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Extraction and Characterization of Essential Oil From Moringa Seed

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Declaration

We hereby declare that the thesis is based on our original work except for flotation's and citations which have been duly acknowledged. We also declare that it has not been previously or currently submitted for any other department at Wolkite university.

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Acronyms

A.V	Acid value
AOA	Antioxidant activity
AOAC	Association of official analytical chemist
FAO	Food and Agriculture Organization
FDA	Food Drug Administration
FFA	Free fatty acid
I.V	Iodine valeu
MAAEE	Microwave-Assisted Aqueous Enzymatic Extraction
MAE	Microwave-Assisted Extraction
M. O	Morniga Oleifera
N. S	Moringa stenopetala
Mc	Moisture Content
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated Fatty Acids
S. V	Saponification value
SFA	Saturated fatty acids
SFE	Supercritical Fluid extraction
Sp.gr	Specific gravity

Abstract

This thesis was targeted to extract an Essential Oil form Moringa Seed using solvent extraction method, to study effect of extraction process and characterizing physio-chemical property of extracted oil. In this thesis, the effect of particle size, extraction time and solvent to solute ratio of solvent on extraction of Moringa Seeds in solvent extraction process was studied.The extraction of essential oil was conducted by using n-hexane as solvent and soxhelet extractor. The physio- chemical property of moringa seed and extracted essential oil was assessed.And the value of moisture content, ash, density, PH, acid value, ester and saponification value were 8%%, 5.3%, 0.7668g/ml,7 ±0.5, 2.242mgNaOH/g,180.08Mg NaOH/g and 182.235mgNaOH/g respectively. Generally, the yield of oil increases from two-hour to four-hour with a maximum yield of 46 %(w/w) at medium particle size and minimum yield of 17 %(w/w) at coarse particle size(2-1)mm. Therefore, from this study, it is possible to generalize; the maximum extraction of essential oil was obtained at medium particle size(1-0.5)mm.

Key words : moringa seed , essential oil, oil yield

Chapter One

1.1 Introduction

1.1.1 Background

Plants have always been serving every humankind irrespective of the era and area to which mankind existed throughout all of his history. Plants give their function according to their nature of existence in different region with respect to various mechanism of their application for different purposes. Some groups of plants are used for daily nutrition whereas, others are wanted by the industry for many applications. For instance, they could be for making medicine, for building, or they are wanted by pulp and paper industry for manufacturing stationary instruments. Anyhow, there function differ. Some plants have only one function, others have only two or three functions. However, there are some plants that have multi-purpose applications.so, they have more than three functions. From among those plants, Moringa oleifera is the typical example. It has a number of applications in different parts of the world. The terms ‘Moringa’ and ‘Oleifera’ imply the genus and species name. The genus name Moringa derives from the Tamil word, murungai, meaning “twisted pod”, alludes to the young fruit[[1]]. The species name oleifera is derived from the Latin words oleum "oil" and ferre "to bear"[2]. The plant has numerous common names across regions where it is cultivated, with drumstick tree, horse radish tree or simply moringa used in English.[3]Anyhow, it is one of the most amazing plant with many packages of applications in our real life situations. However, it is still unfamiliar to many peoples of Ethiopia except for some of our forefathers and foremothers. Our elders know its value traditionally. Its modern application is recent application in different aspects of life. This is why we are now familiar with its modern applications apart from traditional applications of our ancestors.Anyhow,we are hoping all Ethiopian citizens will clearly understand the morale of Moringa seed apart from medicinal use.There are at least thirteen varieties of Moringa species across the globe, but the most common species in our local country are Moringa Oleifera or the “Miracle Tree” which is the source of food and energy for the twenty-first century and Moringa stenopetala which also named as “shiferaw” (Amharic), halako (Gamo/wolayita), kallanki (Benishangul), Shalchade (Konso), Haleko (Burji) and cabbage tree (English).Moringa stenopetala was domesticated in East Africa low lands and indigenous to southern and south-western Ethiopia. Especially during the dry season; areas like Gofa, Konso, Burji and Gamo tribes consume its leaves as a food. Moringa oleifera, known as “Moringa” or “Malunggay” is an Indian tree that also grows in Asia, Africa, South America, the Caribbean and Oceania.

The oil extracted from Moringa is known as Ben oil and reportedly contains 70% of oleic acid, an 18-carbon long monounsaturated fatty acid (MUFA). Since the oleic acid has good oxidative stability when compared with polyunsaturated fatty acids (PUFAs), fatty acids (PUFAs), it has found use in the food industry, as it allows for longer storage and high-temperature frying processing. It also has uses in medicine and water treatment. According with AbdulKarim et al.[4] Ben oil is more stable than canola oil, soybean oil, and palm oil when used in frying. Blending Ben oil with sunflower oil and soybean oil enhances the oxidative stability of the mixture.

1.2 Statement of the problem

According to the present condition of our people, the price of oil has increased to the degree at which we could not buy simply as before unless we have plenty of finance.so, Inflation rate of oil price compared to the past time is as tall as a mountain. The other problem that insisted us to do this project is wastage of time waiting for abroad oil until it is imported from other countries. However,producing essential oil here in our local country is time saving and effect for running process industries that require essential oil. Essential oil is produced from different raw materials like soybean, sun flower, moringa seed. Among these, moringa seed has high composition to be considered as material for essential oil production. The conversion of moringa seed to essential oil needs to put an effort using effective advent technologies. Among those, solvent extraction method is selected and implemented. Inline this, the synergetic effect of particle size and time was studied. Hence, the extraction capacity of essential oil improved and supply-demand ratio was addressed. Subsequently. The market inflation is solved.

1.3 Objective of the study

1.3.1 General objective

To extract essential oil from Moringa seed using solvent extraction method.

1.3.2 Specific objectives

- To characterize the proximate composition analysis of Moringa seed.
- To characterize the Physico-chemical properties of the extracted oil.
- To characterize the final extracted oil.

1.4 Scope of the study

This final project is aimed at how essential oil extracted from morniga seed. It further entails about extraction methods such as solvent extraction method. More-ever, it explains about different parameters that affect the yield of the oil. Generally, it covers collection and preparation of M.stenopetala seed, characterizing the physical and chemical properties of the extracted oil, and study the effect of particle size ,effect of solvent to solute ratio and extraction time on oil yield and beneficiaries of health status for the people.

1.5 Significance of the Study

This study could have benefits to many stakeholders in economy and utilization of these oils can have a great economic impact on local communities and significantly contribute to local farmer's economy. Oils and fats are natural source have extensive applications in our modern industrial world. Global industrialization and increasing demand for environmentally acceptable materials has led to the investigation and exploitation of more vegetable oils as a renewable feed stock in the preparation of oleo chemicals in order to meet the growing needs of human society [5, 6]. The Moringa seeds could be used to obtain high oil yield for multiple applications in the food and cosmetics industries as result of a rich source of fatty acids. Besides the industrial uses such as fine lubricant and perfumery, the fatty acids profile of the oil with its very high content of oleic acid make its oil with high potential for further industrial application which has been shown to be beneficial to the skin. This oil is of excellent quality similar to the olive oil, and is slow to become rancid. It gave high oil yield, which has good antioxidant capacity with potential for industrial, nutritional and Cosmetics applications. The healing properties of Moringa seed oil have been reported by ancient cultures. Moringa oil has tremendous cosmetic value and is used in body and hair care as a moisturizer and skin conditioner [7, 8].

Chapter Two

2.1 Literature review

Moringa is a plant that is often called the drumstick tree, the miracle tree, the ben oil tree, or the horseradish tree. Moringa has been used for centuries due to its medicinal properties and health benefits. Moringa oleifera Lam. is the most widely cultivated species of the tropical flowering plant family Moringaceae containing thirteen diverse species (Shahzad et al., 2013). Moringa oleifera is indigenous to south Asia, where it grows in the Himalayan foothills from North-Eastern Pakistan to North-Western Bengal, India (Sharma et al., 2011). The species was introduced and became naturalized in other parts of the world including East and West Africa (Paliwal et al., 2011). It has a great potential to serve as a high-value food crop, medicinal products, as well as fodder for animals, particularly in developing countries (Shahzad et al., 2013). It also has antifungal, antiviral, antidepressant, and anti-inflammatory properties.The seeds of the moringa specie*s* plant are among the most nutritious and useful botanical and herbal remedies, as nutritional supplements and for industrial and agricultural purposes. Moringa seeds are edible in both fresh and dried forms and, along with the seed pods that contain them, can be prepared in numerous ways as both food and medicinal therapeutic purposes [9]. The medicinal properties of the moringa seed are well documented in the scientific literature and are further supported by the experiences of generations of traditional Ayurvedic practitioners [10]. While many parts of moringa species trees are deemed useful, the seeds are especially prized for their medicinal powers. The seeds have valuable properties that enable them to treat a wide array of illnesses and conditions. The National Charity for Organic Growing has studied the efficacy of moringa species seeds as a medical treatment and found that they provide legitimate relief for many medical problems. These include rheumatism, gout, sexually transmitted diseases, urinary infections, boils, and even epilepsy [11]. Moringa seeds are also used as primary coagulate in drinking water classification and waste water treatment due to the presence of water soluble cationic coagulant protein able to reduce turbidity of the water treated [12]. Shiferaw/Haleko leaves contain high contain of essential amino acids and vitamin A and C. Besides the industrial uses such as fine lubricant and perfumery, the fatty acids profile of the oil with its very high content of oleic acid may make it oil with high potential for further industrial application [13]

Oleic acid is an essential omega-9 fatty acid that was found to be responsible in hindering the occurrence of adrenoleukodystrophy (ADL), a fatal disease that affects the brain and adrenal Sglands [21]. Moringa stenopetala seed powder can be used as effective heavy metal purifying from water and that moringa stenopetala is more effective than oleifera in removal of heavy metals [14]. All parts of the moringa tree such as leaves, immature pods (drumstick), seeds and flowers, and oil pressed from mature seeds are generally used as or in food.

For example, flowers of Moringa Oleifera are rich in calcium, potassium and antioxidants, and are used in various dishes. Leaves are an excellent source of protein with 100 g of fresh raw leaves providing 9.8g of protein. Moringa contains proteins, vitamins, and minerals [15]. Moringa tree has been referred to as the 'Miracle tree' for its versatility and experts all over the world believe Moringa to be an ideal plant to combat under-nutrition because of its nutritional benefits and availability. It has been estimated that fresh leaves of Moringa plant contains four times the vitamin A of carrots, seven times the vitamin C of oranges, four times the calcium of milk, three times the potassium of bananas and two times the protein of yogurt [16]

2.2 Moringa Seed Characteristics

It is three-angled; the kernel is responsible for 70%–75% of the weight. Fruits are tri-lobed capsules, and are frequently referred to as pods. Immature pods are green and, in some varieties, have some reddish color. Pods are brown, triangular, splitting length wise in to 3 parts when dry, 30-120 cm long and 1.8 cm. wide [15]. The Moringa stenopetala seeds are round with a white to brown and Moringa Oleifera seed hulls are generally brown to black. The hulls itself has three white wings that run from top to bottom at 120^{o} intervals. The average weight per seed is 0.3 g for M. Oleifera and 0.42 g for M.stenopetala and the kernel to hull mass ratio is 75:25[8]

M.oleifera seeds are globular, about 1 cm in diameter. They are three-angled, with an average weight of about 0.3 g, 3-winged with wings produced at the base of the seed to the apex 2–2.5 cm long, 0.4–0.7 cm wide; the kernel is responsible for 70%–75% of the weight of the seed [17

 

Figure 2. 1. Seeds without pods (left side) and Moringa tree with pods (right side)

The seeds of M.stenopetala are packed full of fixed oils (over 30% by weight) which are characterized by the predominant oleic acid composition [18]. The seeds are also sources of the cationic proteins which are responsible for the flocculent activity and/or water purification potential of the plant. M. stenopetala is a promising tree for nutrition, water purification and herbal medicine and it has many beneficial qualities, similar to those found in M. oleifera. There is however, still a great need for further detailed chemical and pharmacological evaluation of M.stenopetala. In the future, this tree could have the potential to surpass M. oleifera as an important multipurpose crop [19].

Table 2:1 Chemical composition of Moringa oleifera seeds (g/100 g of dry weight).

Nutrients	**Moringa oleifera Seeds**		
	Mean	SD	Range
Fat	36.7	2.8	4.7–40.4
Protein	31.4	1.3	29.4–33.3
Carbohydrate	18.4	1.4	16.5–19.8
Fiber	7.3	0.5	6.8-8
Ash	6.2	0.9	4.4–6.9
Moisture content	7	1.2	5.7–8.9

2.3 Moringa seed oil

The main components of the oil are oleic acid 78% followed by palmitic acid 7% and behenic acid 4%. Oils and fats are triglycerides with three long chains of fatty acid group randomly esterified with glycerol. The difference between oils and fats is that, oil is liquid whereas fats are solid at ordinary temperature. Improvements Immunity of Moringa oil **is** rich in omega-9 fatty acids, vitamin C, and vitamin E that help seek out free radicals and protect the organs from excess inflammation and strain.

When you analyze essential oils with a chromatograph various organic component are found and the primary ones are as follows: Terpene hydrocarbons, Mono-terpene hydrocarbons, Sesquiterpenes, Oxygenated compounds, Phenols, Alcohols, Monoterpene alcohols, Sesquiterpene alcohols, aldehydes, Ketones. Esters, Lactones, Coumarins and Ethers. In general, pure essential oils can be subdivided into two distinct groups of chemical constituents; the hydrocarbons which are made up almost exclusively of terpenes (mono-terpenes, sesquiterpenes, and di-terpenes), and the oxygenated compounds which are mainly esters, aldehydes, ketones, alcohols, phenols, and oxidesThese nutrients are not only beneficial for heart health, but they also have anti-inflammatory and antioxidant effects [20].

2.4 Moringa seed oil and its application

Oleic acid is an essential omega-9 fatty acid that was found to be responsible in hindering the occurrence of adrenoleukodystrophy (ADL), a fatal disease that affects the brain and adrenal glands [21]. Moringa stenopetala seed powder can be used as effective heavy metal purifying from water and that Moringa stenopetala is more effective than oleifera in removal of heavy metals [22]

Essential oils and their constituents were utilized to treat a large number of human diseases since ancient times. Today, essential oils act as an alternative source with its oral, topical and aromatherapy treatment. Increasing number of scientific investigators has started process of elucidating the specific mode of action of essential oils components. New antibiotic drug has been evolved by targeting novel biochemical pathways that are not targeted by the existing antibiotics. Synergistic effect of existing antibiotic drug with essential oil components may provide an alternative approach to rectify the emerging drug resistance [23]. It is used in wide range of consumer goods such as soaps, detergents, toilet products, cosmetics, pharmaceuticals, confectionery, perfumes, soft drinks, distilled alcoholic beverages, food products, insecticides.A number of workers have carried out a lot of work on the extraction and analysis of Moring seed oils. Primarily on M. oleifera because of distributed large area of the world and extensive demands for oils both for human consumption and for industrial applications.Previous studies on Moringa have focused majorly on its use medicinally and on the nutritional uses of the tree parts specially the leaves as well as on the use of the seed in clarification during wastewater treatment [13]. Perfume manufacturers esteem the oil for its great power of absorbing and retaining even the most fugitive odors. The filtered and pure oil is packed in suitable containers as per our customer's requirement. This oil can be used as preservative in food industries and for cooking. The oil extracted from Moringa seed is known as ben or Behen oil due to the high behenic acid content relative to other oil crops. M. stenopetala can be used as a food, a cosmetic and a lubricant. Seed biomass remaining after the oil extraction can be used as a fertilizer or a flocculating agent for water purification. The oil extracted from Moringa seeds is regarded as having a good commercial interest due to its physical, chemical and pharmacological characteristics [13].

Moringa oil has been mentioned as very useful oil in the medicinal books of Greece and Rome. Even today, this oil is used for a number of industrial applications. It has great significant to use for the skin and hair. Its properties make it suitable for both human consumption and commercial purposes. Indeed, Moringa oil could be a good substitute for olive oil in the diet as well as for non-food applications, like biodiesel, cosmetics, and a lubricant for fine machinery. Moreover, after oil extraction, the seed cake can be used in waste water treatment as a natural coagulant or as an organic fertilizer to improve agricultural productivity [22].

The seeds of M. oleifera have been found to be good antioxidants, able to reduce oxidative damage associated with aging and cancer [24]. Many of the bioactive compounds isolated from M. oleifera seeds have been found to be potential antitumor promoters [25]. However, recent study results found that the ethanol extract of M. oleifera seeds had no significant effect in inhibiting the proliferation of breast and colorectal tumor cells. Nevertheless, a recent study observed a cytotoxic effect of M. oleifera oil in several cancer cell lines [14].

The seeds of the Moringa specie*s* plant are among the most nutritious and useful botanical and herbal remedies, as nutritional supplements and for industrial and agricultural purposes. Moringa seeds are edible in both fresh and dried forms and, along with the seed pods that contain them, can be prepared in numerous ways as both food and medicinal therapeutic purposes [26]. The medicinal properties of the Moringa seed are well documented in the scientific literature and are further supported by the experiences of generations of traditional Ayurvedic practitioners [27].

While many parts of Moringa species trees are deemed useful, the seeds are especially prized for their medicinal powers. The seeds have valuable properties that enable them to treat a wide array of illnesses and conditions.The National Charity for Organic Growing has studied the efficacy of Moringa species seeds as a medical treatment and found that they provide legitimate relief for many medical problems. These include rheumatism, gout, sexually transmitted diseases, urinary infections, boils, and even epilepsy [28]. Moringa seeds are also used as primary coagulate in drinking water classification and waste water treatment due to the presence of water soluble cationic coagulant protein able to reduce turbidity of the water treated [29]. Shiferaw/ Haleko leaves contain high contain of essential amino acids and vitamin A and C. Besides the industrial uses such as fine lubricant and perfumery, the fatty acids profile of the oil with its very high content of oleic acid may make it oil with high potential for further industrial application [13].

Ben oil is used for household cooking, because it is colorless, odorless and resist rancidity, This property of the oil enhances the improvement and retention of taste and natural flavor. This oil also contains fatty acids, vitamin A and C, which is needed in the human body.

Table 2:2.oil of different raw materials

Type of oil	SV	IV	Type of oil	SV	IV
Coconut oil	250-264	7.5-10.5	Safflower oil	189-195	138-146
Cottonseed	190-198	98-115	Sunflower oil	188-194	100-140
Groundnut oil	188-195	87-98	Soybean oil	189-195	125-140
Sesame oil	188-193	103-115	Palm oil	195-205	44-58

Moringa stenopetala oil has higher saturation than soybean, sunflower and rape seed oil, but lower saturation than palm oil, it is important also too remarkable that, the absence poly-saturated fatty acid in Moringa stenopetala oil in comparison to that of soybean, sunflower and rape seed oil. Another interesting fact is they high contain of behenic (docosnic) acid in Moringa stenopetala oil possesses significant resistance to oxidative degradation due to it contain of behenic acid [27]. The Moringa seed extract is used in cosmetics as the oil penetrates deeply into the skin; carrying essential nutrients and helping the skin refresh and rejuvenate it. Beauty companies around the world now uses Moringa oil in perfumes, massages, aromatherapy, because the oil has a property which is nourishing to the skin.

The oil is used in making soaps because of the constituent of the oil which is essential. The oil resembles olive oil and rich in fatty acids and oleic acid, which makes it suitable for edible purposes. If Moringa seeds are to be used for oil production, the seeds are harvested and immediately processed. Furthermore, plant essential oils are widely available and some are relatively inexpensive compared to plant extracts [30]. To extract the essential oil, steam distillation and Soxhlet extraction methods were used. The mature seed of Moringa oleifera is rich in oil containing between 22-40% crude fat. Variation in oil yield may be due to the differences in variety of plant, cultivation climate, ripening stage,

the harvesting time of the seeds and the extraction method used. The high percentage of oil makes this seed a distinct potential for the oil industry. Moringa Oleifera seeds contain between 35-40 % (w/w) of vegetable oil [22]. M. stenopetala seed contains up to 44.9% (w/w) of oil. The oil from Moringa stenopetala seeds will be extracted using three different procedures including cold press, extraction with n-hexane and extraction with a mixture of chloroform and methanol and high oil yield was obtained from n- hexane extraction. The fatty acid composition of M. stenopetala seed oil of the Ethiopian variety investigated and compared with the fatty acid composition of M. oleifera and olive oil. This oil resembles olive oil in its fatty acid composition and is oleic acid-rich, which makes it suitable for edible purposes. Moreover, other applications have been pointed out as preparation of cosmetics, mechanical lubricant, and lately for potential biodiesel fuel elaboration.

2.5 Oil extraction methods

The main point of extraction is the separation of the oil from the oil-bearing material with subsequent purification of the raw oil, but looking at the oil production as a whole not only the oil processing is important for the production of high-quality oil, but also the harvest, the pre treatment of the harvested material, and the storage conditions until processing have to be taken into consideration. The aim of the extraction method is to optimize the oil yield with simultaneous maintenance of the oil quality.

2.5.1 Mechanical oil extraction

Prior to the discovery of any other oil extraction method the oil will be extracted by mechanical or cold pressed. It is the simple process of heating the plant material to low temperatures and then physically pressing the oil out. Today mechanical expression is used mainly for citrus peels and is unpopular due to the low extraction yield. [31]. It can extract 68– 80% of the available oil while the ram presses only achieved 60–65%. The oil extracted by mechanical presses needs further treatment of filtration and degumming in order to produce a pure raw material [32]. It has been also found that pretreatment of seeds before applying mechanical extractor increases the amount of oil recovery.

2.5.2 Solvent extraction

Various solvents have been utilized in the solvent extraction process over the years, including carbon disulfide, petroleum naphtha, benzene, trichloroethylene, alcohols, pentane, supercritical carbon dioxide and hexane. The solvent used in the vast majority of solvent extraction processes around the world today is commercial hexane, a mixture of hydrocarbons generally boiling in the temperature range of 65–69 ^{0}C. Most commercial hexane contains approximately 65% normal hexane, with the remaining 35% of the composition consisting of cyclo-pentane and hexane isomers. Commercial hexane is the preferred solvent today, due to its wide availability, relatively low cost, excellent diffusivity through oilseed cell walls, high solubility with edible oils, low solubility with water, low latent heat of vaporization, low specific heat and moderate boiling range. Commercial hexane also has its downsides. Hexane vapor is three times heavier than air and slight amounts of hexane vapor mixed in air can create an explosive mixture. Special care must be taken in constructing and operating commercial hexane solvent extraction processes. Solvent extraction is the process in which the oil is removed from a solid by means of a liquid solvent, it is also known as leaching [33]. Solvents are compounds that are liquid at room temperature and atmospheric pressure. They are able to dissolve other substances without chemically changing them. When performed at low temperature, solvent extraction has another advantage over screw pressing because it gives better-quality oil [22]. Solvent extraction is a mass transfer operation involving diffusion of a suitable solvent into oil- bearing cells of the raw material, resulting in an oil-solvent solution. The oil is then distilled and the solvent evaporated leaving the oil behind. This process leaves minimum oil residual in the cake compared to mechanical. Soxhlet extraction is required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent passed through the sample; just one batch of solvent is recycled [34]. Extraction of oil using solvents is the most effective method for oil recovery of almost 98%, especially with materials having low oil content. The chemical extraction using n-hexane method results in the highest oil yield which makes it the most commonly used solvent. N- hexane is selected due to its low viscosity, high volatility, simple structure and percentage of saturation. Other factors such as economics high solvency, low boiling point, low toxicity, and low cost also considered [22].

2.5.3 Steam distillation

The percentage of essential oils being extracted by this technique is 93% and the remaining 7% can be further extracted by other methods Basically, the process started by heating of plant material using steam which is supplied from steam generator. Heat is the main factor determining how effectively the plant material structures break down and burst and release the aromatic components or essential oils [35]. Steam distillation extraction technique to increase the isolated essential oil yields and reduce the amount of wastewater produced during the extraction process. The system uses a packed bed of the plant samples, placed above the steam source. Only steam is allowed to pass through the plants and boiling water does not mix with the botanical materials. Therefore, the process requires less steam and the amount of water in the distillate can be reduced [43].

2.5.4 Hydro diffusion

Diffusion of essential oils and hot water through plant membranes is known as hydro diffusion. In steam distillation, the steam does not actually penetrate the dry cell membranes. Therefore, dry plant material can be exhausted with dry steam only when all the volatile oil has been freed from the oil-bearing cells by first thorough combination of the plant material. But, when the plant material is soaked with water, exchange of vapors within the tissue is based on their permeability while in swollen condition. Membranes of plant cells are almost impermeable to volatile oils. Therefore, in the actual process, at the temperature of boiling water, a part of volatile oil dissolves in the water present within the glands, and this oil-water solution permeates, by osmosis, the swollen membranes and finally reaches the outer surface, where the oil is vaporized by passing steam.

2.5.5 Microwave-assisted extraction (mae)

Microwave-assisted extraction (MAE) also called microwave extraction, is a new extraction technique, which combines microwave and traditional solvent extraction (Hao et al., 2002). MAE has been regarded as an important alternative in extraction techniques which have several advantages over other extraction processes, such as reduction of costs, shorter time, less solvent, higher extraction rate, and better products with lower cost, reduce energy consumption and CO2 emissions (Cardoso et al., 2013; Hao et al., 2002). In micro wave assisted aqueous enzymatic extraction (MAAEE) of pumpkin seed oil by using mixtures of cellulose, pectinase and proteinase (w/w/w) (Jiao et al., 2014) obtained the highest oil recovery of 64.17%. The authors also reported that there were no significant differences in physicochemical propertie of MAAEE and Soxhlet extracted oils, and thus, MAAEE is a promising and environmentally friendly technique for pumpkin seed oil extraction.

2.5.6 Supercritical fluid extraction

SFE is used on a large scale for the extraction of some food grade oils and pharmaceutical Products from plants. It is comparatively rapid because of the low viscosities and high diffusivities associated with supercritical fluids. The extraction can be selective to some extent by controlling the density of the medium and the extracted material is easily recovered by simply depressurizing, allowing the supercritical fluid to return to the gas phase and evaporate leaving no or little solvent residues (Mohamed, Abdulamir, & Abas, 2008). Supercritical fluid extraction (SFE) of oils is a modern technique, currently being applied in the process industry. Supercritical carbon dioxide can also be used as a more environmentally friendly solvent for dry cleaning as compared to more traditional solvents such as hydrocarbons and per choro ethylene. Supercritical carbon dioxide is used as the extraction solvent for the creation of essential oils and other herbal distillation. Its main advantages over solvents such as hexane and acetone in this process are that it is non-toxic and nonflammable. Furthermore, separation of the reaction components from the starting material is much simpler than with traditional organic solvents, merely by allowing it to evaporate into the air or recycling it by condensation into a cold recovery vessel. Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. SFE can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). These essential oils can include limonene and other straight solvents. Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. Extraction conditions for supercritical carbon dioxide are above the critical temperature of 31 °C and critical pressure of 74 bar. Addition of modifiers may slightly alter this [36].

2.5.7 Hydro distillation

The procedures start with immersing the plant materials directly into water inside the alembic (vessel), and whole mixture was boiled. The devices include a heating source, vessel (Alembic), a condenser to convert vapor from vessel onto liquid, and a decanter to collect the condensate and to separate essential oils with water. As the oils are surrounded by water, this method is able to protect essential oils to be extracted at a certain degree without being overheated. The main advantage of this extraction technique is its ability to isolate plant materials below.

Chapter Three

3.1 Materials and methods

3.1.2 Material

The main raw materials and chemicals used for this experiment were n-hexane(CAS110-54-3) used as solvent in the extraction process, ethanol(MW46.07) used to determine saponification value of the extracted oil, phenolphthalein indicator() used to change color of the sample solution to pink color, diethyl ether(CID3283), sodium hydroxide(AR PELLET ASSAY) used commonly to characterize acid value, saponification value and ester value of the oil; hydrochloric acid used to prepare volume of blank solution and volume determined solution that contains extracted oil . Moringa Seed samples were collected from gubre.

3.1.3 Equipments

During conducting the project, some equipment's like Soxhlet extractor(LabGeni scientific) used for extracting oil from moringa, water bath used for cleaning the equipment , electric heater used for evaporating process, digital weighting balance(electronic scale) used to measure amount of the sample, beaker that is a flat-bottomed ,jar made of glass used for holding sample of impure moringa oil, round bottom flask is bottle that has a narrow neck which is used to hold fine sample of moringa seed that is going to be extracted in the Soxhlet, thermometer is used check to temperature of oil during separation of n-hexane from moringa oil , condenser is used reducing heat during extracting oil by Soxhlet , crusher is used to reducing size of moringa, separating funnel ,conical flasks is used to measure during preparing the solution that used to titrate,cylinders used to measure the liquid material ,burette is an apparatus used for performing a titration.

3.2 Method

3.2.1 Sample preparation and extraction

The sample was collected from gurage zone gubre . The seeds also had extra seed husk which was separated after sun drying for one day to facilitate the separation of husk and seed kernels. The seed kernels were sun-dried to reduce the moisture content. By using pestle and mortar the sample of Moringa seed was grinded at different particle sizes (i.e. 2-1 mm, 1-0.5mm, and 0.5-0.25mm) and was distinguished by sieve. Powder separated in to different size.15 g of moringa powder each was weighed and placed in the filter paper. Then it was inserted in the center of the Soxhlet apparatus. 127.5 *ml* of the solvent (n-hexane) was measured using a measuring cylinder with a 15 g Moringa seed sample, then poured into a round bottom flask. The Solvent was heated at 69ºC in the Soxhlet apparatus. When the solvent was boiled, the vapor rises through the vertical tube into the condenser at the top. The liquid condensate drops into the filter paper thimble in the center, which contains the oil to be extracted. The extract seeps through the pores of the thimble and fills the siphon tube, where it flows back down into the round bottom flask. That was done for two three and four hours. Next to that miscellany (mixture of solvent and oil to be extracted) obtained. After all the oil was recovered by solvent evaporation using heater evaporator. Miscellany was heated at a low temperature (i.e.40 to 45^0C heating temperature) until the solvent finally evaporates leaving behind the oil extracted by using rotary evaporator. This sample was then weighed and the difference was calculated as weight of sample before extraction – weight of sample after extraction, divided by the initial weight of sample, and multiplied by 100 to give the percentage yield oil. The oil extracted was stored in a plastic container for further use.

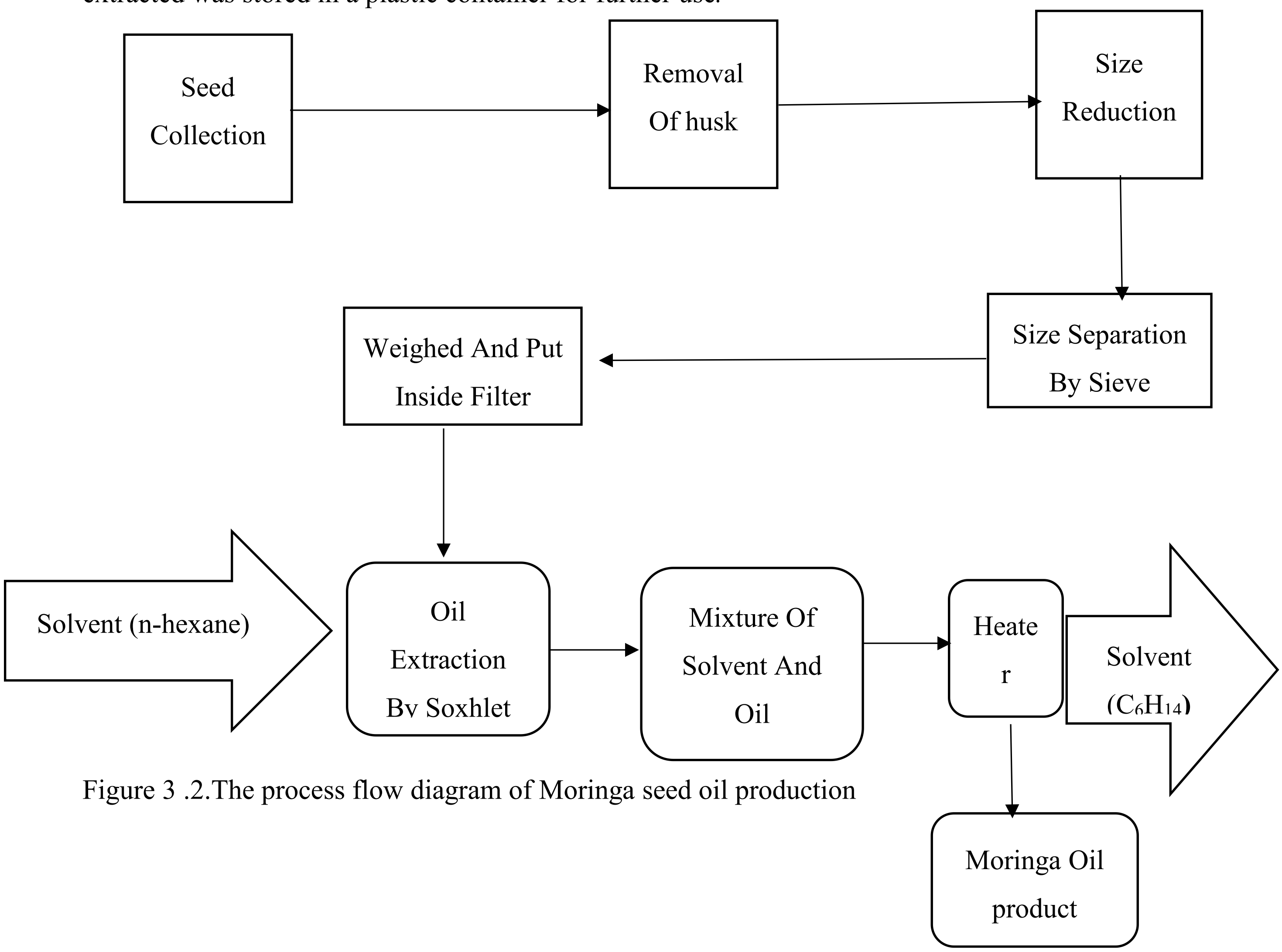

Figure 3 .2.The process flow diagram of Moringa seed oil production

3.3 The working principle of soxhlet extractor

Soxhlet extractor extracts the components using the condensed vapors of the solvent. The condensed vapors come in contact with the sample powder and the soluble part in the powder gets mixed with the solvent. Its working principle detail is as follows. Before getting into the process, first we need to understand that, the desired component from the sample powder must be soluble in the solvent at high temperatures. Then, we would be able to separate it from other components. First we turn on the heat and the metal plate gets heated. The RBF which contains our solvent starts boiling. The vapors from the RBF travel from RBF to the condenser via the distillation tube. The condenser condenses the vapors of solvent and those condensed vapors fall down to thimble. We put our sample powder inside the thimble. The powder has to be covered from the bottom with a cotton ball to avoid powder directly falling into the thimble. And also cover the powder from the top. So, when the condensed vapors fall into the thimble, the powder gets wet with the solvent and the components which are soluble in the solvent gets along with it. Siphon connects the thimble to RBF as we saw earlier.

The solvent mixture starts filling thimble and siphon. A point reaches where the siphon starts overflowing under the influence of gravity. Since the Siphon directly connects RBF, the overflowed liquid falls back to RBF. This marks the first cycle. As mentioned earlier, we can perform as many cycles as we want. One thing to mention is, we don't change the solvent for every cycle. And despite that, when the solvent vaporizes, the components from the sample do not get vaporized. So, each time we get 100% pure solvent vapors. When we think that we have exhausted the sample sufficiently, we stop the cycles. Then, we are left with the mixture of solvent and the components from the sample which are soluble in the solvent. Then, we can separate them by the further procedure. Generally, Soxhlet extraction is a continuous solid -liquid extraction. A solid which contains the material to be extracted is placed in the thimble. A thimble is a made of material which will contain the solid but allow liquids to pass through a lot like filter paper. A thimble containing a material is placed in the Soxhlet extractor. An organic solvent is then heated at reflux. As it boils, its vaporize up and condensed by a condenser. The condensed solvent then fills up the thimble. After it files with enough solvent automatically siphons up back down into the organic solvent. This process took place repeatedly. A solid material containing some of the desired compound is placed inside a thimble made of thick filter paper, which is loaded to the main chamber of the

Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with the condenser. The solvent is heated to the reflux. The solvent vapor is travel up the distillation arm, and floods in to the chamber housing the thimble of solid. The condenser ensures that any solvent vapors cools, and drips back down into the chamber housing the solid materials. The chamber containing the solid materials slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent, When the Soxhlet chamber is full the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times over hours or days. During each cycle a portion of non-volatile compound dissolves in the solvent after many recycles the desired compound is concentrated is in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample as one batch of solvent is recycled [[37]]. After extraction of the solvent is removed by means of a heater evaporator yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble is usually discarded

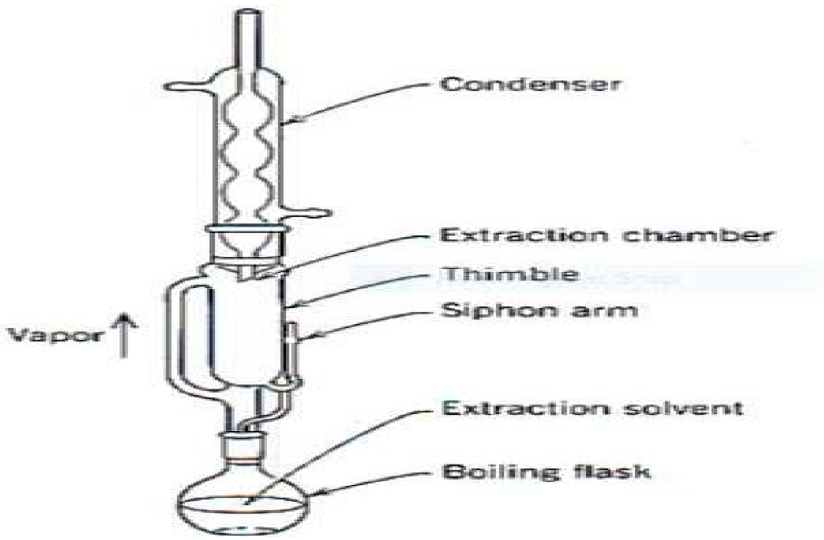

Figure 3. 3 soxhlet extcation

3.4 Proximate composition analysis of moringa seed

- **Determination of moisture content**

The moisture content of the sample of 5gm seed was measured according to AOAC (1990) methods by drying in the oven at 105^oC for three hours until constant mass obtained. Finally, the average value was registered

$$moisture\ content = \left(\frac{m3 - m2}{m1}\right) * 100 \ldots\ldots..(1)$$

Where, m1 is mass of sample

m3 is sampled mass with sample holder(g) and

m2 is final mass of sample with sample holder(g)

- **Determination of crude ash content**

The ash content was analyzed by weighing the samples before and after burning at 550^oC for 4 hours in muffle furnace according to AOAC, 930.22 (2000). A sample of 10gm was weighed (m2). The dried sample was carbonized over a blue flame and ignited in a muffle furnace at 550o C until ash completed (4 hours). After ignition, the sample was cooled in the desiccator to ambient temperature and weighed (m3). Finally, total ash content was calculated as follows:

$$\%\ of\ ash = \left(\frac{m3 - m1}{m2 - m1}\right) * 100 \ldots\ldots\ldots\ldots(2)$$

Where, m_1 is mass of crucible (g)

m_2 is sampled mass with crucible(g)

m_3 is final mass of sample with crucible(g)

3.5 Determination of physico-chemical property of the oil

- **Determination of oil yield**

The percentage of Moringa seed oil yield was calculated using the following equation.

$$\text{\% of oil yield} = \frac{\text{weight of oil}}{\text{weight of sample dry bases}} * 100 \text{..............................}(3)$$

- **Determination of density and specific gravity**

The density and specific gravity of the oil were determined as:-

$$density = \frac{mass\ of\ oil\ in\ gram}{volume\ of\ oil\ in\ mililiter} \text{..............................}(4)$$

$$specific\ gravity = \frac{density\ of\ oil}{density\ of\ water} \text{...................................}(5)$$

- **Determination of acid value**

5g of the given oil was weighed in 250ml conical flask and it was dissolved in alcohol (mixture of 25ml of diethyl ether and 25ml of ethanol). Then, two drops of phenolphthalein indicator were added. The contents were titrated with 0.1M of alcoholic NaOH to the end point with consistent shakes until a dark pink color was observed and the volume of 0.1M NaOH (V0) was recorded **[18].** Finally, the acid value of the oil was calculated as:-

$$AV = 56.1\left(\frac{V * N}{Ws}\right) \text{...................................}(6)$$

Where, 56.1=equivalent weight of NAOH

V= volume of NaOH

Ws= weight of oil

- **Determination of saponification value**

5g of oil was weighed in to conical flask and 50ml of alcoholic 0.1N NaOH was added from burette by allowing it to drain for a definite period of time and a blank test solution was also prepared by allowing it to drain at the same duration of time with continuous stirring. The flask was connected to the condenser and it was boiled gently for about 1 hour. Then the flask and condenser were cooled, the condenser was rinsed with a little distilled water and then the condenser was removed. Finally, 1ml of phenolphthalein indicator was added and it was titrated against 0.5NHCl until the pink color was disappeared. Same procedures were used for other samples and blank [38]. The expression for SV was given by:

$$SV = 56.1N\left(\frac{B-S}{Ws}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (7)$$

Where, B= volume of blank test solution

S= volume of solution containing extracted oil

N= actual normality of HCl

Ws= weight of extracted oil

- **Determination of ester value**

$$\text{Ester value} = \text{Saponification value} - \text{Acid value} \ldots\ldots\ldots\ldots\ldots (8)$$

Chapter Four

4.1 Results and discussions

4.1.1.The physical property of the moringa oil seed

Table 4.1, represents the proximate composition of M. seed oil. As shown from table 4.1, the moisture content of 8 % was obtained. This result is a little bite greater than the range 5.7-8.9 as we have seen from a literature [7.33]. The moisture content of oil seed can determine the storage condition and the effectiveness of downstream processing and also influence the quality of the oil.The density of the M. seed oil were 0.76667 g/ml . which are nearly the same within the range from the literature [7.33] The ash content of the M. seed oil was 5.3% in our work as we have seen from table 4.1 which is in the range found from the literature where the range was from 4.4% -6.94%. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food.The residues after a sample is completely burnt in contrast to the ash remaining after incoete combustion consist mostly of metal oxides. Ash is one of the components in the proximate analmplysis of biological materials, consisting mainly of salty, inorganic constituents. It includes metal salts which are important for processes requiring ions such as Na^+ (Sodium), K^+ (Potassium), and Ca^{2+} (Calcium). It also includes trace minerals which are required for unique molecules, such as chlorophyll and hemoglobin.

Table 4:1.Physical property of Moringa seed crude oil

Physical property	Present Value	Literature range
PH value	7±0.5	7.53
Specific gravity	0.00076	0.9434
Density @25C^0	0.7666 ± 0.5	0.881-0.920[1,2]
Moisture content (%)	8	5.7-8.9[1,2]
Ash content (%)	5.3	4.4 -6.9[1,2]

4.2 Chemical Properties of Moringa Seed Oil

The results of the Physical and chemical properties of the extracted seed oil were presented in the in table 4.2. as follows. The acid value was found to be 2.244mg NaOH/g. According to [Lalas and Tsaknis, (2003)] AV of 3.66 mg NaOH/g for cold pressed and 2.2 mg NaOH/g for solvent extracted M. stenopetala seed oil was documented. The acid value (which is twice the free fatty acid (FFA) value) measures how many fatty acids (a component of oil) are cleaved from their parent molecules (triglycerides or phospholipids). Cleavage of a free fatty acid from a parent molecule shows hydrolytic breakdown and is often used in whole biological systems as an indication of stress.

The test reveals enzymatic activity due to microorganisms in the raw material Low acid value means the oil will be stable over a long period of time without decaying and protects against rancidity and pre-oxidation due to the existence of natural anti- oxidants in seeds of moringa plant like vitamin C and A. The saponification value of the result was 182.325mgNaOH/g of oil found in this study which is in the range between 178.1–191mgNaOH/g of oil for M. seed oil reported through [6]. The oil sample was saponified by refluxing with a known excess of alcoholic sodium hydroxide solution. The alkali required for saponification was determined by titration of excess sodium hydroxide with HCl. It is an index of mean molecular weight of fatty acid glyceride comprising a fat. The lower the saponification value the larger the molecular weight of fatty acids in glycerides and vice versa.

Table4:2.Chemical property of Moringa seed oil

Chemical property of M. seed oil	**preset value**	**Literature value**
Acid value	2.244mgNaOH/g	0.32- 4[3,4]
Saponification value	182.325 mgNaOH/g	178.1–191[3,4]
Ester value	180.081mgNaOH/g	169.2-184.27

4.2.1 Effect of particle size on oil yield at different time

As we have seen from table 4.3 the particle size of the sample has a diverse effect on the yield of M. seed oil at a time of two hours (2hr.) At this time the percentage of oil yield is higher for medium particle size whose range is 0.5mm-1mm. similarly at time three(3hr) the yield is higher for a medium particle size as well as at time of four hours. This is because of the higher diffusivity of solvent through the solute is too much than from the rest type in which whose range is around 2-1mm and 0.5-0.25mm for coarse and fine respectively. The yield of oil on a coarse particle decrease as the surface area of the particle is too high which reduce the solvent penetration through this larger area and the yield of oil in fine particles is high than the coarse particle size due to the lower surface area which allows the solvent to solute penetration effective than a coarse particle.

No	Particle size(mm)	Time(hr.)	Oil yield (gm)	Oil Yield (%)
1	Coarse	2	2.556	17
2	Medium	2	5.052	33
3	Fine	2	4.377	29
1	Coarse	3	5.32	35
2	Medium	3	5.33	35.5
3	Fine	3	5.3212	35.4
1	Coarse	4	6.061	40
2	Medium	4	6.9365	46
3	Fine	4	6.5363	45.5

Table 4:3. Effect of parameters on oil yield

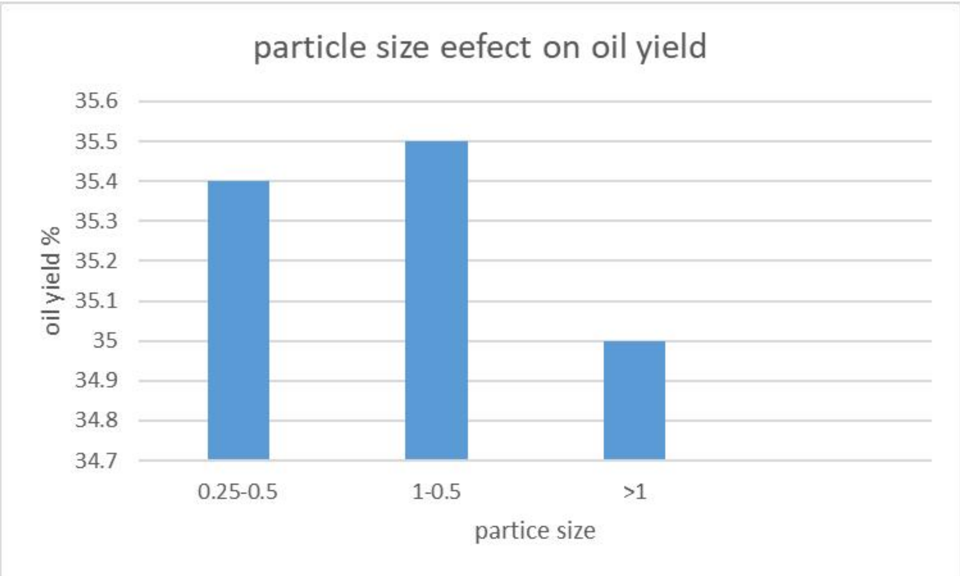

Figure 4. 4 particle size on oil yield

As we have observed on the effect of particle size on the oil extraction. The smaller the size, the greater is the inter-facial area between the solid and liquid. Therefore, the higher is the rate of transfer of material and the smaller is the distance the solute must diffuse within the solid. On the other hand, the surface may not effectively used with a very fine material if the circulation of the liquid is impeded, and separation of the particles from the liquid and drainage of the solid residue are made more difficult. This due to the sticky nature of the oily moringa seed.

4.2.2 The effect of residence time

As we have seen from the above figure it demonstrates the effect of mixing time on the oil yield of Moringa seeds. The result showed that 4hours of extraction resulted in the highest oil yield for Moringa seeds which is 46% of oil yield. The oil yield increased dramatically when the Moringa seed*s* were heated from 1 hour until 6 hours because the diffusivities of the oil and solvent increases, which result in high oil yield. Therefore, the maximum oil yield could be achieved even at shorter residence time with an optimal extraction temperature. The oil yield increases as the mixing time increases until it reaches in equilibrium up to 6 hours. However, the longer the mixing time of extraction did not increase the oil yield of the Moringa seeds. This is due to the finding reported by Mani *et al.* (2007) that any further increase passes 6 hours in extraction time did not increase the oil yield of Moringa seeds from literature. This phenomenon is due to low solvent density contained left in the sample after 6 hours.

The oil yield increases due to the fact that, efficient contact time between the solute and the solvent.

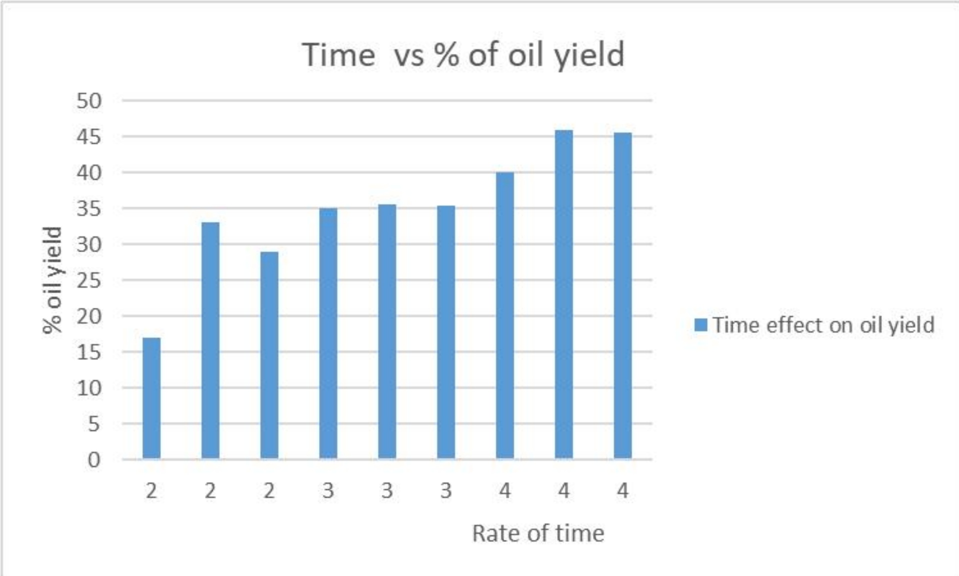

Figure 4. 5 time effct on oil yield

As it has shown from the figure 4.5 as time increases more oil yield is obtained. For three particle sizes as time increases the oil yield increases, since more contact of the solute and the solvent means it leaches more oil by diffusing the membrane of the seed. But increasing time not mean increases the oil yield. A prolonged extraction time results in an increased yield of the oil until equilibrium reached. Thereafter, the concentration of compound will not increase further but there will have greater liability for degradation. The oil yield increases due to the fact that, efficient contact time between the solute and the solvent.

4.2.3 Effect of solvent to solute ratio on oil yield

From table 4.4 we were presented the effect of solvent to solute ratio how it affects the oil extraction from moringa seeds. We obtained the highest oil yield at 130ml of solvent from a constant mass of 15 g of sample of oil was 46.67% of oil yield. As the amount of solvent increases the product of the oil decreases dramatically.

Table 4:4. Effect of solvent solute ratio on oil yield

No	Amount of solvent (ml)	Yield (gm)	% yield
1	130	7	46.67
2	150	6	40
3	170	4.5	30

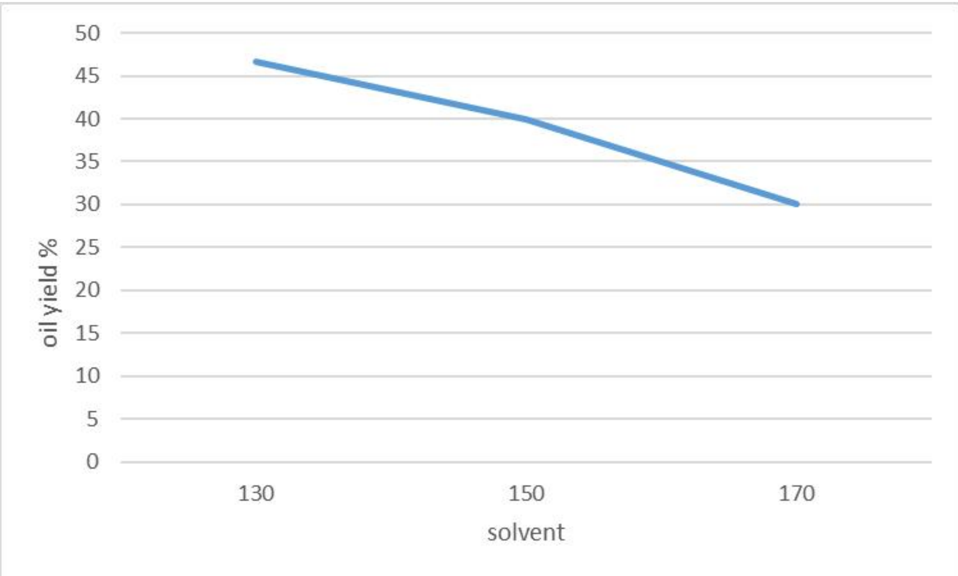

Figure 4. 6 Effect of solvent solute ratio on oil yield

As it can be seen from the graph, as the solvent increases amount of extracted oil or yield decreased. This is because as solvent increases but further increasing of the solvent is not mean always increasing the yield as clearly shown from the graph. Because all oil components are leached out, there is no more oil left in the solute to be extracted.

Chapter five

5.1 Conclusions and recommendations

5.1.2 Conclusions

Our work was focused on the extraction of an essential oil from Moringa seed using a solvent extraction process through the use a Soxhlet apparatus. The extraction process was involved factors which were particle size in range of (2-1, 1-0.5, 0.5-0.25) mm, time (2,3,4,) hours and solvent to solute ratio (8.67:1,10:1,11.33:1) ml/gm as dependent variables at constant mass of sample 15gm and temperature of 69C^0. The maximum oil yield was obtained from process parameters at a time of four hours, 8.67:1 solvent to solute ratio and at (1-0.5) mm particle size. Therefore, the combination to obtained maximum efficiency of yield was under medium particle size, time of four hours was 46.67 oil yield.As we have investigated the effect of particle size on extraction of oil from Moringa seeds with various particles sizes, we found that an intermediate particle size (0.5–1 mm) resulted in the highest oil yields. The reason why this high yield obtained is that, since during sieving Moringa seed is oily it becomes muddy on the sieve when the sieve vibrates if very fine, but when the size is medium it easily passes through the mesh. This makes not agglomeration of the particles if medium sized. Due to this reason the solvent can easily penetrate through the core of the particle and leach the oil easily. The reduction in oil yield observed when Moringa seed particles smaller than 0.5 mm the reason is that, when the particle is fine agglomeration of the particle happens. This leads the hindrance of the solvent to reach the core of the particle to leach the oil component. This results agglomeration of fine particles that reduces the surface area available for solvent flow. When particle size is greater than 1mm surface area to volume ratio decreases. This effect decreases the contact area of the core of the particle, so oil yield becomes not such much as the medium. It takes longer time to leach the oil from its core.

The pysico- chemical property of an extracted essential oil which involves moisture, ash, density,PH,ester and saponification value were8%,5.3%,0.7668g/ml,,7±0.5,2.244mgNaOH/g, 180.086,182.325Mg NaOH/g Generally, the yield of oil increases from two hour to four-hour with maximum yield of 46.67% medium particle size, minimum yield of 17% at coarse particle size.

5.1.2 Recommendations

The detail and further research and investigations should be performed on the source where it is found and its usefulness on different applications by giving awareness for the society in our country to reduce imported oil in some degree.

- ✓ In order to make this study full, it is recommended to further study the iodine content of extracted essential oil via iodine mono chloride method
- ✓ In order to make this study full, it is recommended to further study Deodorizing and neutralizing of the oil should be needed to decrease the risk of health effect by using high technology. Using Nona-neutralization,enzymatic bleaching, short-path distillation, and supercritical processing to get the right quality of the oil.
- ✓ Here we were trying to extract the essential oil by solvent extraction process only using the Soxhlet apparatus. We did not see the effect of other oil extraction techniques like hydro distillation, steam distillation, super critical fluid extraction and solvent free microwave extraction method.so we were informed to do on each extraction method to know the exact efficiency of each extraction process on yield of moringa seed oil.
- ✓ We also recommend to determine the value of peroxide,refractive index and viscosity analysis. Due to material equipment and chemical lack we were unable to perform these analysis.

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Appendix-1

Determination of moisture content

No	Time (min)	Mass of holder with sample(g)
1	0	21.8
2	120	21.4
3	240	21.4
4	320	21.4
5	0	5 **Mass of sample(g)**

Moisture calculation

Where, m1 is mass of sample

m2 is final mass of sample with sample holder(g) and

m3 is sampled mass with sample holder(g)

$$moisture\ content = \left(\frac{m3 - m2}{m1}\right) * 100 \ldots\ldots.(1)$$

$$moisture\ content = \left(\frac{21.8 - 21.4}{5}\right) * 100 \ldots\ldots.(1)$$

$$moisture\ content = 8\%$$

Determination of Ash content

$$\%\ osh = \left(\frac{m3 - m1}{m2 - m1}\right) * 100 \ldots\ldots\ldots\ldots(2)$$

Where, m_1 is mass of crucible (g)

m_2 is sampled mass with crucible(g)

m_3 is final mass of sample with crucible(g)

$$\% \, osh = \left(\frac{301.2 - 300.67}{310.67 - 300.67}\right) * 100$$

$$\% \, osh = 5.3\%$$

Yield calculation

$$\% \text{ of oil yield} = \frac{\text{weight of oil}}{\text{weight of sample dry bases}} * 100 \ldots\ldots\ldots\ldots(3) \% \text{ of oil yield} = \frac{42.1726}{135} * 100$$

$$\% \text{ of oil yield} = 31.123\%$$

Density calculation

$density = \frac{mass\ of\ oil\ in\ gram}{volume\ of\ oil\ in\ mililiter} \ldots\ldots\ldots\ldots(4)$ $density = \frac{42.1726g}{55ml}$, $density = 0.7668\% g/ml$

Specific gravity calculation

$$specific\ gravity = \frac{density\ of\ oil}{density\ of\ water} \ldots\ldots\ldots\ldots(5) specific\ gravity = \frac{0.768}{1000}$$

$$specific\ gravity = 0.000768$$

Determination of acid value

$$AV = 56.1\left(\frac{V * N}{Ws}\right) \ldots\ldots\ldots\ldots(6)$$

Where, 56.1=equivalent weight of NAOH

V= volume of NaOH

Ws= weight of oil

$$AV = 56.1\left(\frac{2 * 0.1}{5}\right)$$

$AV = 2.244mgNaOH/\text{g}$

Determination of saponification value

$$SV = 56.1N\left(\frac{B-S}{Ws}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots . (7)$$

Where, B= volume of blank test solution

S= volume of solution containing extracted oil

N= actual normality of HCl

Ws= weight of extracted oil

$$SV = 56.1N\left(\frac{B-S}{Ws}\right)$$

$$SV = 56.1 * 0.5\left(\frac{81-16}{10}\right)$$

$$SV = 182.325mgNnOH/g$$

Determinatio of ester value

$$\text{Ester value} = \text{Saponification value} - \text{Acid value} \ldots\ldots\ldots\ldots\ldots (8)$$

$$\text{Ester value} = 182.325 - 2.244$$

$$\text{Ester value} = 180.081\text{mgNaOH/g}$$

Appendix -2

Laboratory Equipment's and Samples Photo

Moringa powder

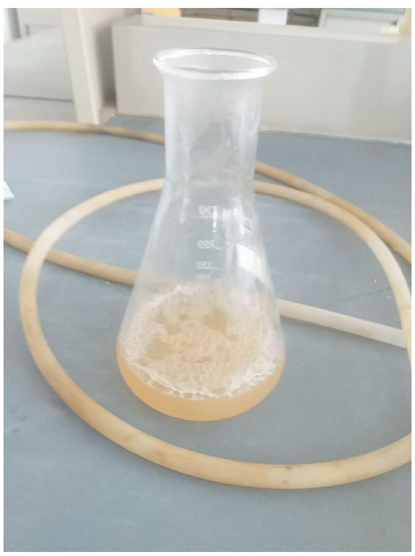
soxhlet extraction

N-hexane with oil

oil sample

Oil on Heater

saponification (before titration)



Saponification (after titration)