-3 drops of oil between the prism.
- \checkmark Allow the light to fall on the mirror.
- ✓ Rotate the dispersion adjustment knob to get a clear image of the reticle.
- ✓ by using the Knob of the Refractive Index, bring the boundary line to the intersection of reticle threads.
- \checkmark Read the refractive index of the oil directly on the scale.
- ✓ 7.To calculate the refractive index at $T^{\circ}C=20^{\circ}C$, we apply the previous formula.



Figure 10 : ABBE refractometer for the determination of the refractive index.

10 Qualitative and quantitative chemical analyses

10.1 GC/MS analyses

The oil of the seeds of *moringa oleifera* was analyzed by gas chromatography-mass spectrometry. GCMS-TQ8040 NX, Helium (purity; 99.99 %) was used as carrier gas. The oven temperature program is given in Table **5**, while other chromatographic conditions are depicted in Table **4**

 Table 4: Chromatographic conditions

Column oven temperature	60.0°C
Injection temperature	250.00°C
Injection mode	Split
Flow control mode	Column Flow
Pressure	56.1 kPa
Total flow	14.0 mL/min
Column flow	1.00 mL/min.
Linear velocity	36.8 cm/sec.
Purge flow	3.0 mL/min.
Spilt ratio	10.0

 Table 5: The oven temperature program

Rate	Temperature(°C)	Hold Time(min)
-	60.00	1.00
10.00	200.00	5.00
5.00	300.00	6.00

10.1.1 Priciple Gas chromatography- Mass spectrometry (GC-MS)

(GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them. GC/MS- is a combination of two different analytical techniques, gas chromatography (GC) and mass spectrometry (MS). It is used to analyze complex organic and biochemical mixtures [46]. The GCMS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility [46], [47] by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column13. Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according to their mass-tocharge ratio (m/z). These spectra can then be stored on the computer and analyzed [47]. A carrier gas is fed from the cylinders through the regulators and tubing to the instrument. It is usual to purify the gases to ensure high gas purity and gas supply pressure. In the instrument, the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column.Gas chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing, the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10-120 m is typical) with an internal diameter of 0.10-0.50 mm, whilst packed GC columns tend to be 1-5 meters in length with either 2 or 4mm internal diameter [48]. Gas chromatography has ovens that are temperature programmable, the temperature of the gas chromatographic ovens typically range from 5°C to 400°C as low as -25°C with cryogenic cooling [49].

10.1.2 Operating mode:

1. SAMPLE PREPARATION:

Samples are generally dissolved or diluted in a solvent and then injected onto the inlet port. Other methods of sample preparation,

2. VAPOURISATION

The liquid sample is vapourised in the hot inlet and becomes a gas.

3. SEPARATIONS

The mobile phase (which is an inert gas such as helium) carries the sample through the column. Different substances in the sample interact differently with the column's stationary phase, depending on their chemistry. This causes them to travel through the column at different speeds, thus separating them.

4. DETECTIONS

The separated compounds then leave the column one after the other, and enter a detector, such as a mass spectrometer (MS). The time taken for a compound to travel through the column is called its retention time.

The GC produces a graph called a chromatogram, where each separated substance is represented by a peak. The number of peaks shows the number of separated compounds in the sample. The position of each peak shows the retention time for each compound. [50]

A MASS SPECTROMETER (MS) IS COMMONLY USED AS A GC DETECTOR.

- 1. The MS will break each separated compound coming from the GC into ionised fragments. To do this, a high energy beam of electrons is passed through the sample molecule to produce electrically charged particles or ions. These fragments can be large or small pieces of the original molecule. Each charged fragment will have a certain mass. The mass of the fragment divided by the charge is called the mass to charge ratio (m/z).
- 2. The fragments then go through a process of acceleration and deflection whilst traveling through a short tunnel and being exposed to a magnetic field.

3. They eventually hit a detection plate at the end of the tunnel, where the mass to charge ratio (m/z) and relative abundance (how much of that fragment was present in the sample) are calculated.

The MS produces a graph called a mass spectrum, which shows the signal intensity or abundance of each detected fragment's mass to charge ratio. The mass spectrum produced by a given chemical compound is the same every time it is analysed, and thus can be considered a "fingerprint" for the molecule, allowing the compound to be identified.

Overall, a compound is identified via GC-MS not only by comparing its retention time to a standard (GC), but also by using its mass spectrum, making this an extremely powerful analytical tool. [50].



Figure 11 : GCMS-TQ8040 NX

CHAPTER V: RESULTS AND DISCUTIONS

1 Percentage (%) Yield of the moringa oleifera

1.1 Soxhlet

After separating by rotary evaporator, The oil yield was 24.437% from 135.45 g of *moringa oleifera* grinded seeds.

In order to calculate the percentage of oil, we use the following equation:

$$\mathbf{24.437(\%)} = \frac{33.1g}{135.45g} \times 100$$

After about 8 and a half hours and with the quantity of 135.45g from the seed, the result of the yield was 24.437 %, this result is more than those obtained from [38] and less than those obtained in other references for different regions, Variation in oil yield may be due to differences in a variety of plants, cultivation climate, ripening stage, and the method of extraction used..

1.2 Cold press

The yield of the *moringa oleifera* seeds oil by the cold press was calculated by the following equation :

$$17(\%) = \frac{17g}{100g} \times 100$$

After half an hour and with 100 g from the seed the extracted oil content of moringa oleifera seed was about 17%, which was a little less than the result reported by Yamuna Devi [39], Variation in oil yield may be due to differences in the type of the machine, variety of plants, cultivation climate, ripening stage, and the method of extraction used, The oil extracted from M. Oleifera seed has an agreeable odor and the color is cream-yellow liquid at room temperature, The cold-press extraction method gives less oil, but in a record period of time compared to the previous extraction method.

2 Physico-Chemical PropertiesDetermination

2.1 Soxhlet

2.1.1 Density

After weighing 3 ml of oil in 17 °C the result of the of the density was 0.9024,

The Density was calculated according to the previously mentioned relationship

 $d^{20} = dt + 0.00068 (T-20^{\circ}C)$ $d^{20} = 0.9024 + 0.00068 (17-20^{\circ}C)$ $d^{20} = 0.90042$

The density of *M. Oleifera* seed oil was **0.90042** and this value is in agreement with the FAO/WHO (2009) international standard for edible oil.[40]

2.1.2 The refractive index

After using the refractometer, the value of the refractive index was determined at 17 $^{\circ}$ C, we obtained 1.4565. And to calculate the refractive index at 20 $^{\circ}$ C we follow the aforementioned relationship

 $\eta^{20} = \eta t + 0,0003 (T-20^{\circ}C)$ $\eta^{20} = 1.4565 + 0,00045 (17-20^{\circ}C)$ $\eta^{20} = 1.4556$

The refractive index 1.4559 was in agreement with theFAO/WHO (2009)[40].

2.2 Cold press

2.2.1 Density

After weighing 1 ml of oil in 17 °C the result of the of the density was 0.9024,

The Density was calculated according to the previously mentioned relationship

 $d^{20} = dt + 0.00068 (T-20^{\circ}C)$

 $d^{20} = 0.9164 + 0.00068 (17-20^{\circ}C)$

$$d^{20} = 0.9143$$

The density of M. oleifera seed oil was 0.9143 and this value differs relative to that obtained by the previous extraction method..(soxhlet)

2.2.2 The refractive index

After using the refractometer, the value of the refractive index was determined at 17 $^{\circ}$ C, we obtained 1.4565. And to calculate the refractive index at 20 $^{\circ}$ C we follow the aforementioned relationship

 $\eta^{20} = \eta t + 0,0003 (T-20^{\circ}C)$ $\eta^{20} = 1.4665 + 0,00045 (17-20^{\circ}C)$ $\eta^{20} = 1.4651$

The refractive index of M. Oleifera seed oil was 1.4651 and this value differs slightly from that obtained by the previous extraction method.(soxhlet)

3 Qualitative and quantitative chemical analyses

3.1 GC and MS Analysis

The oil from *moringa oleifera* seeds was analyzed by GC-MS and the characterization of the constituents was initially accomplished by comparison with the MS library (NIST) and also confirmed by interpretation of the recorded fragmentation pattern.

The GC/MS analysis revealed the presence of 40 components , Including seven major constituent Table 5. GC/MS Chromatogram of *Moringa oleifera*oil shown in Fig.19



Figure12- GC/MS Chromatogram of Moringa oleifera oil

The results pertaining to GC/MS analysis led to the identification of the number of compounds from the oil of the *Moringa oleifera* seed. GC/MS chromatogram showed 40 peaks, indicating the presence of 40 compounds (fig.19).

Table 6 major constituent

Peak	Area%	Name	Formula	Structure chimique
1	61.30	9-Octadecenoic acid, methyl ester, (E)	C ₁₈ H ₃₄ O ₂	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2	4.30	11-Eicosenoic acid, methyl ester	C21H40O2	Pyrrower and the second
3	6.02	Eicosanoicacid, methyl ester	C ₂₁ H ₄₂ O ₂	~~~~~°~
4	9.90	Methyl 20-methyl- heneicosanoate	C ₂₃ H ₄₆ O ₂	Y
5	2.86	Tetracosanoicacid, methyl ester	C ₂₅ H ₅₀ O ₂	-og
6	8.88	Hexadecanoicacid, methyl ester	C ₁₇ H ₃₄ O ₂	Å
7	3.20	Methyl hexadec-9- enoate	C17H32O2	~~~~~°~

The major constituents found in the fixed oil of *M. Oleifera* seed oil are: Oleic acid(9-Octadecenoic acid, methyl ester, (E))(**61.30** %), 11-Eicosenoic acid, methyl ester(4.30%), Eicosanoic acid, methyl ester(6.02%), Methyl 20-methyl-heneicosanoate (9.90%) and Tetracosanoic acid, methyl ester(2.86%). Hexadecanoic acid, methyl ester (Palmitic acid)(8.88%), Methyl hexadec-9-enoate(3.20%).

3.2 Major constituent (Oleic acid (9-Octadecenoic acid, methyl ester, (E))



Figure 13- Mass spectrum of Oleic acid (9-Octadecenoic acid, methyl ester, (E)

Oleic acid (**61.30** %) was the major component of the fixed oil. Oleic acid is a monosaturated omega-9-fatty acid with many health benefits and is safe in the present practices for use and concentrations in cosmetics [41] Oleic acid prevents ulcerative colitis [42], and protects cells from free radical damage [43], reduces blood pressure [44], and increases fat burning [45].

GENERAL CONCLUSION

This review aimed to highlight on the physical and chemical properties of *Moringa oleifera* seed oil grown in Algeria (Tamanrasset Province) and extracted by two different methods (solid-liquid soxhlet) and (cold press), and the comparison between them, the oil extracted from the soxhlet was also analyzed by GC /MS to accurately determine its compounds.

The results showed the yield rate of the two methods 24.43% and 17%, respectively, and although the yield was high in soxhlet, the cold press was giving faster results, and the results of chemical analyzes (density, refractive index) recorded a relative difference between them, as for the GC/MS analyzes, 40 compounds were detected, including 7 essential acids, and the proportion of oleic acid made up the largest share, at a rate of 61.30 %.

In the end, we can say this *M. Oleifera* oil from Algeria could be utilized successfully in cosmetics applications. The seed oil exhibited goodphysicochemical properties and could be useful for industrial applications.

In conclusion, the results of the Algerian *Moringa oleifera* oil still require further studies for further developments and diversification of its fields of use due to its stimulating properties for investment in it.

REFERENCES

[1]- SiliaBoukandoul, Susana Casal, and Farid Zaidi, The Potential of Some Moringa Species for Seed Oil Production, **2018**.

[2]- Nur Zahirah Abd Rani, Khairana Husain and EndangKumolosasi. Moringa Genus: A Review of Phytochemistry and Pharmacology .2018

[3]- Washim Khan, Rabea Parveen, Karishma Chester, Shabana Parveen and Sayeed Ahmad ,.Hypoglycemic Potential of Aqueous Extract of *Moringa oleifera* Leaf and *In Vivo* GC/MS Metabolomics, 12 September **2017**.

[4]- Anwar, F.; Ashraf, M.; Bhanger, M.I. Interprovenance variation in the composition of Moringa oleifera oilseeds from pakistan. J. Am. Oil Chem. Soc. 2005, 82, 45–51.

[5]- Ndabigengesere, A.; SubbaNarasiah, K. Quality of water treated by coagulation using Moringa oleifera seeds. Water Res. 1998, 32, 781–791

[6]- Emmanuel, S.A.; Emmanuel, B.S.; Zaku, S.G.; Thomas, S.A. Biodiversity and agricultural productivity enhancement in nigeria: Application of processed Moringa oleifera seeds for improved organic farming. Biol. J. N. Am. **2011**, 2, 867–871.

[7]- Bianca Aparecida de Marco, Bárbara SaúRechelo, ElianeGandolphoTótoli, Ana Carolina Kogawa,Hérida Regina Nunes Salgado,. Evolution of green chemistry and its multidimensional impacts: A review, .2018.

[8]- Dhakar RC et al. "Moringa: The herbal gold to combat malnutrition." Chronicles of Young Scientists 2011;2(3):119-25.

[9]- Z.F. Ma, J. Ahmad , H. Zhang , I. Khan , S. Muhammad , Evaluation of phytochemical and medicinal properties of Moringa (*Moringa oleifera*) as a potential functional food , 2018

[10]-Paula García Milla, RocíoPeñalver ,Gema Nieto , Health Benefits of Uses and Applications of *Moringa oleifera* in Bakery Products , 2021

[11]-Orhevba BA, Sunmonu MO, and Iwunze HI, Extraction and Characterization of *Moringa oleifera* Seed Oil , 2013

[12]-Carla Trigo, María Luisa Castelló, María Dolores Ortolá, Francisco José García-Mares and María Desamparados Soriano, *Moringa oleifera*: An Unknown Crop in Developed Countries with Great Potential for Industry and Adapted to Climate Change,2020 [13]-Andrew B. Falowoa, Felicitas E. Mukumboa, Emrobowansan M. Idamokoroa, JoséM. Lorenzob, Anthony J. Afolayanc, VosterMuchenje, Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products, 2018.

[14]-Ahmad Faizal AbdullRazisMuhammad Din Ibrahim ,SaieBrindhaKntayya , healthy benefits of *Moringa oleifera* , 2014

[15]-Agrawal, B., Mehta, A.,. Anti-asthmatic activity of *Moringa oleifera* Lam: a clinical study. Ind. J. Pharmacol. 2008 40, 28–31.

[15]-Mercè Vilaseca, Víctor López-Grimau , and Carmen Gutiérrez-Bouzán , Valorization of Waste Obtained from Oil Extraction in *Moringa Oleifera* Seeds: Coagulation of Reactive Dyes in Textile Effluents , 2014

[16]-Foidl, N., Makkar, H.P.S. and Becker, KThe potential of *Moringa oleifera* for agricultural and industrial uses. 2001.

[17]-Francis, John K.; Liogier, Henri A. Naturalized exotic tree species in Puerto Rico. Gen.Tech. Rep. SO-82. New Orleans, LA: U.S. Department of Agriculture, Forest Serveice,Southern Forest Experiment Station. 1991. 12 p

[18]-Khawaja Tahir Mahmood TahiraMugal and Ikram Ul Haq ,*Moringa oleifera*: a natural gift-A review , 2010

[19]- Stadtlander, T.; Becker, K. Proximate composition, amino and fatty acid profiles and element compositions of four different Moringa species. J. Agric. Sci. 2017, 9, 46–57.

[20]- Farm Africa in partnership with SUGECO, Trainer's guide for Production, Harvest, Post-Harvest Handling and Value Addition for Moringa in Tanzania , 2019.

[21]-Chase, M.W.; Christenhusz, M.J.M.; Fay, M.F.; Byng, J.W.; Judd, W.S.; Soltis, D.E.; Mabberley, D.J.; Sennikov, A.N.; Soltis, P.S.;Stevens, P.F.An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc. 2016, 181, 1–20

[22]- W. Wardencki, J. Curyo, J. Namieœnik,. Green Chemistry Current and Future Issues, **2004**.

[23]-Emmanuel O. Aluyor, Kessington O. Obahiagbon and Mudiakeoghene Ori-jesu . Biodegradation of vegetable oils: A review . 2009 [24]- MUTIU IDOWU KAZEEM, ISIAKA A. OGUNWANDE. Role of Fixed Oil and Fats in Human Physiology and Pathophysiology, 2012

[25]- Illés, V.; Daood, H.G.; Biacs, P.A.; Gnayfeed, M.H.; Mészáros, B. Supercritical CO₂ and subcritical propane extraction of spice red pepper oil with special regard to carotenoid and tocopherol content. *J. Chromato. Sci.* **1999**, *37*, 345–352.

[26]- John Duncan, COSTS OF BIODIESEL PRODUCTION, 2003

[27]- Rudolph Gibson, lipids fixed oils and waxes, slideplayer.

[28]- The basics of medicinal plants, 3, faculty of agriculture, green university of al-qasim.

[29]-Eiman Ahmed Ali, Layla Mustafa Ali, Yasmin Mohammed Yousif, Extraction of Fixed Oil from Seeds of ammomumSublatum (Black Cardamom), 2016.

[30]-Daniel Pioch.Les huiles végétales : diversité d'usages et filières en compétition,LeDéméter 2018.

[31]- G.Baskar G.Kalavathy R.Aiswarya I.Abarnaebenezer Selvakumari ,.7 - Advances in bio-oil extraction from nonedible oil seeds and algal biomas.,2019

[32]- Divine Bup Nde and Anuanwen Claris Foncha., Optimization Methods for the Extraction of Vegetable Oils: A Review ., 2019

[33]- Hasene, K.Ç.; Derya, K.Y.; Uğur, G.; Fahrett, G. Optimisation of microwave-assisted extraction of pomegranate (Punicagranatum L.) seed oil and evaluation of its hysicochemical and bioactive properties. Food Technol. Biotechnol. 2017, 55, 86–94.

[34]- Mutiu Idowu Kazeem, IsiakaAjanaiOgunwande.role of fixed oil and fats in

Human physiology and pathophysiology. 2012. pp:102: 17-100, RPMP Vol. 33: Food Oils, lagos state university.

[35]- Chabane Djamila, Importance of ethnobotanic survey in Tamanrasset (Saharan region) in the south of Algeria face to climate changes,2017

[36] - Calvo, A.; Morante, J.; PlanderSzSzekely, E. Fractionation of biologically active components of grape seed *(vitisvinifera)* by supercritical fluid extraction. *Actaahmentaria* **2017**, *46*, 27–34

[37] - Juan Cuesta, Dimitri Edouart, Mohamed Mimouni, Pierre H. Flamant, Claude Loth, et al..Multiplatform observations of the seasonal evolution of the Saharan atmospheric boundary layer in Tamanrasset, Algeria, in the framework of the African Monsoon Multidisciplinary Analysis field campaign conducted in 2006. Journal of Geophysical Research: Atmospheres, American Geophysical Union, 2008,.

[38] - OUBIRI Maissoune, OUAFIANE Amel ., Extraction des huiles fixes à partir des plantessahariennes : *Moringa oleifera* . , 2018.,

[39] -Yamuna Devi., EXTRACTION OF COLD PRESS MORINGA OIL , April 2015.,

[40] -FAO/WHO (2009). Report of the 21st session of the Codex Alimentarius Committee on fats and oils. Kola Kinabalu, Malaysia, 16 – 20 February 2009

[41] -Liebert MA. Final Report on the Safety Assessment of Oleic acid, Laurie acid, Palmitic acid, Myristic acid and Stearic acid. Journal of American College of Toxicology. 1987. 6(3): 321-402.

[42] - De Silver PS, Luben R, Shrestha SS, Khaw KT, Hart AR..Dietary arachidonic and oleic acid intake in ulcerative colitis etiology: A prospective cohort study using 7-days food diaries. Eur. J. Gastroenterol Hepatol ,2014, 26 (1): 11-8

[43]- Haug A, Hestmark AT and Harstad OM. Bovine milk in human nutrition-a review. Lipid Health and Disease. (2007). 6:25.

[44] -Ruiz-Gutirrez V, Munana FJ, Guerrero A, Cert AM, Villar J. Plasma Lipids erythrocytes membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic from two different sources. J. Hypertens. (1996). 14: 1483-1490.

[45]- Lim JH, Gerhart-Hines Z, Doming JE, Lee Y, Kim S, Tabata M, Xiang YK, Puigserver P. Oleic acid stimulates complete oxidation of fatty acids through protein kinase A-dependent activation of SIRT1-PGC1α-complex-J. (2013). Biol Chem. 288:7117-26.

[46]- Skoog, DA. Holler, FJ. "Principles of Instrumental Analysis" 6 th Edition. Brooks/Cole Cengage Learning, 2007. 11, 20, 26, 27

[47]- Baker, M. Pattenden G, "Mapping the structural requirements of inducers and substrates for decarboxylation of weak acid preservatives by the food spoilage mould", Aspergillus niger. International Journal of Food Microbiology, (2012),157, 375.

[48]- Eiman Abdallah Abdelmarouf Mohamed .,GC-MS Studies on Seeds of Pithecellobium dolce Fixed Oil, . August, 2016.

[49]- Wauschkuhn, C. ,Fügel D, Wrany U, Anastassiades M, Hancock P, Dunstan J, "Application of GC-MS/MS for Pesticide Residues Analysis" (2006).

[50]- Shimadzu corporation, Shimadzu's Fundamental Guide to Gas Chromatography-Mass Spectrometry (GCMS), 2020.

[51]- Vaknin, Y.; Mishal, A. The potential of the tropical "miracle tree" *Moringa oleifera* and its desert relative *Moringa peregrina* as edible seed-oil and protein crops under Mediterranean conditions. Sci. Hortic. **2017**, 225, 431–437.

[52]- Mercè Vilaseca, Víctor López-Grimau, and Carmen Gutiérrez-Bouzán, Valorization of Waste Obtained from Oil Extraction in *Moringa Oleifera* Seeds: Coagulation of Reactive Dyes in Textile Effluents **2014**.

[53]- Díaz-Domínguez, Yosvany; Tabio-García, Danger; Rondón-Macias, Maylin;
FernándezSantana, Elina; Muñoz - Rodríguez, Susana; Ameneiros - Martínez, José María;
PilotoRodríguez, Ramón Extraction and characterization of *Moringa oleifera* Lam var.
Supergenius seed oil from Cuba. 2017

[54]- Martin J. Mulvihill, Evan S. Beach, Julie B. Zimmerman, and Paul T. Anastas,. Green Chemistry and Green Engineering: A Framework for Sustainable Technology Development,.**2011.**

[55]- LEEROY BARNETE ,. DESIGN OF A PROCESS TO EXTRACT 1.4TPD OF MORINGA OIL FROM MORINGA SEEDS USING COLD PRESS EXTRACTION METHOD,. **2018.**

[56]- Thurber, M.D.; Fahey, J.W. Adoption of Moringa oleifera to combat under-nutrition viewed through the lens of the "diffusion of innovations" theory. Ecol. Food Nutr. 2009, 48, 212–225.

ANNEXES

Sample Information

Method

[Comment]

------ Analytical Line 1 ------

[AOC-201+5]			
# of Rinses with Presolvent		:3	
# of Rinses with Solvent(post)	:2	
# of Rinses with Sample		:2	
Phinger Speed(Suction)		High	
Viscosity Comp. Time		:0.2 sec	
Plunger Speed(Injection)		High	
Syringe Insertion Speed		High	
Injection Mode		Normal	
Pumping Times		:5	
Inj. Port Dwell Time		:0.3 sec	
Tenninal Air Gap		:No	
Phinger Washing Speed		High	
Washing Volume		SuL.	
Syringe Suction Position		:0.0 mm	
Syringe Injection Position		:0 0 mm	
Solvent Selection		only A	
Column Oven Tenno	-60 0 °C		
Injection Temp	-250.00 °C		
Injection Mode	Split		
Flow Control Mode	Column Flow		
Pressure	-56 1 kPa		
Total Flow	-14 0 mJ/min		
Column Flow	-1.00 mI /mm		
Linear Valocity	-36.8 cm/sec		
Purge Flow	-3 0 mL min		
Split Ratio	10.0		
High Pressure Injection	OFF		
Carrier Cas Saver	OFF		
Calify Uaid	OFF		
Oran Tana Draman	.011		
Pata Pata	Tananatara/0(1)		Hald Time (min)
Rale	remperature(-C)		Loo
10.00	200.0		5.00
10.00	200.0		5.00
5.00	300.0		0.00
Oven Cooling Rate	Middle		
< Ready Check Heat Unit >			
Column Oven	Yes		
SPI 1	· Yes		
MS	Ves		
< Ready Check Detector (FTT)/BID)>		
< Ready Check Baseline Drif	1>		
< Ready Check Injection Flox	v>		
SPL1 Carrier	- Yes		
SPI 1 Purce	·Ves		
< Ready Check APC Flow>			
< Ready Check Detector APC	Flow >		

External Watt Equilibrium Time Anto-flame On Anto-flame Off Reignite Auto-zero after Ready PrepRim Start No 3.0 min OFF ON OFF ON Auto [GC Program] [GCMS-TQ8040 NX] IonSourceTemp Interface Temp Solvent Cut Time Detector Gain Mode Detector Gain Threshold Interstold [MS Table] --Group 1 - Event 1--Compound Name Start Time Fand Time Acq Mode Event Time Scan Speed Start m/z Resolution Q3 Resolution Sample Inlet Unit

200 00 °C 280 00 °C 3 00 min Relative to the Tuning Result 0.83 kV+0 00 kV 0

3.00min 46.00min Q3 Scan 0.300sec 166.6 45.00 500.00 GC

[MS Program] Use MS Program

OFF








	Peak Report TIC									
Peak#	R. Time	1.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	12.490	12.470	12.535	238826	0.03	183867	0.20	1.30	MI	
2	13.859	13.825	13.905	88380	0.01	45912	0.05	1.92	MI	
3	14,525	14.495	14.555	181310	0.02	122100	0.13	1.48	MI	
4	14.583	14.560	14 615	41058	0.00	27350	0.03	1.50	MI	
5	14.675	14.635	14.705	34247	0.00	23314	0.03	1.47	MI	
ó	14.804	14.740	14.855	3301365	0.35	2154360	2.35	1.53	MI	
7	15.657	15.615	15.705	93257	0 01	50076	0.05	1.86	MI	
8	15.781	15.750	15.820	93418	0.01	51802	0.06	1.80	MI	
9	15.981	15.935	16.020	215865	0.02	119818	0.13	1.80	МІ	
10	17.179	17.020	17.260	29852421	3.20	9120477	9.96	3.27	MI	
11	17.302	17.250	17.370	1226774	0.13	532004	0.58	2.31	MI	
12	17.572	17.400	17.780	82884509	8.88	14216222	15.53	5.83	MI	
13	17.904	17.860	17.945	201177	0.02	89680	0.10	2.24	MI	
14	18.950	18.845	19.035	1349870	0.14	362442	0.40	3.72	MI	
15	19.456	19.375	19.560	2534719	0.27	688431	0.75	3.68	MI	
10	21.795	21.035	22.300	572438413	61.30	16545924	18.07	34.60	MI	
17	22.350	22.300	22385	273829	0.03	121195	0.13	2.26	MI	
18	22.420	22.385	22.475	306352	0.03	136831	0.15	2.24	MI	
19	22.902	22.855	22.960	161157	0.02	55759	0.06	2.89	MI	
20	23.256	23.185	23.310	130091	0.01	40640	0.04	3.20	MI	
21	23.490	23.430	23.550	1088961	0.12	367049	0.40	2.97	MI	
22	23.580	23.540	23.630	147855	0.02	65789	0.07	2.25	MI	
23	23.677	23.640	23,710	54886	0.01	23373	0.03	2.35	MI	
24	24.064	23.960	24.130	474520	0.05	129715	0.14	3.66	MI	
25	25.421	25.365	25.465	642210	0.07	227405	0.25	2.82	MI	
26	25.643	25 470	25.700	40114821	4.30	8508444	9.29	4.71	MI	
27	25.736	25.695	25.810	2818239	0.30	1106508	1.21	2.55	MI	
28	26.179	26.025	26.260	56262557	6.02	10478049	11.44	5.37	MI	
29	27.834	27.785	27.900	940522	0.10	328236	0.36	287	МІ	
30	27.973	27.920	28.030	1568516	0.17	532836	0.58	2.94	MI	
31	29.298	29.240	29.350	2865106	0.31	1031926	1.13	2.78	MI	
32	29.427	29.350	29.485	998021	0.11	272712	0.30	3.66	MI	
33	29.859	29 660	29.960	92438321	9.90	13236841	14.46	6.98	MI	
34	31.379	31.325	31.425	2058894	0.22	768093	0.84	2.68	MI	
35	32.651	32.610	32.705	1498248	0.16	564216	0.62	2.66	MI	
36	32.973	32.870	33.065	26682317	2.86	7152277	7.81	3.73	MI	
37	34.410	34.345	34.470	464909	0.05	157653	0.17	2.95	MI	
38	35.828	35.780	35.885	1610510	0.17	580293	0.63	2.78	MI	
39	40.199	40.135	40.355	1705230	0.18	420161	0.45	4.06	MI	
40	40.977	40.895	41185	3810920	0.41	925723	1.01	4.12	MI	















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