Sudan University of Science and Technology

College of Graduate Studies

Mineral content of *Moringa Oleifera* Leaves and Its Utilization in Controlling Blood Glucose Level in Diabetes Mellitus Patients.

المحتوى المعدني لأوراق المورينجا أوليفيرا واستخدامها في السيطرة على مستوى جلوكوز الدم في مرضى داء السكري.

A Thesis Submitted in Fulfillment for The Ph. D. Degree in Chemistry.

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DEDICATION

Dedicated to:

my parents,

my brother and sisters,

my friends.

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I wish to especially express my gratitude to my supervisor Professor **Abd Alsalam Abdalla Dafalla,** College of Science Department of Chemistry, Sudan University of Science and Technology, for permitting me in working with such kind of project and also for his total guidance and support in this project.

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Abstract

Moringa oleifera Lam. tree grows in many tropical and subtropical countries. The seed, leaves, root and flowers of *M. oleifera* are often used in traditional medicine, while the immature pods, leaves and seed are used as nutrition products in human food. This study carried out proximate analysis for mineral elements assessment in dried *M. oleifera* leaves. Fresh leaves of *M. oleifera lam* were plucked from *M. oleifera* trees in River Nile State (Atbara Town). Analyzed for the mineral element content using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and Atomic Absorption Spectrophotometer (AAS). Leaves powder of *M. oleifera* digested with nitric acid solution before determining concentrations of mineral elements. By using (AAS) mineral elements concentrations decrease in the order: "Ca (585.10ppm) > Mg (117.0ppm) > K (62.39ppm) > Na (24.05ppm) > Fe (22.94ppm) > Zn (1.854ppm) > Mn (1.015ppm) > Cu (0.336ppm) Cr (0.017ppm)" Pb and Ni not detected. By using (ICP) mineral elements concentrations decrease in the order: "Ca (46560ppm) > Mg (14140ppm) > K (12620ppm) > Al (1491ppm) > Fe (1401ppm) > Na (913ppm) > Si (526.00ppm) > Sr (113.30ppm) > Ti (71.65ppm) > Mn (61.69ppm) > Ba (14.78ppm) > Zn (13.76ppm) > Cu (13.43ppm) > Li (7.128ppm) > Mo (4.88ppm) > V (3.342ppm) > Cr (2.086ppm) > Ni (1.919ppm) > Pb (1.547ppm) > Be (0.0207ppm)". Mineral elements content of leaves powder determining using inductively coupled plasma into the microwave digestion of plant tissue in an open vessel. Concentrations of mineral elements decrease in the order: "Ca (54450ppm) > K (16370ppm) > Mg (16250ppm) > Al (1426ppm) > Fe (1402ppm) > Si (1804ppm) >Na (960.6ppm) > Sr (118.3ppm) > Mn (76.52ppm) > Ti (25.95ppm) > Zn (19.21ppm) Ba (17.21ppm) > Cu (10.54ppm) > Pb (6.463ppm) > Mo (5.436ppm) > Li (4.190ppm) > V (3.022ppm) >Cr (2.484ppm) > Ni (2.081) > Be

(0.0257ppm)". The results showed no difference for mineral elements concentrations by two different methods when using (ICP), but by using (AAS) mineral elements concentrations were less compared to concentrations by using (ICP). On the other hand this study aims to investigate the effects of *M. oleifera* leaves powder on the blood glucose in humans**,** and determine suitable quantity of powder to use. The study targeted patients who their blood glucose not lowering by drugs. Eighty three volunteers of diabetic patients were divided into three groups. Three doses 0.25, 0.5, 1.0 g of *M. oleifera* leaves powder were taken by group 1, 2 and 3, respectively for a month. Blood sugar levels for all patients was determined before and after using *M. oleifera* leaves powder. Results showed blood sugar levels decreased statistically significant ($p<0.001$) for all patients. Results indicated *M. oleifera* leaves powder is promising to reduce the diabetic complications in diabetic patients, and suitable dose to consume to reduce blood glucose for diabetic patients who their blood glucose levels not lowering with drugs is 0.5g. This study investigated side effect for using *M. oleifera* leaves powder to liver and kidney. Urea, creatinine, ALT and AST enzyme for diabetic patients was determined before and after using *M. oleifera* leaves powder. The results indicated no statistically significant difference in the mean values of urea creatinine, ALT and AST before and after taken *M. oleifera* leaves powder ($p> 0.001$). This study indicated the leaves of *M. oleifera* is rich in essential minerals needed by the body's health.

المستخلص

تنمو شجرة المورينجا اوليفيرا في العديد مِنْ البلدانِ الإستوائيةِ والشبه إستوائية . تستعمل في أغلب الأحيان بذور وأوراق وجذور وزهور هذه الشجرة في الطبِّ التقليدي ، بينما تستعمل الجذور الغير ناضجة والأوراق والبذور كمُنتَجات تغذيةٍ في الغذاءِ الإنساني . في هذه الدراسةِ نفّذ تحليلاً مباشراً لتقدير تركيز العناصر المعدنية في الأوراق المُجَفَّفة للمورينجا اوليفيرا . قطفت الأوراقُ الناضجة لشجرة المورينجا اوليفيرا من ولاية نهر النيل (مدينة عطبرة). َ استخدما جهازي طيف الامتصاص الذري و طيف كتلة البلازما المتولدة بالحث لتحلّيل محتوي العناصر المعدنية . استخلصت العناصر المعدنية لمسحوق أوراق المورينجا بمحلول حامض النتريك . باستخدام جهاز طيف الامتصاص الذري (AAS) تركيز العناصر المعدنية حسب الترتيب التنازلي :

"Ca (585.10ppm) > Mg (117.0ppm) > K (62.39ppm) > Na (24.05ppm) > Fe (22.94ppm) > Zn (1.854ppm) > Mn (1.015ppm) > Cu (0.336ppm) Cr (0.017ppm)"

عنصري Pb و Ni لم يتم لهما تسجيل تركيز بهذا الجهاز .

باستخدام جهاز طيف كتلة البلازما المتولدة بالحث (ICP) تركيز العناصر المعدنية حسب الترتيب التنازلي :

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استخلصت العناصر المعدنية لمسحوق أوراق المورينجا أوليفيرا بالمايكروويف. باستخدام جهاز طيف كتلة البلازما المتولدة بالحث (ICP) تركيز العناصر المعدنية حسب الترتيب التنازلي :

"Ca (54450ppm) > K (16370ppm) > Mg (16250ppm) > Al (1426ppm) > Fe (1402ppm) > Si (1804ppm) >Na (960.6ppm) > Sr (118.3ppm) > Mn (76.52ppm) > Ti (25.95ppm) > Zn (19.21ppm) Ba (17.21ppm) > Cu (10.54ppm) > Pb (6.463ppm) > Mo (5.436ppm) > Li (4.190ppm) > V (3.022ppm) >Cr (2.484ppm) > Ni (2.081) > Be (0.0257ppm)".

أوضحت النتائج أنه لا يوجد اختلاف كبير في تركيز العناصر المعدنية لمسحوق الأوراق بطريقتين مختلفتين باستخدام جهاز (ICP) , لكن عند مقارنة تركيزها باستخدام جهاز(AAS) أوضح أنه أقل من تركيزها باستخدام جهاز (ICP) .

من جانب آخر تهدف هذه الدراسة لإثبات تأثير مسحوق أوراق المورينجا اوليفيرا على مستوى الجلوكوز في دمَّ البشر و تقدير كمية المسحوق المناسبة للاستعمال . استهدفت الدراسةُ المرضى الذين كمية السُكّر فيَ دمِّهم لا يَنخفضُ بتعاطي الأدوية . ثلاثة و ثمانون مِنْ المتطوعين من مرضى السكري قسموا إلي ثلاث مجموعات . أخذت ثلاث جرع 0,25 , 0,5 , 1,0جرام من مسحوق أوراق المورينجا للمجموعات 1, 2 و3 علي التوالي لمدة شهر . قيست كمية السكر في الدم قبل و بعد استعمال مسحوق أوراق المورينجا . أوضحت النتائج لكل المرضي انخفاض معنوي في كمية السكر في الدم, الجرعة المناسبة لخفض مستوى الجلوكوز في الدم للمرضى الذين مستوى الجلوكوز في دمهم لا ينخفض بالدواء هي 0,5 جرام, مما يدل علي أن مسحوق أوراق المورينجا يَعِدُ بتَخفيض التعقيداتِ السُكّريةِ لمرضى السكري .

هذه الدراسة تحقق فيها من الأثر الجانبي لتناول مسحوق أوارق المورينجا علي الكبد و الكلي . قيست لمرضي السكري إل urea و creatinine وإنزيمات AST وALT قبل وبعد تعاطي مسحوق أوراق المورينجا أوليفيرا , أثبتت النتائج عدم وجود تأثير معنوي علي الكلي وإنزيمات الكبد. أشارت هذه الدراسة إلي أن مسحوق ورق المورينجا غني بالمعادن الضرورية التي تحتاجها صحة الجسم.

Contents

Abberviation

M.	*Moringa*
AAS	Atomic Absorption Spectroscopy
ICP	Inductively Coupled Plasma atomic
AST	Aspartate Transaminase
ALT	Alanine Transaminase
TAS	Total Antioxidant Status
DM	Diabetic Mellitus
CVD	Cardio Vascular Disease
LDL	Low Density Lipoprotein
VLDL	Very Low Density Lipoprotein
ROS	Reactive Oxygen molecules
HSV	Herpes Simplex Virus type 1
CCBs	Calcium Channel Blockers
GLV	Green Leafy Vegetables
T2DM	Type-2 Diabetes Mellitus
PPBG	Post-Prandial Blood Glucose
AUCs	Area Under the Curves
HbA1c	glycated Hemoglobin

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Introduction

Moringa Oleifera " has 14 species of family *Moringaceae*, native to India, Africa, Arabia, South Asia, South America and pacific and Caribbean islands. Because *M. oleifera* has been naturalized in tropic and sub tropic regions worldwide, the plant is referred to number or names such as horesdish tree, drumstick tree, ben oil tree, miracle tree and " Mothers best friends"(Juali, 2008). *M. oleifera* is a small, fast growing evergreen or deciduous tree that usually grows upto 10 to 12 m. in height, open crown of dropping fragile branches, feathery toliage of tripinnate leaves and thicky corky, whitish bark (Roloff *et al*., 2009). *M. oleifera* is used as a highly nutritive vegetable in many countries. Its young leaves, flowers, seeds and tender pods are commonly consumed and they are having same medicinal properties. Traditionally its roots are applied as plaster to reduce the swelling and rheumatism .The root, flower, fruit and leaf have analgesic and anti-inflammatory activity. *M. Oleifera* leaves contain phytochemical having potent anticancer and hypotensive activity and are considered full of medicinal properties and used in siddha medicine (Monica premi *et al*., 2010). The whole plant of *M. Oleifera* is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac, aqueous extract of root and bark were found to be effective in preventing implantation , aqueous extract of fruit have shown significant anti inflammatory activity, methanolic extract of leaves have antiulcer activity and ethanolic extract of seed exhibited antitumor activity (Patel Rameshwar *et al*., 2010). *M. Oleifera* is used as drug in many ayurvedic practitioners for the treatment of asthama and evaluate the anthelmentic activity of methanolic extract of *M. Oleifera* in adult Indian earthworms pheretima posithuma at different doses(Ishwar Chandra *et al*., 2010). The *M. Oleifera* plant provides a rich and rare combination of zeatin, quarcetin, kaemferon and many other

phytochemicals. Various parts of the plant such as leaves, root, seed, flower, fruits and immature pod acts as cardiac and circulatory stimulant, posses antitumor, antipyretic, antiepileptic, anti inflammatory , antiulcer (Pal *et al*., 1995). Other important medicinal properties of the plant includes antispasmodic(Caceres *et al*., 1992), diuretic(Morton, 1991), antihypertensive(Dahot,1988), cholesterol lowering (Mehta *et al*., 2003), antioxidant, anti diabetic, hepatoprotective (Ruckmani *et al*., 1998), antibacterial and antifungal activity(Nickon *et al*., 2003). *M. Oleifera* grown and used in many countries around the world is a multi-purpose tree with medicinal, nutritional and socio-economic values. In Senegal and Benin, *M. oleifera* leaves are dispensed as powder at health facilities to treat moderate malnutrition in children. It established the medicinal uses of *M. oleifera* leaves by local communities in Uganda and identified phytochemicals present in *M. oleifera* leaves extracts(Josephine *et al*., 2010). The leaves of this plant contain a profile of important trace elements, and are a good source of proteins, vitamins, beta-carotine, amino acids and various phenolics (Anwar, 2007). In Indian traditional system of medicine, *M. Oleifera* Lam. *M. Oleifera* is commonly used as healing herb to treat diabetes. Different parts of this plant are used in the indigenous systems of human medicine for the treatment of a variety of human ailments. The leaves of *M. Oleifera* are reported to be used as a hypocholesterolemic agent, and hypoglycemic agent(Dangi *et al*., 2002., Ghasi *et al*., 2000). *M. Oleifera* leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage(Sreelatha, 2009). This oxidative damage is a crucial etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in the ageing process (Gutteridge, 1995., Pong, 2003). Oxygen free radicals and other “reactive oxygen species” are constantly produced in the

human body. Multiple studies have shown that type 2diabetes is accompanied by increased oxidative damage to all biomolecules in body. Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid per oxidation. An increased oxidative stress has been observed in diabetic patients as indicated by high free radical production (Giugliano *et al*., 1996). Oxidative damage due to free radicals was associated with vascular disease in people with types 1 and 2 diabetes mellitus(Oberley, 1988). There are several potential resources of free radical production in diabetics including autoxidation of plasma glucose (Pieper *et al*., 1995), activation of leucocytes, and increased transition metal bioavailability (Wolff *et al*., 1991). The Total Antioxidant Status (TAS) in type 1 or 2 DM was lower than that of age-matched controls, and this might be attributed to lower levels of vitamin C, vitamin E (Maxwell *et al*., 1997), or other factors including micronutrients(Mooradian *et al*., 1994, Anderson, 1995 Anderson *et al*., 1997 and Cunningham, 1998) in blood. Green leafy vegetables (GLV) offer a cheap but rich source of a number of micronutrients and other phytochemicals having antioxidant properties. The potential of 30 GLV in the raw and cooked form as natural antioxidant supplements for vegetarian diets was assessed. There was a large variability in the values of antioxidant activity of various GLV extracts in the lipid micelles (1.5-5.6 mM vitamin E/100 g for raw samples and 1.6-3.8 mM vitamin E/100 g for cooked samples). Leaves of coriander, *Amaranthus viridis*, colcasia green and drumstick showed high values (Tarwadi and Agte, 2003).

Objectives of the Study:

The main objectives for this study:

- To analyze some mineral elements in *M. oleifera* leaves.
- To study the effect of *M. oleifera* leaves powder on blood sugar of diabetic patients, and determine the appropriate dose.

The secondary objective for this study:

- To study safe side effect on liver enzymes and kidney for diabetic patients after taking *M. oleifera* leaves powder.

CHAPTER ONE

Literature Review

1.1 *Moringa Oleifera* Tree:

Moringa oleifera is the most widely cultivated species of a monogeneric family, the *Moringaceae*, that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians., it is now widely cultivated and has become naturalized in many locations in the tropics. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. It is already an important crop in India, Ethiopia, the Philippines and the Sudan, and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific islands. All parts of the *M. oleifera* tree are edible and have long been consumed by humans. According to Fuglie, 1999 the many uses for *M. oleifera* include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedlng damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds). *M. oleifera* seed oil (yield 30-40%, by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil, that resists rancidity. It has been used in salads, for fine machine lubrication, and in the

manufacture of perfume and hair care products. In the West, one of the best known uses for *M. oleifera* is the use of powdered seeds to flocculate contaminants and purify drinking water, but the seeds are also eaten green, roasted, powdered and steeped for tea or used in curries. This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so-called "developing" regions of the world where undernourishment is a major concern (Fahey, 2005).

1.2 Taxonomy :

Binomial	Name *Moringa oleifera*.
Kingdom	Plantae – Plants .
Subkingdom	Tracheobionta - Vascular plants.
Superdivision	Spermatophyta - Seed plants.
Division	Magnoliophyta - Flowering plants.
Class	Magnoliopsida – Dicotyledons .
Subclass	Dilleniidae .
Order	Capparales .
Family	*Moringaceae* – Horse-radish tree family .
Genus	*Moringa* Adans. – moringa .
Species	*Moringa oleifera* Lam. – horseradishtree

(Ganatra *et al.*, 2012) .

1.3 Description of Tree:

Moringa oleifera is a fast-growing, evergreen, deciduous tree. It can reach a height of 10–12 m (Parotta and John, 1993). The trunk can reach a diameter of 45 cm (Plant Resources of Tropical Africa, 2013). The bark has a whitish-grey colour and is surrounded by thick cork. Young shoots have purplish or greenish-white hairy bark. The tree has an open crown of drooping, fragile

branches and the leaves build up a feathery foliage of tripinnate leaves. The flowers are fragrant and bisexual, surrounded by five unequal thinly veined yellowish-white petals. The flowers are approximately 1-1.5 cm long and 2 cm broad. They grow on slender hairy stalks in spreading or drooping later flower clusters which have a longitude of 10–25 cm. Flowering begins within the first six months after planting. In seasonally cool regions, flowering will only occur once a year between April and June. In more constant seasonal temperature and with constant rainfall, flowering can happen twice or even all year-round (Parotta and John, 1993). The fruit is a hanging, three-sided brown capsule of 20–45 cm size which holds dark brown, globular seeds with a diameter of approximately 1 cm. The seeds have three whitish papery wings and are dispersed by wind and water (Plant Resources of Tropical Africa, 2013). In cultivation, it is often cut back annually to 1–2 meters and allowed to regrow so the pods and leaves remain within arm's reach (Verzosa and Caryssa, 2012).

Figure 1: *Moringa oleifera*Tree.

1.4 Nutritional Value :

M. oleifera is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics'. The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa (Anwar and Bhanger, 2003., Anwar *et al.*, 2005). *M. oleifera* leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Dillard and German, 2000., Siddhuraju and Becker, 2003). In the Philippines, it is known as 'mother's best friend' because of its utilization to increase woman's milk production and is sometimes prescribed for anemia (Estrella *et al.*, 2000., Siddhuraju and Becker, 2003).

1.4.1 Malnutrition and Disease:

Many people, believe it or not, are not fully aware of the connection between malnutrition and disease. The body intrinsically has the ability to both prevent disease as well as fight disease as long as it has the nutrients it needs to do this work. The body its organs and its immune system need certain nutrients in certain amounts in order to function properly. If the body does not have these nutrients it full and most efficient functioning is deteriorated and even lose. For instance, many children in the so-called "developing nations" suffer from night blindness and other eye diseases and afflictions simply because they do not get enough vitamin A. Due to the high vitamin A content of *M. oleifera*, this could be alleviated by mixing a few tablespoons of *M. oleifera*, into the food of these children. Many disease and afflictions affecting millions of people, especially children around the world

due to nutrient poor diets can be alleviated by just adding *M. oleifera*, leaves powder to their foods (Fahey, 2005).

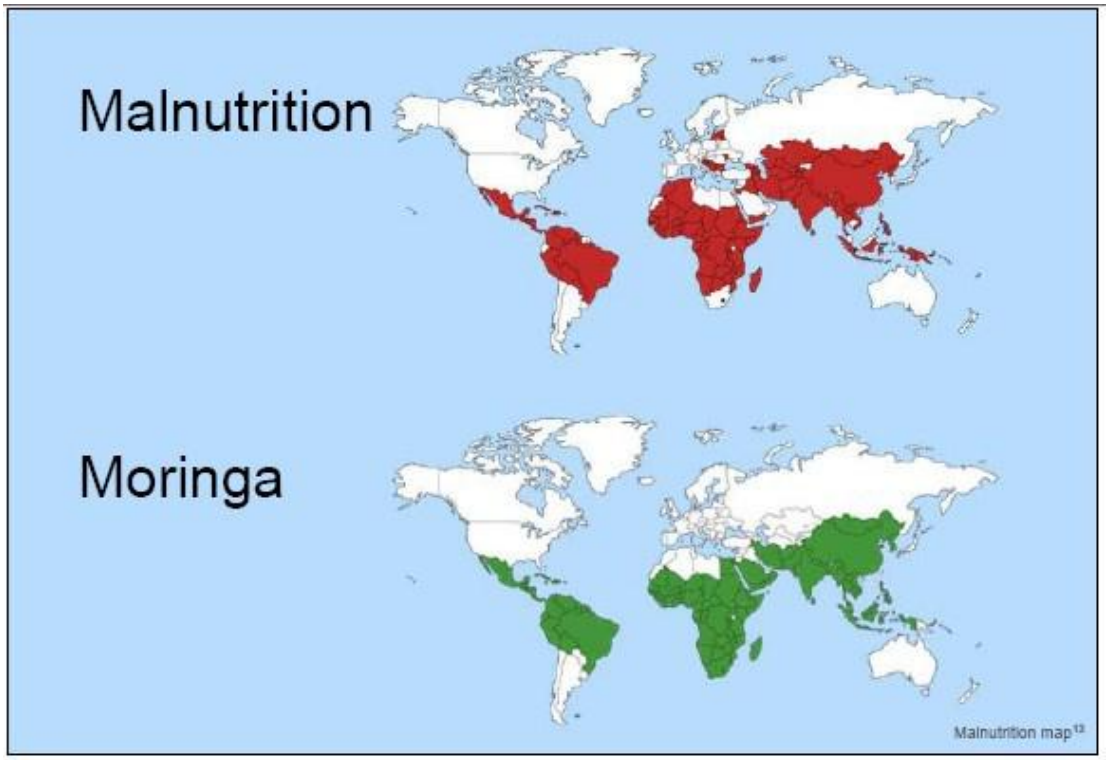

Figure 2: The map of the places where malnutrition is a major issue is the same as the map of where *Moringa oleifera* grow wild.

1.4.2 *Moringa oleifera* leaves:

Moringa oleifera, leaves contain all the essential amino acids to build strong healthy bodies. Example of some few nutritional value of *M. oleifera*: 2 times – the protein of yogurt, 3 times – the potassium of Bananas, 4 times – the calcium of milk, 4 times – the vitamin A of carrots and 7 times of – the vitamin C of Oranges (Belay and Sisay, 2014). When carefully dried, gram for gram *M. oleifera* leaves contain 24 times the iron of spinach, vitamin B, and minerals (Janick *et al*., 2008).

Fig 3: *Moringa oleifera* leaves.

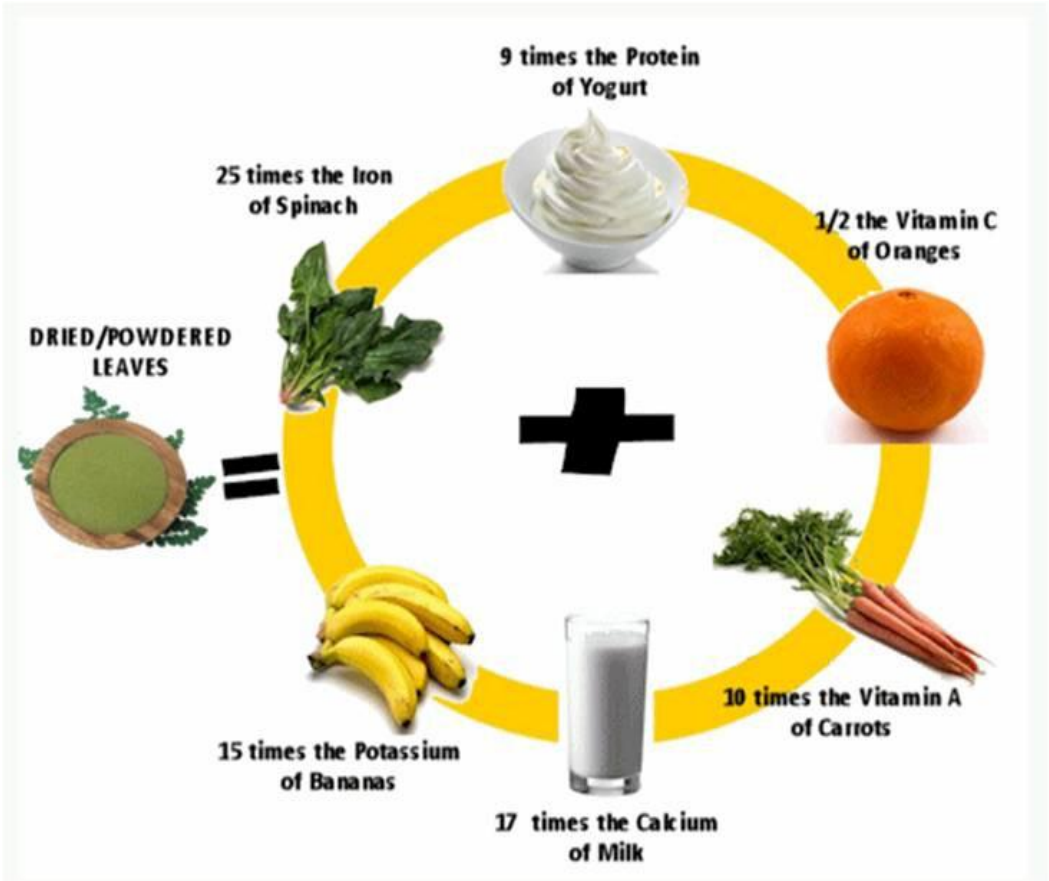

Figure 4: Nutrients in dried / powdered leaves.

1.5 Phytochemical Constituents:

Phytochemicals are chemical compounds that are naturally found in plant. They are responsible for the color and organoleptic properties of the plant (Liu, 2004). It is also referred to as those chemicals that may have biological significance but are not established as essential nutrients in plant. Phytochemicals could be available as a dietary supplement, but the potential health benefits of phytochemicals are derived from consumption of the whole plant(Rao and Rao, 2007). This plant contains a considerable amount of various nutrients, and has been suggested as a good supplement for such nutrients as protein, fiber and minerals (Oduro *et al*., 2008., Jongrungruangchok *et al*., 2010). Also, including it in diets to supplement daily nutrient needs could help to fight against many diseases as nutraceuticals (Sharma *et al*., 2012). It is a viable supplement for dietary minerals (Aslam *et al*., 2005). "Nutraceuticals" can restore, correcting or modifying physiological function that may help to prevent diseases in human being. Nutraceutical mostly obtained from natural sources exception is synthetic vitamins (Rajasekaran *et al*., 2008).

1.5.1. Vitamins:

Moringa oleifera is said to cure about three hundred diseases and almost have all the vitamins found in fruits and vegetable even in larger proportions. *M. oleifera* has vitamin A (Beta carotene), vitamin B1 (Thiamine), vitamin B2 (Riboflavin), vitamin B3 (Niacin), Vitamin B6 (Phyrodixine), vitamin B7 (Biotin), vitamin C (Ascorbic acids), vitamin D (Cholecalciferol), vitamin E (Tocopherol) and vitamin K (Belay and Sisay, 2014).

Table (1): Vitamin content of *Moringa Oleifera* leaves:

Vitamin	Fresh Leaves	Dried Leaves
Carotene (Vit. A)	6.78 mg	18.9 mg
Thiamin (B1)	0.06 mg	2.64 mg
Riboflavin (B2)	0.05 mg	20.5 mg
Niacin (B3)	0.8 mg	8.2 mg
Vitamin C	220 mg	17.3 mg

(All Things *Moringa*, 2010).

1.5.2. Mineral supplements:

The dried leaves had the following mineral: calcium, phoshorus, magnesium, potassium, sodium, zinc, copper, manganese, iron and selenium(Moyo *et al*., 2011). Mineral supplements are often chelated, or bound, with bioavailable compounds that may improve absorption. There are twenty two well known minerals essential to human health, they are divided into "major" minerals and "trace" minerals present in the body. Minerals include calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, sodium and zinc. Deficiency of either a major or trace mineral can produce equally harmful effects. Minerals play remarkable role in different physiological function e.g. participate in muscle contraction, blood formation, building protein, energy production and numerous other body functions. Sodium, potassium and chloride are electrically charges "Electrolytes" that helps for conduction of electrical impulses along nerves which transport substances in and out of the cells. In addition minerals regulate pH balance of blood and other fluids as well as fluid pressure between cells and the blood (Soma, 2013). *Moringa oleifera* have nutritional potential because their leaves contain a high concentration of energy, nutrients, and minerals. Therefore, the therapeutic potential of *M. oleifera* may be due to the presence of these constituents (Journal of Food and Nutrition Sciences, 2015). Previous studies indicated that *M.*

oleifera leaves powder its rich with essential trace elements that have clinical property e.g. hyperglycemia may be associated with raised plasma chromium and increased urinary excretion, without reflecting tissue level. Chromium concentrations in urine, hair, and other tissues or body fluids have also been reported not to reflect chromium status. The role of chromium supplementation was investigated in special subgroups of patients with diabetes(Entsar, 2017). Zinc plays an important role in the proliferation, differentiation, and metabolic function of mammalian cells. Mutations that activate H-Ras are monogenic in most cells and lead to malignant transformation and this Ras signaling pathway is inhibited by zinc(Franklin and Costello, 2007). Copper plays a very important role in our metabolism largely because it allows many critical enzymes to function properly(Harris, 2001). Iron is involved in the binding, transporting, and release of oxygen in higher animals (Lingamaneni Prashanth *et al*., 2015). Calcium is the most abundant mineral found in the human body, the majority (99%) is stored in the bones and teeth, the rest is stored in muscle tissue and blood. In addition to bone building and remodeling, calcium is also responsible for muscle contraction, central nervous function and hormone secretion. By providing abundant calcium in *M. oleifera* helps prevent: Anemia, Osteoporosis, Bone weakness and damage, Muscle damage and Abnormal heartbeat and functioning (All Things *Moringa*, 2010). Elements such as iron, zinc, and selenium are essential components of enzymes where they attract or subtract molecules and facilitate their conversion to specific end products. Some of the trace elements control important biological processes by facilitating the binding of molecules to their receptor sites on cell membrane, by alternating the structures or ionic nature of membrane to prevent or allow specific molecules to enter or leave a cell and in inducing gene expression resulting in the formation of protein involved in life processes (Nielsen, 1990). Essential trace elements: Boron, cobalt, copper, iron, manganese,

molybdenum, and zinc. Probable essential trace elements are chromium, nickel, selenium, and vanadium. Physically primitive trace elements are Bromine, lithium, silicon, tin and titanium (Frieden,1985).

Table (2): Mineral content of *M. Oleifera* leaves Macro-elements(gm/kg of dried *M*.) Micro , elements (mg/kg of dried *M*.):

Ca	P	Mg	Na	K	Fe	Mn	Zn	Cu
26.4	1.36	0.11	2.73	21.7	175	51.8	13.7	7.1

(Paliwal *et al*., 2011).

1.5.3. Protein and amino acid:

In addition to these vitamins and minerals, one of the most significant benefits of *M. oleifera* is the ability of this plant to provide as much as 27.1 grams of protein (nearly 1/3 of the edible portion) including 19 of the 20 prominent protein amino acids (Table 3). The roles that amino acids play in the fundamental processes of tissue formation, regeneration and function are so distinctive that this class of substances is considered to be the primary component of all living matter. In contrast to fats and carbohydrates, which are also essential for life and which function primarily as energy sources, proteins vary widely in composition not only between living organisms but also among the various tissues and cellular fluids within a particular living organism. In addition to their roles as building blocks of proteins, the amino acids are precursors of many other important biomolecules, including various hormones, vitamins, coenzymes, alkaloids and porphyrins. The aromatic amino acids are especially versatile precursors. From the amino acids are made alkaloids, such as morphine, codeine and papaverine, and a number of hormones. Some of these hormones include the thyroid hormone, thyroxine, the plant hormone, indole acidic acid and the adrenal hormone, epinephrine (Gennaro *et al*., 1975). The nutritional value of proteins in our

diet involves understanding something about both the quality and the quantity of proteins consumed. Humans do not have the ability to synthesize all of the amino acids required for normal, good health. Those that must be supplied in our diets are called essential amino acids. *M. oleifera* contains all of the eight amino acids considered essential. Although proteins found in meat, eggs and milk are considered to have the best nutritional value, such foods are those which should be limited due to their negative effect on serum cholesterol. Moreover, persons who either cannot or who choose not to consume these foods (persons who are lactose intolerant or those who are vegetarians) may run the risk of developing a protein deficiency. Adequate protein nutrition obviously requires the consumption of sufficient protein to meet daily requirements. The protein must include the essential amino acids. Therefore, protein deficiency may be caused by either a reduced intake, or the use of low-quality protein. Symptoms of deficiency can include weight loss, nutritional edema, skin changes and may be associated with conditions such as nephritis and colitis. Deficiency may also result in a lowered immune system since adequate protein intake is necessary for the formation of antibodies. Daily stress and pregnancy may also cause a deficiency of amino acids, and greater consumption of protein is required for these conditions for optimal health. For such individuals, *M. oleifera* is an important source of these vital nutrients (Brett *et al*., 2005).

Table (3): Amino acids composition of dried *M. oleifera* leaves:

Amino acid	Quantity (mean+/- %)	Standard error
Arginine	1.78	0.010
Serine	1.087	0.035
Aspartic acid	1.43	0.045
Glutamic acid	2.53	0.062
Glycine	1.533	0.060
Threonine*	1.357	0.124
Alanine	3.033	0.006
Tyrosine*	2.650	0.015
Proline	1.203	0.006
HO-Proline	0.093	0.006
Methionine*	0.297	0.006
Valine*	1.413	0.012
Phenylalanine*	1.640	0.006
Isoleucine*	1.177	0.006
Leucine*	1.960	0.010
Histidine*	0.716	0.006
Lysine*	1.637	0.006
Cysteine	0.010	0.00
Tryptophan*	0.486	0.001

*General essential amino acids (Busani *et al*., 2011).

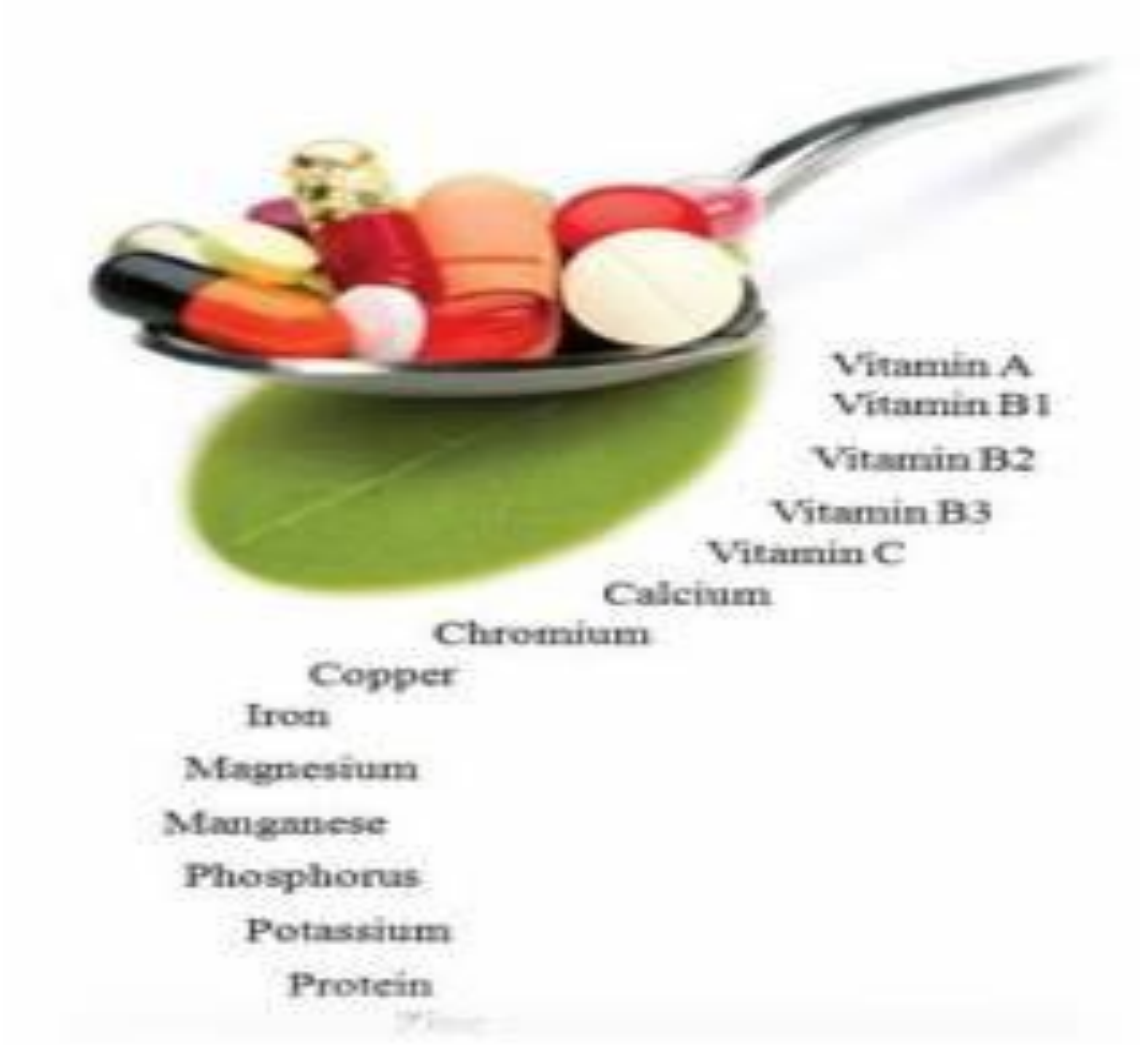

Fig 5: Nutritional contents in *M. Oleifera l*eaves.

1.5.4. Other Supplements Constituents:

M. oleifera grown and used in many countries around the world is a multi-purpose tree with medicinal, nutritional and socio-economic values. It established the medicinal uses of *M. oleifera* leaves by local communities in Uganda and identified phytochemicals present in *M. oleifera* leaves extracts. Phytochemicals present included: tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars. The local communities in Uganda use *M. oleifera* leaves to treat common ailments. Presence of phytochemicals in the extracts, indicate possible

preventive and curative property of *M. oleifera* leaves. There is need to standardize *M. oleifera* leaves use for nutrition and herbal medicine (Josephine *et al*., 2010).

1.5.5 Phytochemical Constituents and Their Specific Advantage :

1.5.5.1Tannins:

Are a group of polymeric phenolic compounds and cause local tumours (Kapadia *et al.*, 1978). They are able to inactivate and kill microorganisms. They used in the treatment of varicose ulcers, hemorrhoids, minor burns, frostbite as well as inflummantation of gums, in recent years, these compounds have demonstrated their antiviral diseases (Cowan, 1999).

1.5.5.2 Flavonoids:

Are strong antioxidants and are effective antibacterial substances *in vitro* against a large number of microorganisms by inhibition of the membrane-bound enzymes (Cowan, 1999). They also showed substantial anticarcinogenic and antimutagenic activities due to their antioxidant and anti-inflammatory properties [(Nandakumar *et al*., 2008), (Li-Weber, 2009)] and also they are an important class of natural products, are the main bioactive constituents of a lot of medicinal or dietary plants, they have been reported to show extensive benefits to human health, including antioxidant, anti-inflammatory, and anti-cancer activities in most cases, Flavonoids are present in plants as a series of analogues with similar structures and physicochemical properties (Belay and Sisay, 2014).

1.5.5.3 Alkaloids:

Are group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Alkaloids have antimicrobial properties by intercalating with bacterial DNA such as nicotine, are used in pesticides and others are used as chemicals reagents. Quinine, is a natural white crystalline

alkaloid having antipyretic (fever reducing), analgesic (painkilling), and anti-inflammatory properties and a bitter taste used in treating malaria (Manske, 1965).

1.5.5.4 Steroids:

Are drugs that are structurally related to the cyclic steroid ring system and have similar effects to testosterone in the body. They increase protein within cells, especially in skeletal muscles. Anabolic steroids also have androgenic and virilizing properties, including the development and maintenance of masculine characteristics such as the growth of the vocal cords, testicles (primary sexual characteristics) and body hair (secondary sexual characteristics) (Raju *et al*., 2004).

1.5.5.5 Saponins:

Any of a class of glycosides, found widely in plants, that have detergent properties and form a lather when shaken with water. which are stable both in alkaline and acidic media. Color reaction can be used to characterize saponins (and sapogenins) in order to verify the identity of drugs. Possess antioxidant, anti-inflammatory, antiapoptosis and immunostimulant properties which were found in *M. oleifera* (Galeotti *et al*., 2008).

1.5.5.6 Anthraquinones:

Are a group of naturally occurring phenolic compounds and are present in *M. oleifera* leaves which showed laxative properties (Belay and Sisay, 2014).

1.5.5.7 Terpenoids:

Were detected in *M. oleifera* which were reported to be active against *Staphylococcus aureus* (Cowan, 1999). These compounds also have anticarcinogenic properties (Yun *et al*., 1996).

1.6 Medical Benefits of *Moringa oleifera*:

M. oleifera is an edible plant. Awide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits, and seeds(Ramachandran *et al*.,1980., Anwar *et al*., 2007., Kumar *et al*., 2010).

1.6.1 Medicinal Plants:

Medical plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavondoids, and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes [(Okwu , 1999), (Okwu, 2001)]. Traditional knowledge of medicinal plants has always guided the search for new-cures. In spite of the advent of modern high throughout drug discovery and screening techniques. Traditional knowledge systems have given clues to the discovery of valuable drugs (Buenz *et al*., 2004). Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal practices form an integral part of complementary or alternative medicines. Although their efficacy and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Park and pezzutto, 2002). Traditional medicines are used by about 60 per cent of the world population. The nursery for the introduction of food, crop and medicinal plants was created in 1823. And before the introduction of chemical medicines, man relied the healing properties of medicinal plants. Medicinal plants have been indentified and used throughout human history in this case medicinal plants are of great importance to the health of

individuals and communities (Belay and Sisay, 2014). The importance of medicinal plants, are extremely useful for us on the one hand they provide us with the oxygen we need to be able to breathe for edible landscaping, a *M. oleifera* tree is hard to beat. *M. oleifera* plants produce special substances in their roots, leaves, flowers or seeds that help them to survive and healing with medicinal plants old as mankind itself. *M. oleifera* is considered to be effective in the treatment of many diseases (Caceres *et al*., 1991). *M. oleifera* tree is mainly grown in the semi-arid tropical and sub-tropical areas. It grows best in dry sandy soil and can tolerate any other type of soil. It is a fast growing drought resistant tree that is native to the Southern foothills of Himalayans in Northern India. It is considered as one of the world's most useful tree, as almost every part of the plant could be used for food or has some other beneficial properties (Anamika *et al*., 2010).

1.6.2 *Moringa oleifera* and It's Medicinal Usage:

Free radicals, produced as a result of normal biochemical reactions in the body, are implicated in contributing to cancer, atherosclerosis, aging, immunosuppression, inflammation, ischemic heart diseases, diabetes, hair loss and neurodegenerative disorders such as Alzheimer's disease and parkinson's disease [(Beal,1995),(Maxwell,1995), (Poulson *et al*., 1998)]. The human body possesses innate defence mechanisms to counter free radicals in the form of enzymes such as superoxides dismutate, catalose, and glutathione peroxidase. Vitamin C, vitamin E, selenium, β – carotene, lycopene, lutein and other carotenoids have been used as supplementary antioxidants. A part from these, plant secondary metabolites such as flavonoids and terpeniods play important role in the defence against free radical [(Park and pezzutto, 2002), (Devasagayun and sainis, 2002), Govindarajs *et al*., 2005)]. Medicinal plants parts are commonly rich in phenolic compounds such as flavonoids, stillbenes, tannis, coumarins,

lignans and lignins [(Larson, 1988), Kahkonen *et al*., 1999)]. There have been several studies on the antioxidant activities of various herbs/ plants with medicinal values. Phytochemicals in fruits, vegetable, spices and traditional gerbul medicinal plants have been found to play protective role against many human chronic diseases including cancer and cardio vascular disease (CVD). Phytocomponents including phenolics, flavonoids, tannis proanthocyanidins and various plants or herbal extracts have been reported to be radical scavengers and inhibitors of lipid peroxidation (Xie *et al*., 1992).When Phytochemicals compounds react with a free radical, it is the delocalization of the guined electron over the phenolic antioxidant and the aromatic nucleus that prevents the continuation of the free radical chain reaction. This is often called "Radical scavenging". But polyphenolic compounds inhibit oxidation through a variety of mechanisms (Cuvelier *et al*., 1992). The plant possesses valuable medicinal properties but most of the advantages are still confined to tribal areas because of raw knowledge and absence of proper scientific standardization. For the useful application of the plant parts in modern medicine, Physico-chemical and phytochemical standardization is very important (Belay and Sisay, 2014) .

1.7 Pharmacological Properties:

M. oleifera also has numerous medicinal uses, which have long been recognized in the Ayurvedic and Unani systems of medicine (Mughal *et al*., 1999).

1.7.1 Antihypertensive, diuretic and cholesterol lowering activities:

The widespread combination of diuretic along with lipid and blood pressure lowering constituents make this plant highly useful in cardiovascular disorders. *M. oleifera* leaves juice is known to have a stabilizing effect on blood pressure(Dahot, 1988). Nitrile, mustard oil glycosides and thiocarbamate glycosides have been isolated from *M. oleifera* leaves, which

were found to be responsible for the blood pressure lowering effect (Faizi *et al.*, 1994a., 1994b., 1995). Most of these compounds, bearing thiocarbamate, carbamate or nitrile groups, are fully acetylated glycosides, which are very rare in nature (Faizi *et al.*, 1995). Bioassay guided fractionation of the active ethanol extract of *M. oleifera* leaves led to the isolation of four pure compounds, niazinin A , niazinin B, niazimicin and niazinin A + B which showed a blood pressure lowering effect in rats mediated possibly through a calcium antagonist effect (Gilani *et al.*, 1994a). Another study on the ethanol and aqueous extracts of whole pods and its parts, i.e. coat, pulp and seed revealed that the blood pressure lowering effect of seed was more pronounced with comparable results in both ethanol and water extracts indicating that the activity is widely distributed (Faizi *et al.*, 1998). Activity-directed fractionation of the ethanol extract of pods of *M. oleifera* has led to the isolation of thiocarbamate and isothiocyanate glycosides which are known to be the hypotensive principles (Faizi *et al.*, 1995). Methyl phydroxybenzoate and β-sitosterol , investigated in the pods of *M. oleifera* have also shown promising hypotensive activity (Faizi *et al.*, 1998). *M. oleifera* roots, leaves, flowers, gum and the aqueous infusion of seeds have been found to possess diuretic activity (Morton, 1991., Caceres *et al*., 1992) and such diuretic components are likely to play a complementary role in the overall blood pressure lowering effect of this plant. The crude extract of *M. oleifera* leaves has a significant cholesterol lowering action in the serum of high fat diet fed rats which might be attributed to the presence of a bioactive phytoconstituent, i.e. β-sitosterol (Ghasi *et al.*, 2000). *M. oleifera* fruit has been found to lower the serum cholesterol, phospholipids, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) cholesterol to phospholipid ratio, atherogenic index lipid and reduced the lipid profile of liver, heart and aorta in hypercholesteremic rabbits and increased the excretion of fecal cholesterol (Mehta *et al.*, 2003).

1.7.2 Antispasmodic, antiulcer and hepatoprotective activities:

M. oleifera roots have been reported to possess antispasmodic activity (Caceres *et al.*, 1992). *M. oleifera*, leaves have been extensively studied pharmacologically and it has been found that the ethanol extract and its constituents exhibit antispasmodic effects possibly through calcium channel blockade (Gilani *et al.*, 1992., 1994a., Dangi *et al.*, 2002). The antispasmodic activity of the ethanol extract of *M. oleifera* leaves has been attributed to the presence of 4-[α-(L-rhamnosyloxy) benzyl]- o-methyl thiocarbamate (*trans*), which forms the basis for its traditional use in diarrhea (Gilani *et al.*, 1992). Moreover, spasmolytic activity exhibited by different constituents provides pharmacological basis for the traditional uses of this plant in gastrointestinal motility disorder (Gilani *et al.*, 1994a). The methanol fraction of *M. oleifera* leave extract showed antiulcerogenic and hepatoprotective effects in rats. Aqueous leaf extracts also showed antiulcer effect (Pal *et al.*, 1995a) indicating that the antiulcer component is widely distributed in this plant. *M. oleifera* roots have also been reported to possess hepatoprotective activity. The aqueous and alcohol extracts from *M. oleifera* flowers were also found to have a significant hepatoprotective effect (Ruckmani *et al.*, 1998), which may be due to the presence of quercetin, a well known flavonoid with hepatoprotective activity (Gilani *et al.*, 1997).

1.7.3 Antibacterial and antifungal activities:

M. oleifera roots have antibacterial activity (Rao *et al.*, 2001) and are reported to be rich in antimicrobial agents. These are reported to contain an active antibiotic principle, pterygospermin, which has powerful antibacterial and fungicidal effects (Ruckmani *et al.*, 1998). A similar compound is found to be responsible for the antibacterial and fungicidal effects of its flowers (Das *et al.*, 1957). The root extract also possesses antimicrobial activity attributed to the presence of 4-α-L-rhamnosyloxy benzyl isothiocyanate

(Eilert *et al.*, 1981). The aglycone of deoxy-niazimicine (N-benzyl, S-ethyl thioformate) isolated from the chloroform fraction of an ethanol extract of the root bark was found to be responsible for the antibacterial and antifungal activities (Nikkon *et al.*, 2003). The bark extract has been shown to possess antifungal activity (Bhatnagar *et al.*, 1961), while the juice from the stem bark showed antibacterial effect against *Staphylococcus aureus* (Mehta *et al.*, 2003). The fresh leaves juice was found to inhibit the growth of microorganisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*), pathogenic to man (Caceres *et al*., 1991).

1.7.4 Antitumor and anticancer activities:

Makonnen *et al.* (1997) found *M. oleifera* leaves to be a potential source for antitumor activity. *O*-Ethyl- 4-(α-L-rhamnosyloxy)benzyl carbamate together with 4(α-L-rhamnosyloxy)-benzyl isothiocyanate niazimicin and 3-*O*-(6′-*O*-oleoyl-β-D-glucopyranosyl)- β-sitosterol have been tested for their potential antitumor promoting activity using an *in vitro* assay which showed significant inhibitory effects on Epstein– Barr virus-early antigen. Niazimicin has been proposed to be a potent chemopreventive agent in chemical carcinogenesis (Guevara *et al.*, 1999). The seed extracts have also been found to be effective on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice (Bharali *et al.*, 2003). A seed ointment had a similar effect to neomycin against *Staphylococcus aureus pyodermia* in mice (Caceres and Lopez, 1991). It has been found that niaziminin , a thiocarbamate from the leaves of *M. oleifera*, exhibits inhibition of tumor-promoter-induced Epstein–Barr virus activation. On the other hand, among the isothiocyanates, naturally occurring 4-[(4′-*O*-acetyl-α-i-rhamnosyloxy) benzyl], significantly inhibited tumor-promoterinduced Epstein–Barr virus activation, suggesting that the

isothiocyano group is a critical structural factor for activity (Murakami *et al.*, 1998).

1.7.5 Antioxidants:

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is now directed towards natural antioxidants originated from plants due to safe therapeutics. *M. oleifera* is used in Indian traditional medicine for a wide range of various ailments. To understand the mechanism of pharmacological actions, antioxidant properties of the *M. oleifera* leaves extracts were tested in two stages of maturity using standard in vitro models. The successive aqueous extract of *M. oleifera* exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical, superoxide, nitric oxide radical and inhibition of lipid per oxidation. The free radical scavenging effect of *M. oleifera* leaves extract was comparable with that of the reference antioxidants. The extracts of *M. 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 damages (Sreelatha and Padma, 2009). The viability and functionality of a cell partly depends on a favor- able redox state, i.e., on its ability to prevent excessive oxidation of its macromolecules, including DNA, proteins, and lipids (Ryter *et al*., 2007., Limon-Pacheco and Gonsebatt, 2009). ROS (reactive oxygen molecules) and free radicals are the major mediators of the oxidative process. Cellu- lar inability to reduce ROS leads to oxidative stress. All cells are variably capable of endogenous self-protection against this stress through the actions of enzymes such as catalase, superoxide dis- mutase, and glutathione peroxidase, as well as through reducing molecules such as glutathione. Nutritional antioxidants such as vitamins A, C, and E provide additional

protection from the stress (Limon-Pacheco and Gonsebatt, 2009). Oxidative stress is widely accepted as a major contributing factor in the pathogenesis of CVD and diabetes (Dhalla *et al*., 2000., Kaneto *et al*., 2007., Rodrigo *et al*., 2011). Arecurring explanation for the therapeutic actions of *M. oleifera* medication is the relatively high antioxidant activity of its leaves, flowers ,and seeds (Chumark *et al*., 2008., Sreelatha and Padma, 2009., Verma *et al*., 2009., Atawodi *et al*., 2010). Among the major classes of phytochemicals found in the plant, flavonoids appear to carry most of this activity.

1.7.6 Anti diabetes:

In Indian traditional system of medicine, *M. oleifera* Lam. Syn. *M. oleifera* pterygosperma Gareth is commonly used as healing herb to treat diabetes. Different parts of this plant are used in the indigenous systems of human medicine for the treatment of a variety of human ailments. The leaves of *M. oleifera* are reported to be used as a hypocholesterolemic agent, and hypoglycemic agent (Dangi *et al*., 2002)., (Ghasi *et al*., 2000). Moreover, an antidiabetic property is also included among the medicinal benefits of *M. oleifera* (Anwar *et al*., 2007). Aqueous extract *M. oleifera* leaves shows anti-diabetic activity on glucose tolerance in Goto-Kakizaki and wistar rats (Suzuki, 2007). According (Mishra, 2011) aqueous extract of *M. oleifera* leaves shows anti diabetic control and thus exhibit glycemic control.

1.7.7 Other diverse activities:

M. oleifera has also been reported to exhibit other diverse activities. Aqueous leaves extracts regulate thyroid hormone and can be used to treat hyperthyroidism and exhibit an antioxidant effect (Pal *et al.*, 1995a., 1995b., Tahiliani and Kar, 2000). A methanol extract of *M. oleifera* leaves conferred significant radiation protection to the bone marrow chromosomes in mice (Rao *et al.*, 2001). *Moringa oleifera* leaves are effective for the regulation of thyroid hormone status (Tahiliani and Kar, 2000). A recent report showed

that *M. oleifera* leave2 may be applicable as a prophylactic or therapeutic anti-HSV (Herpes simplex virus type 1) medicine and may be effective against the acyclovir-resistant variant (Lipipun *et al.*, 2003). Table 4 depicts some common medicinal uses of different parts of this plant. The flowers and leaves also are considered to be of high medicinal value with anthelmintic activity (Bhattacharya *et al.*, 1982). *M. oleifera* is coming to the forefront as a result of scientific evidence that *M. oleifera* is an important source of naturally occurring phytochemicals and this provides a basis for future viable developments. Different parts of *M. oleifera* are also incorporated in various marketed health formulations, such as Rumalaya and Septilin (the Himalaya Drug Company, Bangalore, India), Orthoherb (Walter Bushnell Ltd, Mumbai, India), Kupid Fort (Pharma Products Pvt. Ltd, Thayavur, India) and Livospin (Herbals APS Pvt. Ltd, Patna, India), which are reputed as remedies available for a variety of human health disorders (Mehta *et al.*, 2003). *M. oleifera* seeds have specific protein fractions for skin and hair care. Two new active components for the cosmetic industry have been extracted from oil cake. Purisoft consists of peptides of the *M. oleifera* seed. It protects the human skin from environmental influences and combats premature skin aging. With dual activity, antipollution and conditioning/strengthening of hair, the *M. oleifera* seed extract is a globally acceptable innovative solution for hair care (Stussi *et al.*, 2002).

Table(4): Some common medicinal uses of different parts of *M. oleifera*:

Plant part	Medicinal uses
Leaves	Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh, leaf juice is believed to control glucose level, applied to reduce glandular swelling [Morton, 1991; Fungile, 2001; The wealth of India, 1962., Dahot,1988].
Root	Antilithic, rubefacient, vesicant, carminative, antifertility, anti inflammatory, stimulant in paralytic afflictions; acts as cardiac, circulatory tonic, used as a laxative, abortifacient, treating rheumatism, inflammations, articular pain, lower back of kidney pain and constipation [The wealth of India, 1962., Dahot,1988., Rukmani *et al*., 1988].
Flower	High medicinal value as stimulant, aphrodisiac, abortifacient, cholagogue used to cure inflammations, muscle diseases, hysteria, tumors and enlargement of spleen, lower the serum, cholesterol, phospholipid, triglycerides, decrease lipid profile of liver, heart and aorta in hypercholesterolaemic and increased the excretion of faecal cholesterol [Bhattacharya *et al*., 1982., Siddhuraju and Becker, 2003., Mehta *et al*., 2003].
Stem Bark	Rubefacient, vesicant and used to cure eye diseases and for treatment of delirious patients, prevent enlargement of the spleen and formation of tuberculous gland of the neck, to destroy tumors and heal ulceres. The juice from the root bark is put into ears to relieves ear aches and also placed in a tooth cavity as a pain killer, and has anti tubercular activity [Bhatnagar *et al*., 1961., Siddhuraju and Becker, 2003].
Seed	Seed extract exerts its protective effect by decreasing liver lipid peroxide, antihypertensive , compounds thiocarbamate and isothiocyanate glycosides have been isolated from the acetate phase of ethanolic extract of *Moringa Oleifera* pods [Faizi *et al*., 1998].
Gums	Used for dental caries, and is astringent and rubecient, gum, mixed with sesame oil is used to relieve headache, fevers, intestinal complaints, dysentery, asthama and some times used as an abortifacient and to treat sysphilis and rheumatism. [Fuglie, 2001].

1.8 Future Prospects of *M. Oleifera*:

So far numerous studies have been conducted on different parts of *M. oleifera*, but there is a dire need to isolate and identify new compounds from different parts of the tree, which have possible antitumor promoters as well as inhibitory properties. Although preliminary studies are under way in different laboratories to use the antispasmodic, antiinflammatory, antihypertensive and diuretic activities of *M. oleifera* seed, these studies should be extended to humans in view of the edible nature of the plant. *M. oleifera* roots and leaves have been used traditionally to treat constipation. Studies to verify these claims need to be carried out in the light of the reported antispasmodic activities, which are contrary to its medicinal use as a gut motility stimulant. Earlier studies on the presence of a combination of spasmogenic and spasmolytic constituents in different plants used for constipation (Gilani *et al.*, 2000., 2005a., Bashir *et al.*, 2006) might be of some guidance in designing experiments in which the presence of antispasmodic constituents at higher doses are explained as a possible mode to offset the side-effects usually associated with high dose of laxative therapy. Similarly, the known species differences in the pharmacological actions of medicinal plants (Ghayur *et al.*, 2005., Ghayur and Gilani, 2006) may also be taken into account when planning studies involving contradictory results. Food plants are considered relatively safe as they are likely to contain synergistic and/or side effect neutralizing combinations of activities (Gilani and Atta-ur- Rahman, 2005). *M. oleifera*, known to be rich in multiple medicinally active chemicals, may be a good candidate to see if it contains effect enhancing and/or side-effects neutralizing combinations. Medicinal plants are relatively rich in their contents of calcium channel blockers (CCBs) which are known to possess a wide variety of pharmacological activities such as antihypertensive, hepatoprotective, antiulcer, antiasthmatic, antispasmodic and antidiarroeal (Stephens and

Rahwan, 1992., Gilani *et al.*, 1994b., 1999., 2005b., Yaeesh *et al.*, 2006., Ghayur *et al.*, 2006) and it remains to be seen whether such activities reported to be present in *M. oleifera* have a direct link with the presence of CCBs. Niazimicin, a potent antitumor promoter in chemical carcinogenesis is present in the seed, its inhibitory mechanism on tumor proliferation can be investigated by isolating more pure samples. The mechanism of action of *M. oleifera* as prophylactic or therapeutic anti- HSV medicines for the treatment of HSV-1 infection also needs to be examined. The available information on the α-, β- and γ - tocopherol content in samples of various parts of this edible plant is very limited. β-Carotene and vitamins A and C present in *M. oleifera*, serve as an explanation for their mode of action in the induction of antioxidant profiles, however, the exact mechanism is yet to be elucidated. β-Carotene of *M. oleifera* leaves exerts a more significant protective activity than silymarin against antitubercular induced toxicity. It would be interesting to see if it also possesses hepatoprotective effect against other commonly used hepatotoxic agents such as CCl_4 and galactosamine, which are considered more suitable models and close to human viral hepatitis (Gilani and Janbaz, 1995., Yaeesh *et al.*, 2006). Although *M. Oleifera* leaves are considered a best protein source, it still has to be shown whether or not this protein source could compete with the more common protein sources in highly productive growing or milk producing ruminants. Since this plant naturally occurs in varying habitats, it is naive to expect a great magnitude of variation in the concentration and composition of chemical ingredients in different parts of the tree. However, the extent to which the chemical composition varies in populations adapted to varying habitats is not known. Thus, detailed studies are required to examine this aspect. In view of its multiple uses, the *M. oleifera* plant needs to be widely cultivated in most of the areas where climatic conditions favor its optimum growth. In this way, a maximum yield of its different useable parts could be achieved to derive the

maximal amount of commodities of a multifarious nature for the welfare of mankind (Anwar *et al*., 2007).

1.9 Toxicity Study on *M. Oleifera*:

According one acute toxicity study of various extracts of *M. Oleifera* roots, results of that study showed a safe range. The lethal dose of 50% (LD_{50}) for the aqueous extract was 15.9g/kg body weight while that of ethanol extract was 17.8g/kg .The results were supported by the work done by Adedapo *et al*., 2009. Using commonly used terms for toxicities along the dose equivalent for rats/mice, *M. Oleifera* root peels are relatively harmless ≥1kg probable lethal single dose for man. Kasolo *et al*., 2010 *M. Oleifera* root peel is relatively non- toxic when given as single dose. Awodele, 2012 reported no significant effects were observed with respect to hematological or biochemical parameters or sperm quality. The authors conclude that aqueous extract was safe to use. The consumption of *M. oleifera* leaves powder seem to the relatively safe as shown by the acute toxicity in rats. This conclusion appears to be supported by other studies as indicated above. A sub chronic study of the administration of *M.oleifera* leaves powder need to e undertaken (JMPS, 2017).

1.10 Hyperglycemia:

An individual is diagnosed as diabetic when his blood glucose level is chronically ≥ 126 mg/dL after an overnight fast, and ≥ 200 mg/dL 2h after an oral glucose load of 75g (oral glucose tolerance test, OGTT., Alberti and Zimmet, 1998). Age, genetics, environment, and lifestyle influence the development of this pathology. The relative importance of these factors and their combinatorial effects are not yet fully understood. Two types of DM are commonly recognized: type1 DM (T1DM) results from autoimmune destruction of pancreatic β cells and represents only 5% of all cases., type-2 DM (T2DM)is the most common form of the disease. In its early stages,

T2DM is characterized by chronic hyperglycemia and hyperinsulinemia, due to loss of tissue sensitivity to insulin, and compensatory secretion of the hormone by islet β cells. Its progression involves a complex network of interacting cellular and physiological alterations leading to β cell failure. Glucotoxicity and lipotoxicity are the most commonly invoked mechanisms for this failure(Robertson *et al*., 2004). Glucotoxicity arises from excessive uptake of glucose by islet β cells. The excess sugar drives glycation reactions and the mitochondrial electron transport chain, producing macromolecule-damaging ROS, at levels beyond the antioxidation capacity of the cell. The ensuing oxidative stress impairs insulin synthesis and secretion, and initiates a cascade of cellular events that ultimately lead to apoptosis (Kaneto *et al*., 2007).

1.11 Evidence of Anti-Hyperglycemic Properties of *M. Oleifera*:

William *et al*., 1993 examined in a controlled study with untreated T2DM patients, how *M. oleifera* addition to a standardized meal, taken after an overnight fast, affected the1- and 2-h PPBG, relative to the standard meal alone or a 75-g oral glucose load. *M. oleifera* was compared to bitter gourd and curry leaves. Compared to the glucose load, standard meals with or without vegetable supplements induced a significantly lower rise in PPBG (glycemic response) as derived from area under the curves (AUCs). However, when leaves-supplemented meals were compared to standard meals, only the *M. oleifera* leaves-supplemented meal elicited a lower response(−21%, $P < 0.01$). Plasma insulin AUCs, did not differ significantly between the two meals, suggesting that the hypoglycemic effect of *M. oleifera* leaves supplementation was not due to increased insulin secretion. **(**Kumari, 2010) examined the hypoglycemic effect of *M. oleifera* leaves dietary consumption over a 40-day period in T2DM patients, 30–60 years of age, not on anti-hyperglycemic medication. The experimental group

included 46 subjects ,32men, and 14women, the control group of 9 subjects included 4 men and 5 women. Daily meals were comparable among these groups in terms of relative content of food types(e.g. ,cereals, green leafy vegetables, fruits, etc.) and nutrients (e.g., proteins ,fat, fiber, minerals, etc.) as well as calories. The experimental group received a daily dose of 8g *M. oleifera* leaves powder. At the end of the protocol (final) were compared to baseline levels. Final values did not differ much from baseline in the control group. They were significantly reduced in the experimental group ($P < 0.05$). More recently, (Ghiridhari *et al*., 2011) studied a group of 60 T2DM patients, age 40–58 years, on sulfonylurea medication and a standardized calorie-restricted diet. The patients were equally divided into an experimental and a control groups. Patients in the experimental group were prescribed two *M. oleifera* leaves tablets/day, one after breakfast, the other after dinner for 90 days. *M. oleifera* leaves powder constituted 98% (w/w) of the tablet content, but the average weight of tablets was not specified, making the total daily dose unclear. Blood glycated hemoglobin (HbA1c) was measured before and after the regimen. PPBG was determined before the regimen and every 30 days afterward. In the control group, HbA1c progressed downwardly with time, but the change was not significant. In the experimental group, in contrast, relative to the baseline, HbA1c decreased by 0.4% point(from7.8 ± 0.5 to 7.4 ± 0.6., $P<0.01$). Compared to the starting levels(210 ± 49 mg/dl), indicating that *M. oleifera* medication can induce with time better glucose tolerance. However, it should be noted that treatment allocation to patients appear to have not been randomized as baseline values for the two parameters were higher in the experimental group than in the control group, 7.8 ± 0.5 vs. 7.4 ± 0.6% for HbA1c. (Rosario *et al*., 2014) determined the changes in glucose, lipid profile and antioxidant capacity in humans with moderately raised serum glucose and cholesterol levels after consumption of *M. oleifera* leaves supplemented-food products. Test foods are buns, fish

sausage and veggie soup with and without *M. oleifera* leaves powder. Thirty-eight participants were randomly grouped into control and experimental, given foods without and with *M. oleifera*. The total amount of dietary fiber containing *M. oleifera* leaves powder was 14.4 g while without *M. oleifera* was 9.3 g. Results: Serum blood glucose from baseline to end line for the control group was still considered moderately raised (6.2 to 5.6 mmol/L) while in the experimental group resulted from moderately raised (5.8 mmol/L) to normal serum glucose (5.0 mmol/L., $P<0.05$). The results indicated that *M. oleifera* leaves supplemented-food products decreased fasting blood sugar. (Kumar and Mandapaka, 2013) concluded supplementation of the powder of *M. Oleifera* leaves decreased serum glucose and LDL. These values were also found to be statistically significant. And it is concluded that the leaves of *M. oleifera* have definite hypoglycemic and hypocholesterolemic activity in type II diabetes mellitus in obese people. The increased susceptibility of tissues such as the liver and kidney of diabetic animals to diabetic complications may be due to increased lipid peroxidation. In addition, increased lipid peroxidation under diabetic conditions resulted due to excessive oxidative stress. From this view point, prevention of oxidative damage was considered to play a crucial role in diabetes and / or its complications resulting from lipid peroxidation (Stanely and Menon, 2001). Isoflavones are phytoestrogens have a structural/functional similarity to human estrogen and have been consumed by humans world-wide. Among all the phytoestrogens, soy isoflavones have been studied most. A high isoflavone intake (20–100 mg/day) is associated with lower incidence and mortality rate of type II diabetes. Good magnesium status reduces diabetes risk and improves insulin sensitivity., chromium picolinate, calcium and vitamin D appear to promote insulin sensitivity and improve glycemic control in some diabetics, extracts of bitter melon and of cinnamon have the potential to treat and possibly prevent diabetes. However it has been

suggested that nutraceuticals with meaningful doses of combinations may substantially prevent type II diabetes (McCarty, 2005).

1.12 Proven Benefits of *M. oleifera* Leaves Powder:

M. oleifera has gained a reputation for fighting inflammation and combating various effects of malnutrition and aging, earning the nickname "the miracle plant." Here are six topics proven *M. oleifera* benefits to show that nickname is well-deserved(Levy and CHHC., 2018).

Fig 6: *Moringa oleifera* fresh leaves.

Fig 7: *Moringa oleifera* dried leaves powder.

1.12.1 Balances Hormones and Slows the Effects of Aging:

A 2014 study published in the Journal of Food Science and Technology tested the effects of *M. oleifera* along with amaranth leaves on levels of inflammation and oxidative stress in menopausal adult women. Knowing that levels of valuable antioxidant enzymes get affected during the postmenopausal period due to deficiency of "youthful" hormones, including estrogen, researchers wanted to investigate if these super foods could help slow the effects of aging using natural herbal antioxidants that balance hormones naturally.

1.12.2 Helps Improve Digestive Health:

Due to its anti-inflammatory properties, *M. oleifera* has been used in ancient systems of medicine such as Ayurveda to prevent or treat stomach ulcers, liver disease, kidney damage, fungal or yeast infections (such as candida), digestive complaints, and infections. A common use of *M. oleifera* oil is helping to boost liver function and therefore detoxifying the body of harmful substances, such as heavy metal toxins. It might also be capable of helping to fight kidney stones, urinary tract infections, constipation, fluid retention/edema and diarrhea.

1.12.3 Protects and Nourishes the Skin:

M. oleifera contains natural antibacterial, antifungal and antiviral compounds that protect the skin from various forms of infections. Some of the common ways *M. oleifera* is used on the skin include: reducing athlete's foot, eliminating odors, reducing inflammation associated with acne breakouts, treating pockets of infection or abscesses, getting rid of dandruff, fighting gum disease (gingivitis), and helping heal bites, burns, viral warts and wounds. *M. oleifera* oil is applied directly to the skin as a drying, astringent agent used to kill bacteria, but at the same time when used

regularly it's known to act like a lubricant and hydrate the skin by restoring its natural moisture barrier.

1.12.4 Helps Stabilize Your Mood and Protects Brain Health:

As a high protein food and a rich source of the amino acid tryptophan, *M. oleifera* benefits neurotransmitter functions, including those that produce the "feel good" hormone serotonin. *M. oleifera* is also rich in antioxidants and compounds that improve thyroid health, which makes it beneficial for maintaining high energy levels plus fighting fatigue, depression, low libido, moods swings and insomnia.

1.12.5 Provides Antioxidants and Anti-Inflammatory Compounds:

According to a report published in the Asian Pacific Journal of Cancer Prevention, *M. oleifera* contains a mix of essential amino acids (the building blocks of proteins), carotenoid phytonutrients (the same kinds found in plants like carrots and tomatoes), antioxidants such as quercetin, and natural antibacterial compounds that work in the same way as many anti-inflammatory drugs. *M. oleifera* leaves are high in several anti-aging compounds that lower the effects of oxidative stress and inflammation, including polyphenols, vitamin C, beta-carotene, quercetin, and chlorogenic acid.

1.12.6 Balances Blood Sugar Levels, Helping Fight Diabetes:

M. oleifera contains a type of acid called chlorogenic acid, which has been shown to help control blood sugar levels and allow cells to take up or release glucose (sugar) as needed. This gives *M. oleifera* natural antidiabetic and hormone-balancing properties. Aside from chloregnic acid, compounds called isothiocyanates that are present in *M. oleifera* have also been tied to natural protection against diabetes (Levy and CHHC., 2018).

1.13 Biochemical parameter measures which responsible to human health:

1.13.1 The Blood Glucose:

Glucose is a major fuel for animal cells. It is supplied to the organism through dietary carbohydrates and, endogenously, through hepatic gluconeogenesis and glycogenolysis. Glucose absorption from the gastrointestinal tract(GIT) into blood is regulated by a variety of neuronal signals and enterohormones(incretins), as well as by meal composition and the intestinal flora. Glucose homeostasis reflects a balance between glucose supply and its utilization. Physiologically, this balance is determined by the level of circulating insulin and tissue responsiveness to it. Insulin is secreted by pancreatic islet β cells. It stimulates glucose uptake and utilization by tissues, especially by liver, skeletal muscle, and adipose tissue. It also suppresses gluconeogenesis in hepatocytes, while stimulating lipogenesis and inhibiting lipolysis in adipocytes(Gerich, 2000).

1.13.2 The Blood Urea:

The liver produces urea in the urea cycle as a waste product of the digestion of protein. Normal human adult blood should contain between 6 to 20 mg of urea nitrogen per 100 mL (6–20 mg/dl) of blood (Deepak *et al*., 2007). The normal kidney can excrete large amounts of urea (Mayne, 1994). The measurement of urea is an important investigation in diagnosing kidney damages (Cheesbrough, 1987).

1.13.3 Creatinine:

Creatinine is a nitrogenous wasted product formed from the metabolism of creatine in skeletal muscle by irreversible non-enzymatic dehydration of creatine phosphate. Creatinine diffuses freely throughout the body water. It is filtered from the extracellular fluid by the kidney and excreted in the urine (Murray *et al*., 1988). Creatine is not converted directly to creatinine. The

rate of creatinine excretion is relatively constant from day to day. Indeed, creatinine output is sometimes measured as a check on the accuracy of the urine collection in metabolic studies; an average daily output is calculated, and the values for the daily output of other substances are corrected to what they would have been at this creatinine output (Ganong, 1993). The amount of creatinine excreted from the body is proportional to the total creatine phosphate content of the body, and thus can be used to estimate muscle mass. When muscle mass decreases for any reason, the creatinine content of the urine falls. In addition, any rise in blood creatinine is a sensitive indicator of kidney malfunction, because creatinine is normally rapidly removed from the blood and excreted. A typical adult male excretes about 15 mmol of creatinine per day. The consistency of this excretion is sometimes used to test the reliability of collected 24-hours urine samples-too little creatinine in the total urine may indicate an incomplete sample (Champe and Hervey, 1994).

1.13.4 Serum alanine aminotransferase (ALT) and Serum aspartate transaminase (AST):

AST and ALT are considered to be two of the most important tests to detect liver injury, although ALT is more specific to the liver than is AST. Sometimes AST is compared directly to ALT and an AST/ALT ratio is calculated. This ratio may be used to distinguish between different causes of liver damage and to help recognize heart or muscle injury. ALT values are often compared to the results of other tests such as alkaline phosphatase (ALP), total protein, and bilirubin to help determine which form of liver disease is present. ALT is often used to monitor the treatment of persons who have liver disease, to see if the treatment is working, and may be ordered either by itself or along with other tests for this purpose (AACC, 2013).

1.13.5 The Effect of taken *M. oleifera* leaves powder on Biochemical parameter measures:

(Prasanna and Ravi, 2013) concluded that supplementation of the leaves powder of *M. oleifera* decreased serum glucose. (Das *et al.*, 2012) have shown that in mice fed with a high-fat diet, an aqueous extract of *M. oleifera* leaves protects against liver damage as demonstrated by reductions in tissue histopathology and serum activities of marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) as well as reduced lipid peroxidation and increases in reduced glutathione. (Pari and Kumar, 2002) showed that an ethanol extract of *M. oleifera* leaves protected rats against the hepatotoxicity of various antitubercular drugs including isoniazid, rifampicin, and pyrazinamide. The extract decreased drug-induced levels of AST, ALT, ALP, and bilirubin, and inhibited drug-induced lipid peroxidation in the liver. (Sharifudin *et al*., 2013) also reported on the ability of hydroethanol extracts of *M. oleifera* leaves and flowers at doses of 200 and 400 mg/kg given intraperitoneally to inhibit acetaminophen-induced hepatotoxicity. No changes were observed with respect to markers of kidney function (Urea and Creatinine).

1.14 Uses of *M. Oleifera* to flocculate contaminants and purify drinking water:

In the Sudan, dry *M. oleifera* seeds are used in place of alum by rural women to treat highly turbid Nile water (Jahn, 1986). Studies by Eilert *et al.*, 1981 identified the presence of an active antimicrobial agent in *Moringa oleifera* seeds. The active agent isolated was found to be 4a Lrhamnosyloxy-benzyl isothiocyanate, at present the only known glycosidic mustard oil. Madsen *et al.*, 1987 carried out coagulation and bacterial reduction studies on turbid Nile water in the Sudan using *Moringa oleifera* seeds and observed turbidity

reduction of 80-99.5% paralleled by a bacterial reduction of 1- 4 log units (90-99.9%) within the first one to two hours of treatment, the bacteria being concentrated in the coagulated sediment. Many studies have also been conducted on the performance of *M. oleifera* seeds as an alternative coagulant, coagulant aid and in conjunction with alum for treating waste water. Therefore, it is important to identify the active constituents of *M. oleifera* seed for a better understanding of the coagulation mechanism. Reports on the antimicrobial effects of the protein purified from *M. oleifera* are very rare (Anwar *et al*., 2007).

Fig 8: *Moringa oleifera* seeds and observed turbidity reduction.

1.15 Common ways to use *M. oleifera* to get the best benefits possible:

M. oleifera dried leaves or *M. oleifera* powder: It takes roughly seven pounds of *M. oleifera* leaves to make one pound of dried *M. oleifera* powder. The leaves are considered the most potent parts of the plant, containing the most antioxidants and available macronutrients. In regard to the concentration of phenolic compounds, amino acids and volatile oils, the stem and root portions of the plant appear to have the least bioactive nutrients compared to the leaves. *M. oleifera* tea: This type of *M. oleifera* is made from dried leaves steeped in hot water, just like many other beneficial herbal teas. The most nutrient-dense types are organic and dried slowly under low temperatures, which helps preserve delicate compounds. Avoid boiling the leaves to help retain the nutrients best. *M. oleifera* seeds: *M. oleifera* seeds, pods and flowers appear to have a high phenolic content along with proteins and fatty acids. These are the parts of the plant used to purify water and add protein to low-nutrient diets. The seeds inside the pods are removed and roasted or dried just like nuts to preserve their freshness. *M. oleifera*: The oil from *M. oleifera* seeds is sometimes called Ben oil. Look for it in natural creams or lotions. Keep the oil in a cool, dark place away from high temperatures or the sun(Levy and CHHC., 2018).

CHAPTER TWO

Materials and Methods

2.1Materials:

2.1.1 Plant material:

The plant leaves were collected from River Nile State (Atabra Town) in 2015 .The leaves were harvested green then washed by distilled water, air-dried at room temperature, and milled into powder.

2.1.2 Instruments:

- Inductively Coupled Plasma at Best Price in India Ocean Series 2060T.
- Atomic Absorption Spectrophotometer (AAS) the model of instrument AA -6800 , SHIMADZU , Kyoto, Japan.

2.2 Methods for determination mineral elements concentrations:

2.2.1 By using ICP and AAS:

Equipment:

- Analytical balance, 250-g capacity, resolution k l.0 mg.
- Porcelain crucibles, 30-mL capacity.
- Muffle furnace capable of 500^{o}C.
- Repipette, 10.0 k 0.2 mL.
- Volumetric labware, 50 mL, plastic.
- AAS and ICP-AES.

Reagents:

- Deionized water, ASTM Type I grade.
- l.0N HC1 Solution: dilute 83.5 ml concentrated HC1 to 1.0 L with deionized water.
- Standard Calibration Solutions (K**,** Ca, Al, Na, Zn, Mn, Cu, Ni, Mo, Cr, Sr, Ba,V and Fe): from 1,000 mg/L reference solutions, prepare five multi element standards of K, Ca, and Mg ranging from 5.0 to 500 mg/L, Na

ranging from 1.0 to 100 mg/L., and Na, Zn, Mn, Fe, and Cu ranging from 0.10 to 10.0mg/L. Dilute standard calibration solutions with 0.1N HCl.

Procedure :

- 1.00g plant leaves was weighed into a porcelain crucible.
- Crucible was placed in a muffle furnace and slowly were increased the ramp temperature to 500^{o}C over 2 hours. Samples were ash for 4 hours at 500^{o}C.

-were cooled to room temperature in muffle furnace, slowly door was opened, and samples ash were removed. Sample ash was took caution not to disturb while transferring from the furnace.

- Ash was dissolved with 10.0 mL 0.1N HC1 solution. Dissolution of the ash was heated "to recovery of some elements were facilitated" (Munter and Grande, 1981).
- The contents of the crucible quantitatively were transferred into a 50-mL volumetric flask, were diluted to volume with deionized water, cap, and was inverted three times.
- (AAS) and (ICP-AES) were used to analysis elemental of plant digests. The method chosen will determine specific matrix modifications, calibration standard range, and the need for instrument-specific sample preparations and dilutions. Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using standard calibration solutions. The analyst concentrations of a method blank, unknown samples and record analyst concentrations determined in mg/L.

Calculations:

For P, K, Ca, Mg, and Na, report results to the nearest 0.001%:

$$\% \text{ analyte} = \frac{(\text{mg/L - method blank}) \times (25) \times (0.0001)}{\text{dry matter (\%)}/100}$$

For B, Zn, Mn, and Fe, report results to the nearest 1 mg/kg, and for Cu, the nearest 0.1 mg/kg:

$$\text{mg/kg analyte} = \frac{(\text{mg/L - method blank}) \times (25)}{\text{dry matter (\%)}/100}$$

2.2.2 By using ICP into the microwave digestion of plant tissue in an open vessel:

Equipment:

- A commercially available laboratory microwave drying digestion oven, such as Model MDS-81 DTM (CEM Corp., Indian Trail, NC).
- Teflon digestion vessels (with Teflon screw caps) of 120-mL capacity (CEM Corp., Indian Trail, NC).
- Brinkrnann dispensette acid dispensers, adjustable from 0 to 10 mL, for nitric acid (HNO_3) and hydrochloric acid (HC1).
- Auto-pipette for hydrogen peroxide (H_2O_2).
- Filter funnels.
- Whatman No. 42 filter paper.

Procedure:

- 1.00 g (0.01 g accuracy) plant leaves sample were transferred (20-mesh) into the microwave digestion vessel. 10 mL HNO_3 70% (specific gravity 1.42) were added , and the vessel gently were swirled so that all the tissue comes in contact with the acid.
- Digestion vessels were loaded on the turntable and the turntable were put in the oven. Center wheel of turntable were sit inside the tabs on the drive lugs. Switched were checked to ensure that assembly rotates smoothly.
- was entered in time (30 minutes) and was powered (90%), press was started, was made sure that the exhaust is on full power and fume hood is on fast function.

- At the end of the digestion cycle, were stopped the turntable rotation. The digestion vessels were left in the microwave oven for about 5 minutes to exhaust fumes.
- Digestion vessels was took out of microwave oven, carefully the cap under a fume hood was removed, and slowly 1.0 mL H_2O_2(30%) were added, and stand was let for about 5 minutes.
- The digestion vessels was placed back into the microwave oven, the turntable were started , and was digested at 90% was powered for 15 minutes.
- Then was cooled for about 5 minutes, the digestion vessel was removed from the microwave oven, the cap under a fume hood was removed, 2.0 mL HCl 37% (specific gravity 1.18) were added , and sit was let for about 5 minutes.
- The digestion vessels was placed back into the microwave oven, the turntable was started , digest at 30% was powered for 10 minutes.
- The digestion vessels were removed from the microwave oven, the cap was removed (in a fume hood), and was rinsed with water. Were rinsed down sides of container.
- Sample solutions were filtered (using Whatman No. 42 filter paper) into 100mL volumetric flasks (in a fume hood).
- Digestion vessels were rinsed three times to ensure that material is quantitatively were transferred to funnels (make sure that it was filtered before second and third additions). Was made up to 100 mL with deionized water.
- After thorough were mixed, and were transferred an aliquot into a 60-ml Nalgene bottle for determination of calcium (Ca), magnesium (Mg), potassium (K), sodium(Na), manganese (Mn), iron (Fe) and aluminum (Al) by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Note: The ICP-AES has its own computer. The weight and volume of each sample were entered and internal calibration and calculation are done with the blank subtracted (Hogan and Maynard, 1984).

2.3 Plant collection and preparation for studing the effects of *M. oleifera* leaves powder on the blood sugar in humans:

The plant leaves were collected from River Nile State (Atabra Town). The leaves were harvested green then was washed by distilled water, air-dried at room temperature, and milled into powder. They were stored in well-dried plastic containers at room temperature.

2.4 Selection of Patients:

Eighty three volunteers diabetic patients visiting Atabar hospital were selected for this study. The study targeted patients who their blood sugar is not lowering by drugs. They were asked to take *M. oleifera* leaves powder beside drugs. The subjects age between 30-80 years. Selected diabetic patients were divided into three experimental groups each containing 30 subjects. Three doses 0.25, 0.5, 1.0 g of *M. oleifera* leaves powder were taken by group one, group 2 and group 3, respectively. Prepared *M. oleifera* leaves powder was supplied to all subjects in 40 g plastic containers were taken to every one of patients. All subjects were asked to take this powder after breakfast regularly for 30 days.

2.5 Samples collection:

Two ml of venous blood samples were collected from each diabetic patients by laboratory staff of the hospital. The blood samples were centrifuged for 15 minutes at 1000 rpm, and then the top layers, which contain the serum, used for measuring glucose parameter before and after using of *M. oleifera* leaves powder.

2.6 Biochemical methods:

Kits used in biochemical measures of glucose, urea, creatinine, ALT and AST were obtained from biosystem laboratory products, these parameter were determined by Kinetic-Spectrophotometeric methods which were described by Young ,1997.

2.6.1Requirements for biochemical tests:

Spectrophotometer-Biosystem BTS 310 instrument, automatic pipettes 10-100 µl, yellow and blue tips, distill water (dH_2O) for wash and base line, biosystem kits, plastic rack, glass tubes and stop watch.

2.6.2 Estimation of serum glucose:

Principle:

The glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to a red-violet quinoneimine dye as indicator, a coloured complex that can be measured by spectrophotometry.

$$\text{Glucose} + {}^{1}/_{2}\,O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminofenazone} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Procedure:

The reagent was brought to room temperature. 1000 µl from reagent A (Phosphate 100mmol/L, phenol 5 mmol/L, glucose oxidase >10 U/ml, peroxidase > 1U/ml, 4-aminoantipyrine 0.4 mmol/L, pH 7.5) was pipetted into the test tube and 10 µl from sample was add, then the tube was mixed well and incubated for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C, the concentration of glucose standard already was calibrated and saved for further use was 100 mg/dl. Biosystem BTS-310 was prepared for use, the location of urea that mentioned in instrument sheet

could be changed. Filter of glucose was already calibrated in the instrument was 500 nm, the test sample was aspirated then the result was read directly from the instrument. **(**Reference range : 70-110 mg/dl).

2.6.3 Estimation of urea:

Principle:

Urea in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{Salicylate} + \text{NaClO} \xrightarrow{\text{nitroprusside}} \text{Indophenol}$$

Procedure:

The reagent was brought at room temperature. 1000 µl from reagent A (A1 + A2) was pipette into the test tube, and 10 µl from sample was added, the tube was mixed well and incubated for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C, then 1000 µl from reagent B (Sodium hypochlorite 7mmol/L, Sodium hydroxide 150 mmol/L) was pipetted into the same test tube, which was mixed well and incubated for 10 minutes at room temperature or for 5 minutes at 37°C. Urea standard (50 mg/dl) already was calibrated and saved for further reading. Biosystem BTS-310 was prepared for use, the filter of urea colour was already calibrated in the instrument was 600 nm. The tested sample was aspirated then the result was read directly from the instrument.

(Reference range: 6 – 20 mg/dl).

2.6.4 Estimation of creatinine:

Principle:

Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex. The complex formation rate is measured in a short period to avoid interferences.

Procedure:

The working reagent was brought at 37°C. 1000 µl from working reagent (mix equal volumes of reagent A and B) was pipette into the test tube, and 100 µl from sample was added, then test tube was mixed and aspirated immediately. Standard of creatinine (1.0 mg/dl) was already calibrated and saved for further use. Biosystem BTS-310 was prepared for use which include: enter location of creatinine test which was already determined and saved in the instrument, waited 5 minutes to bring the Biosystem BTS-310 at 37°C, washed with dH_2O then washed with air, enter Base line (dH_2O) which was acted as blank and at end pressed enter. The filter of creatinine colour was already calibrated in the instrument was 500 nm and the reading of concentration was obtained from instrument after 90 second.

Reference range: Male (0.9 - 1.3 mg/dl) Female (0.6 – 1.1 mg/dl)

2.6.5 Measurement of alanine aminotransferase (ALT):

Principle:

Alanine aminotransferase catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction.

$$\text{Alanine} + \text{2- Oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

Procedure:

In test tube was add 200 µl from reagent A (Tris 150 mmol/L, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L, pH 7.3) and800 µl from reagent B(NADH 1.3 mmol/L, 2-oxoglutarate 75 mmol/L, Sodium hydroxide 148 mmol/L Sodium azide 9.5 g/L), the ratio of reagent A to B was 1: 4. The instrument location of ALT was prepared and wait to bring instrument at

37°C, the filter of ALT (340 nm) was already calibrated, then the base line with D.W was inter. 50 µl from sample was pipetted in test tube and mixed well, which immediately was aspirated into the instrument, then the result was read from the screen of instrument after five minutes. Reference range: 6 – 37 U/L.

2.6.6 Measurement of Aspartate aminotransferase (AST):

Principle:

Aspatae aminotransferase catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction.

$$\text{Aspatate} + \text{2- Oxoglutarate} \longrightarrow \text{Oxalacetate} + \text{Glutamate}$$

$$\text{Oxalacetate} + \text{NADH} + H^{+} \xrightarrow{\text{MDH}} \text{Malate} + NAD^{+}$$

Procedure:

In test tube was add 200 µl from reagent A (Tris 121 mmol/L, L-aspartate 362 mmol/L, maltate dehydrogenase > 460 U/L, lactate dehydrogenase > 660 U/L, Sodium hydroxide 255 mmol/L, pH 7.8) and800 µl from reagent B (NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, Sodium hydroxide 148 mmol/L Sodium azide 9.5 g/L), the ratio of reagent A to B was 1: 4. The instrument location of AST was prepared and wait to bring instrument at 37°C, the filter of AST (340 nm) was already calibrated, then the base line with D.W was inter. 50 µl from sample was pipetted in test tube and mixed well, which immediately was aspirated into the instrument, then the result was read from the screen of instrument after five minutes. Reference range: 6 – 37 U/L.

CHAPTER THREE

Results and Discussion

3.1 Characteristics of Study:

The results showed mineral elements in *M. oleifera* powder leaves, and determined mineral elements concentrations that existed in leaves tissues. 83 volunteers diabetic patients(37 man and 46 woman) choose to evaluate effect of *M. oleifera* powder leaves on blood sugar, and investigated side effect for taken *M. oleifera* powder leaves on liver enzymes and kidney, the results showed measurement of biochemical parameters in the patients before and after taken *M. oleifera* leaves powder for a month.

3.2 Determination of mineral elements content in *M. oleifera* leaves:

The results of this study showed that concentrations for the same mineral element in *M. oleifera* leaves powder was difference when using different analytical methods (AAS and ICP).

3.2.1 Using Atomic Absorption Spectrophotometer:

Results showed *M. oleifera* leaves powder contains essential mineral elements with different concentrations. Mineral elements concentrations by using (AAS) decrease in the order Ca (585.10ppm) > Mg (117.0ppm) > K (62.39ppm) > Na (24.05ppm) > Fe (22.94ppm) > Zn (1.854ppm) > Mn (1.015ppm) > Cu (0.336ppm) Cr (0.017ppm) Pb and Ni not detected. Pb and Ni not detected (Table 5).

Table (5): The mineral elements content in 1.00g of *M. oleifera* leaves powder by using (AAS):

Mineral elements	Concentrations (ppm)
Ca	585.10
Mg	117.10
K	62.39
Na	24.05
Fe	22.94
Zn	1.854
Mn	1.015
Cu	0.336
Cr	0.017
Ni	N.D
Pb	N.D

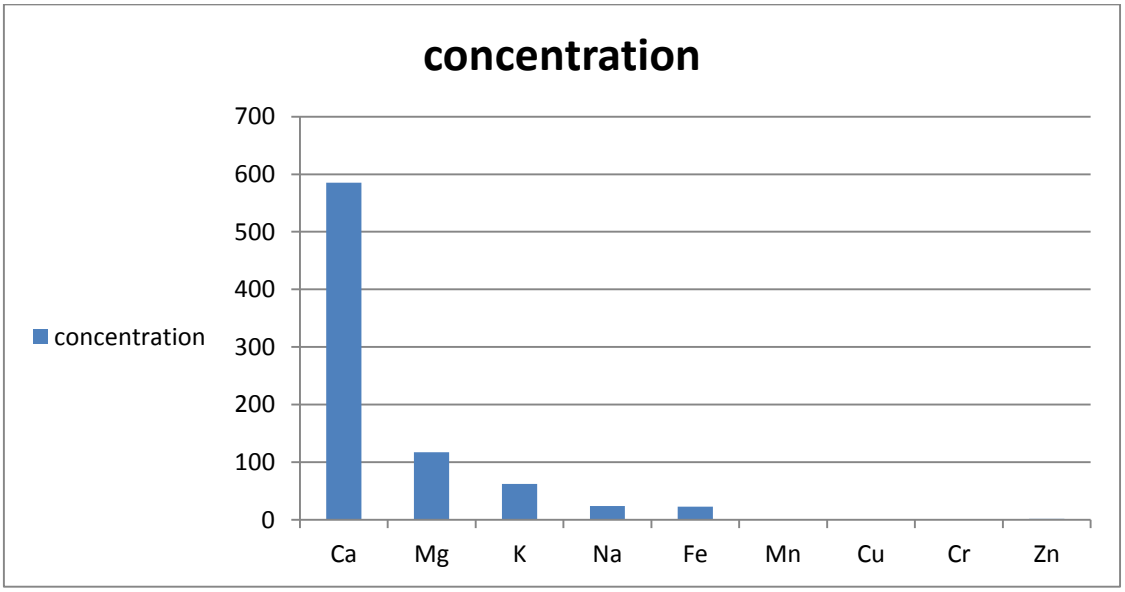

Fig 9: Concentrations of mineral elements using (AAS).

3.2.2 Using Inductively Coupled Plasma:

Table (6) presented concentrations of mineral elements in *M. oleifera* leaves powder using (ICP). Concentrations of mineral elements decrease in the order: Ca (4656ppm) > Mg (14140ppm) > K (12620ppm) > Al (1491ppm) > Fe (1401ppm) > Na (913ppm) > Si (526.00ppm) > Sr (113.30ppm) > Ti (71.65ppm) > Mn (61.69ppm) > Ba (14.78ppm) > Zn (13.76ppm) > Cu (13.43ppm) > Li (7.128ppm) > Mo (4.88ppm) > V (3.342ppm) > Cr (2.086ppm) > Ni (1.919ppm) > Pb (1.547ppm) > Be (0.0207ppm).

Table (6) The mineral elements content in 1.00g of *M. oleifera* leaves powder by using (ICP):

Mineral elements	Concentrations (ppm)
Ca	46560
Mg	14140
K	12620
Al	1491
Fe	1401
Na	913
Si	526
Sr	113.3
Ti	71.65
Mn	61.69
Ba	14.78
Zn	13.76
Cu	13.43
Li	7.128
Mo	4.88
V	3.342
Cr	2.086
Ni	1.919
Pb	1.547
Be	0.0207

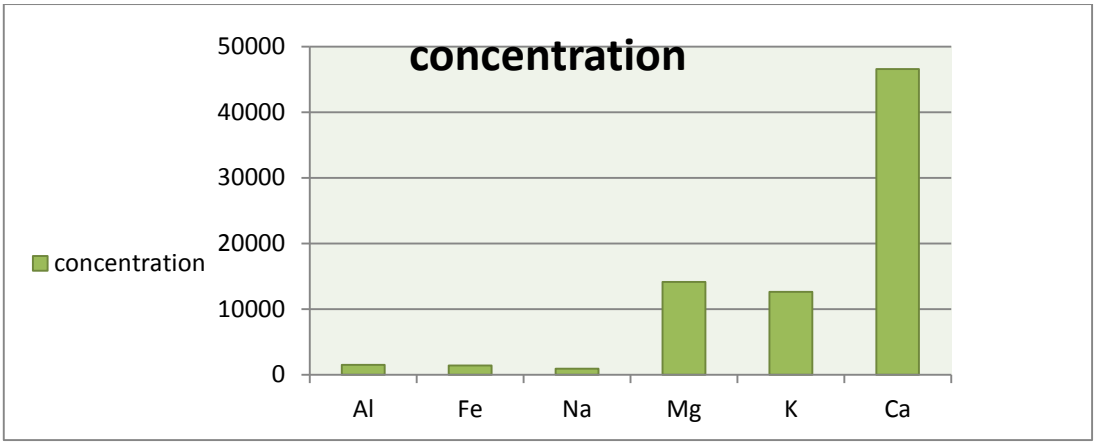

Fig 10: Concentrations of mineral elements(of high conc.) using (ICP).

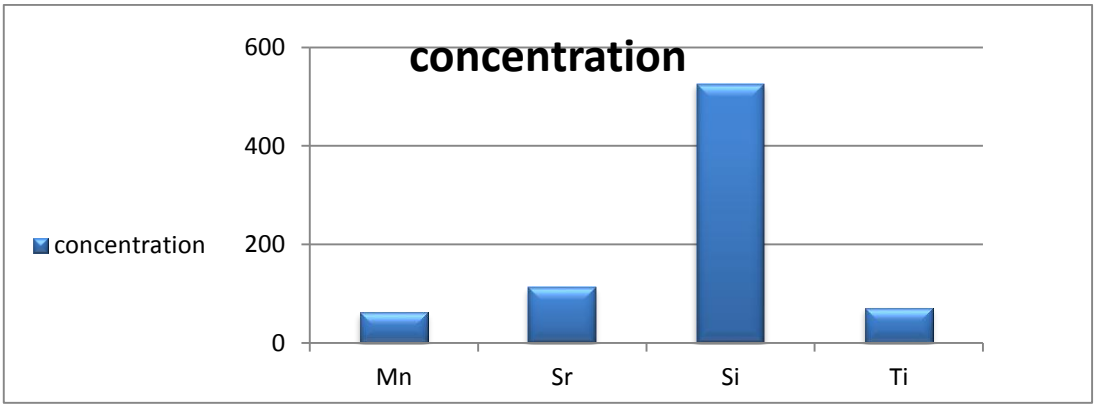

Fig 11: Concentrations of mineral elements(of medium conc.) using (ICP).

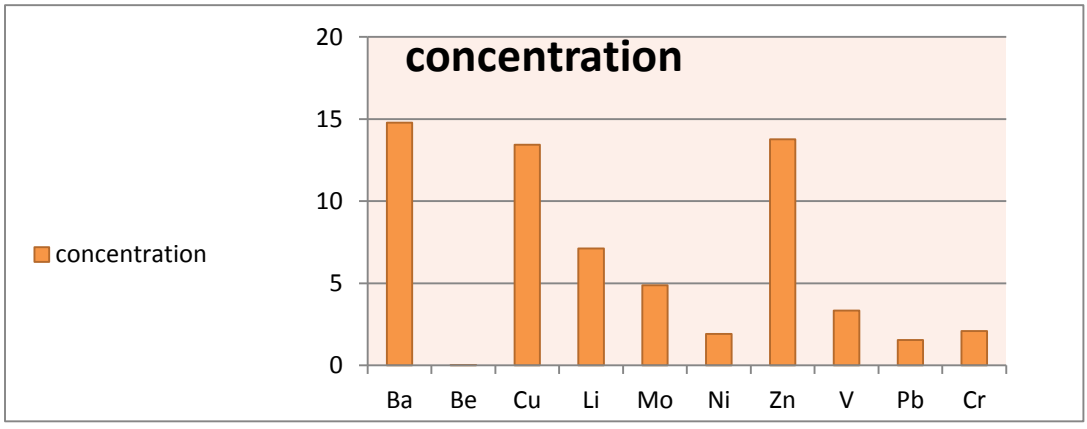

Fig 12: Concentrations of mineral elements(of trace conc.) using (ICP).

3.2.3 Using ICP (Microwave digestion of plant tissue in an open vessel):

Table (7) showed the average values of mineral elements concentrations in *M. oleifera* leaves powder by using(ICP)(leaves powder digested with microwave). Concentrations of mineral elements decrease in the order: Ca (54450ppm) > K (16370ppm) > Mg (16250ppm) > Al (1426ppm) > Fe (1402ppm) > Si (1804ppm) >Na (960.6ppm) > Sr (118.3ppm) > Mn (76.52ppm) > Ti (25.95ppm) > Zn (19.21ppm) Ba (17.21ppm) > Cu (10.54ppm) > Pb (6.463ppm) > Mo (5.436ppm) > Li (4.190ppm) > V (3.022ppm) >Cr (2.484ppm) > Ni (2.081) > Be (0.0257ppm).

Table (7) The mineral elements content in 1.00g of *M. oleifera* leaves powder by using ICP (microwave):

Mineral elements	Concentrations (ppm)
Ca	54450
K	16370
Mg	16250
Al	1426
Si	1804
Fe	1402
Na	960.6
Sr	118.3
Mn	76.52
Ti	25.95
Zn	19.21
Ba	17.21
Cu	10.54
Pb	6.463
Mo	5.436
Li	4.190
V	3.022
Cr	2.484
Ni	2.081
Be	0.0257

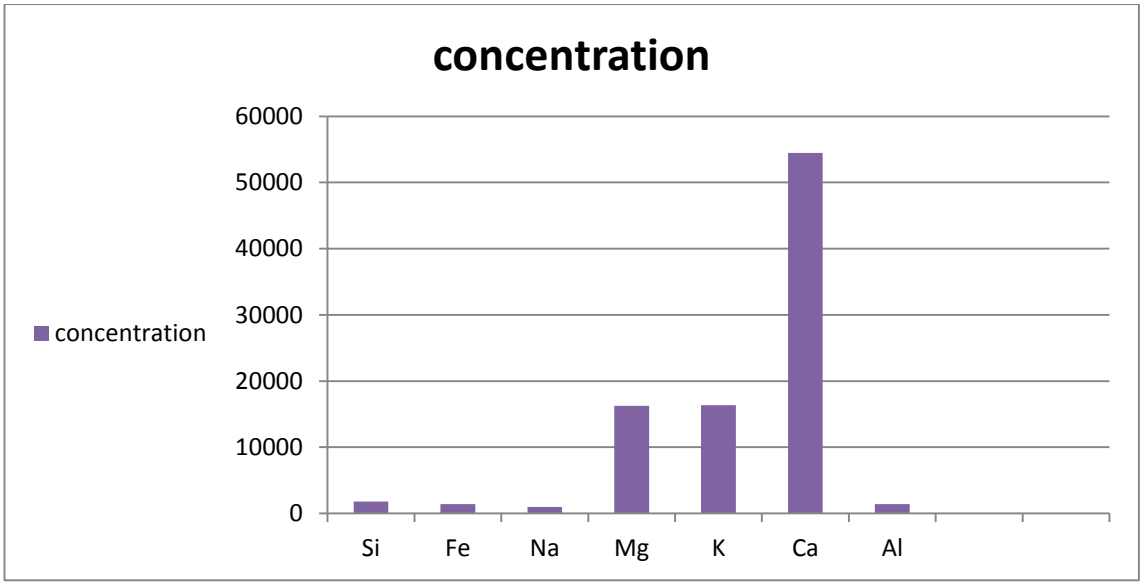

Fig 13: Concentrations of mineral elements(of high conc.) using (ICP) with Microwave.

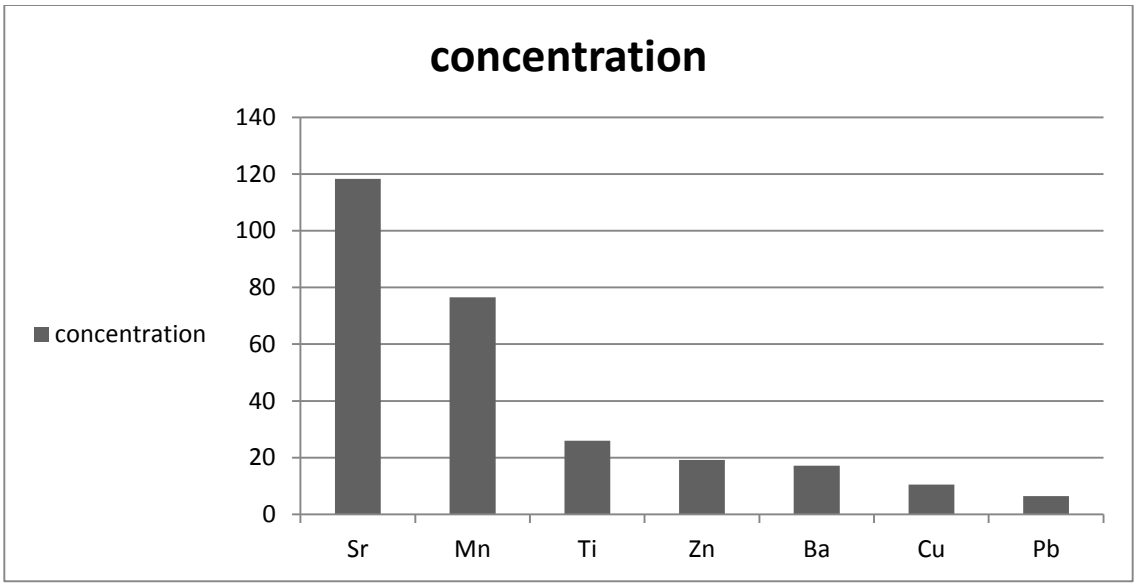

Fig 14: Concentrations of mineral elements(of medium conc.) using (ICP) with Microwave.

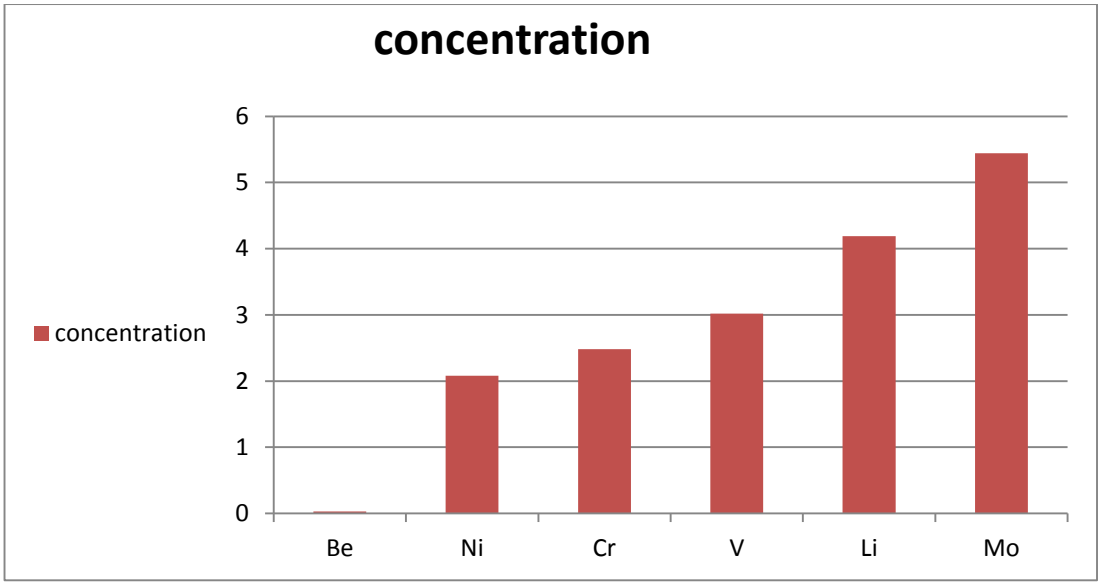

Fig 15: Concentrations of mineral elements(of trace conc.) using (ICP) with Microwave.

Results of study conducted in several other countries. Fakankun *et al*., 2013 study mineral composition and some heavy metal contents in different parts of *M. oleifera*. Parts of the plant were obtained from Badagry in Lagos State, Nigeria. Analyzed for the mineral element content using atomic absorption spectrophotometer. The observed mean concentrations of the mineral elements were 26000, 643, 8210, 2980, 69.9, 169 and 15.3 mg/kg for Ca, Mg, K, Na, Mn, Fe and Zn, respectively. Co and Se and the heavy metals Pb and Cd were not detected. There seemed to be no significant differences in the overall levels of the mineral elements in the different parts (P> 0.05). Journal of Food and Nutrition Sciencese, 2015 evaluated the mineral elements in *M. oleifera* leaves powder collected from supermarket in Mekelle according to (ICP-MS) Ca (2016.5–2620.5 mg/100 g), K (1817–1845 mg/100 g), and Mg (322.5–340.6 mg/100 g). Concluded that *M. oleifera* could be employed in edible and commercial application. (C. Limmatvapirat *et al*., 2015) determined eleven heavy metals in *M. oleifera* products using (ICP-AES). For various products from *M. oleifera* the average concentrations of heavy metals decrease in the order: Al (255.718

mg/kg)>Fe (247.608 mg/kg)>Zn (78.373 mg/kg)>Mn (73.135 mg/kg)>Ni (26.408 mg/kg)>Cu (8.54 mg/kg)>Pb (2.449 mg/kg)>Cr (1.164 mg/kg)>As (0.362 mg/kg)>Cd (0.122 mg/kg)>Hg (0.087 mg/kg). Among *M. oleifera* products, tea leaves had the highest average concentrations of As (0.509 mg/kg), Hg (0.142 mg/kg), Cr (1.597 mg/kg), and Ni (43.6313 mg/kg) while leaves capsules had the highest average concentrations of Cd (0.188 mg/kg), Pb (4.681 mg/kg), Cu (12.176 mg/kg), Fe (414.576 mg/kg), Al (354.351 mg/kg), and Mn (109.536 mg/kg). Moreover, leaves powders had the highest average concentration of Zn (92.778 mg/kg). (Mulyaningsih, and Yusuf,, 2018) determine the mineral content in the leaves of the *M. oleifera* taken from Indonesia. Mineral content in the samples is determined using Neutron Activation Analysis (NAA). The results obtained *M. oleifera* is rich in essential minerals Ca, Mg, K, Zn, Fe and Cl. Content in dried leaves include: calcium (3.45 %), magnesium (0.66 %), potassium (3.35 %), chloride (0.25%), iron (147.20 mg/kg), sodium (152.52 mg/kg), zinc (35.71 mg/kg), and manganese (102.10 mg/kg). *M. oleifera* also contains other minerals such as chromium (4.76 mg/kg), bromine (4.82 mg/kg), cobalt (0.16 mg/kg), and aluminium (150.40 mg/kg). The results of the proximate and mineral analyses of the whole leaves extract revealed the presence of appreciable amount of nutrients in leaves of *M. oleifera* which is in line with the observations of Krishnaiah *et al*., (2009) and A. O. Oluduro, (2012). This proves why leaves of this plant are used as food supplement and essential for infants and nursing mothers (Krishnaiah *et al*., 2009., A. O. Oluduro, 2012). Compared with the results of existing studies, it shows that mineral composition in *M. oleifera* leaves varies depending on the location where the plant is grown.

3.3 The effects of *M. oleifera* leaves powder on the blood sugar in humans:

Blood sugar levels for all diabetic patients is decreased after taking *M. oleifera* leaves powder for a month. The high percentage of the decrease (59%) is observe in young diabetic patients who medicated with insulin, and low percentage of the decrease (5%) is observe in young diabetic patients who medicated with tablets. For the men high percentage of the decrease is (54%), and low percentage of the decrease is (5%). For the women high percentage of the decrease is (59%), and low percentage of the decrease is (11%), so percentage of the decrease for the women is double of the percentage of decrease for the men (table 8).

Table (8) Measurement of blood sugar levels two hours after breakfast for all patients before and after taking (1.00g) *M. oleifera* leaves powder:

Sex	Age	Drugs	Blood sugar before uses (mg/dl)	Blood sugar after uses (mg/dl)	Percentage of decrease %
Man	40	tablets	259	120	54
Man	41	tablets	190	180	5
Man	45	insulin	161	80	50
Man	45	insulin	300	161	46
Man	54	insulin	300	140	53
Man	55	tablets	166	125	25
Man	58	tablets	320	204	36
Man	65	tablets	224	164	27
Man	67	tablets	193	175	9
Woman	22	insulin	255	131	49
Woman	26	tablets	254	122	52
Woman	36	insulin	165	80	52
Woman	43	tablets	186	136	27
Woman	45	insulin	211	172	19
Woman	45	insulin	390	162	59
Woman	46	insulin	287	177	38
Woman	48	tablets	241	148	39
Woman	50	insulin	396	260	34
Woman	50	tablets	225	171	24
Woman	50	insulin	270	160	41
Woman	52	tablets	201	179	11
Woman	55	tablets	224	181	19
Woman	57	tablets	272	235	14
Woman	57	tablets	250	180	28
Woman	57	insulin	265	141	47
Woman	60	insulin	293	245	16
Woman	75	tablets	313	155	51

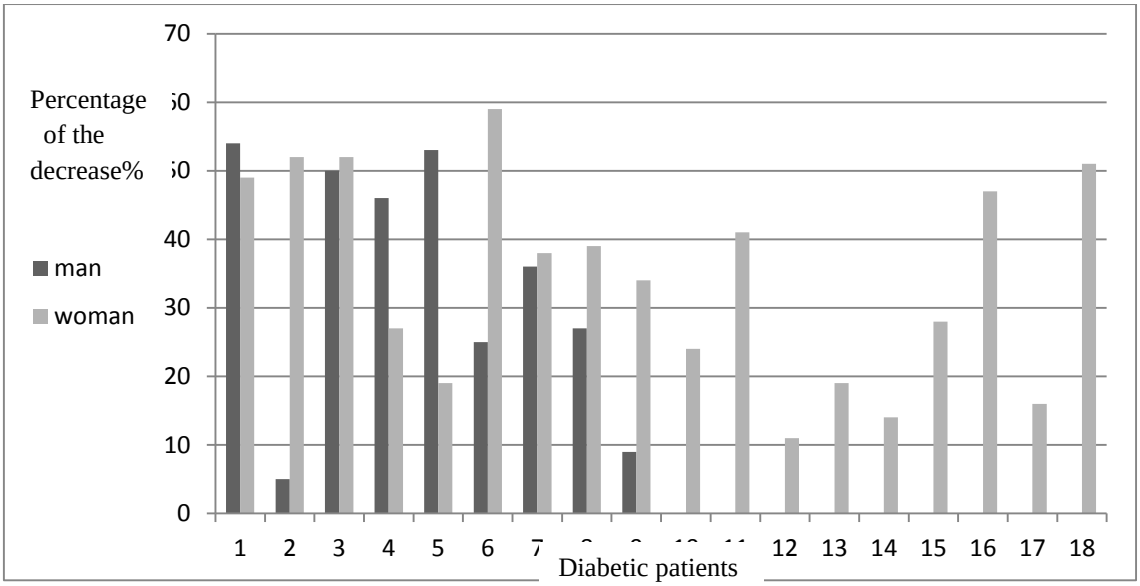

Fig 16: Percentage of the decrease for the men compared to the women (after taking 1.00g).

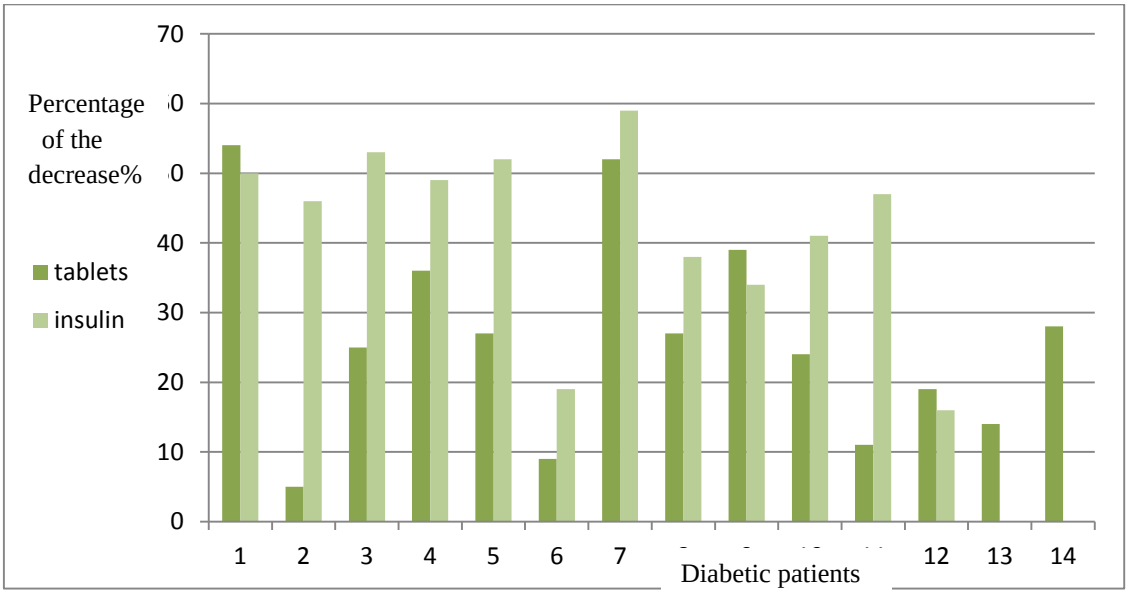

Fig 17: Percentage of the decrease for patients who medicated with tablets compared to patients who medicated with insulin(after taking 1.00g).

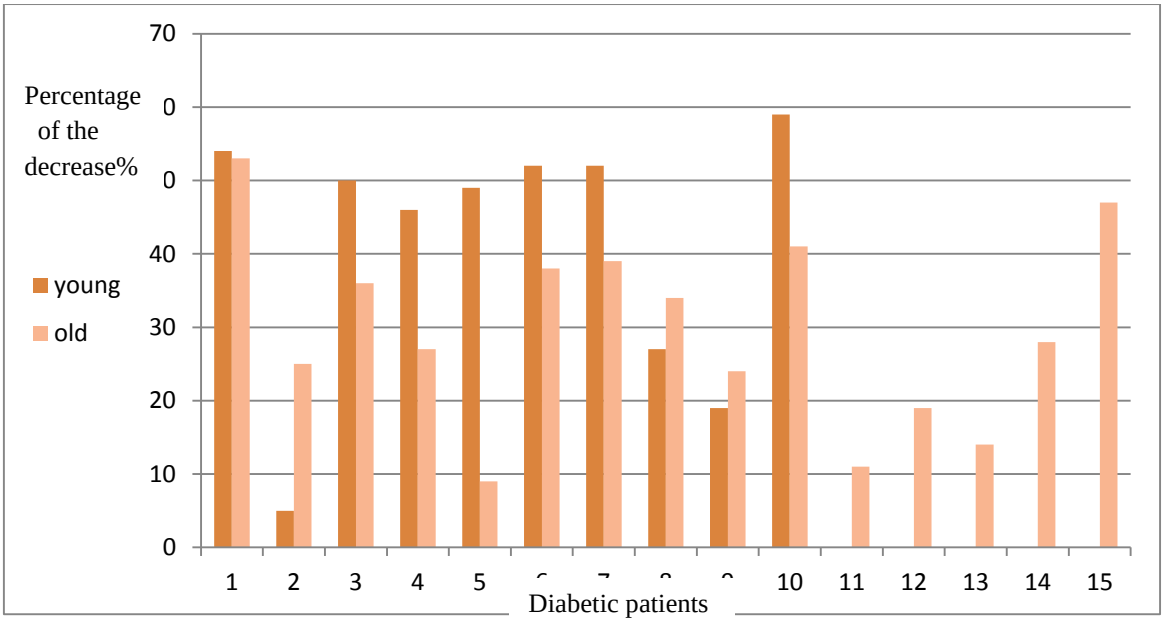

Fig 18: Percentage of the decrease for young diabetic patients compared to old (after taking 1.00g).

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The results showed decrease on blood glucose for all diabetic patients. The high percentage of the decrease (63%) is observe in young diabetic patients who medicated with insulin, and low percentage of the decrease (2%) is observe in old diabetic patients who medicated with tablets. The high percentage of the decrease for the men is (51%), and low percentage of the decrease is (2%). For the women the high percentage of the decrease is (63%), and low percentage of the decrease is (10%), so percentage of the decrease for the men is lowest than percentage of the decrease for the women (table 9).

Table (9) Measurement of blood sugar levels two hours after breakfast for all patients before and after taking (0.5g) *M. oleifera* leaves powder:

Sex	Age	Drugs	Blood sugar before taking (mg/dl)	Blood sugar after taking (mg/dl)	Percentage of decrease %
Man	19	insulin	240	150	38
Man	45	tablets	200	154	23
Man	45	insulin	318	224	28
Man	45	tablets	175	130	26
Man	47	insulin	388	190	51
Man	48	insulin	312	280	10
Man	50	tablets	195	152	22
Man	51	insulin	290	220	24
Man	52	tablets	222	216	2
Man	57	tablets	203	148	27
Man	58	insulin	200	183	8
Man	60	tablets	188	166	12
Man	62	tablets	272	202	26
Man	66	insulin	333	215	35
Man	70	tablets	200	120	40
Man	73	tablets	216	145	33
Woman	25	tablets	300	251	16
Woman	29	tablets	358	200	44
Woman	35	insulin	200	154	23
Woman	35	insulin	216	81	63
Woman	35	insulin	340	188	45
Woman	39	insulin	270	200	26
Woman	41	tablets	300	266	11
Woman	44	tablets	400	190	51
Woman	48	tablets	200	180	10
Woman	50	tablets	170	150	10
Woman	50	tablets	243	145	40
Woman	56	insulin	340	225	34
Woman	62	insulin	213	142	33
Woman	66	tablets	225	114	49
Woman	70	tablets	240	170	29

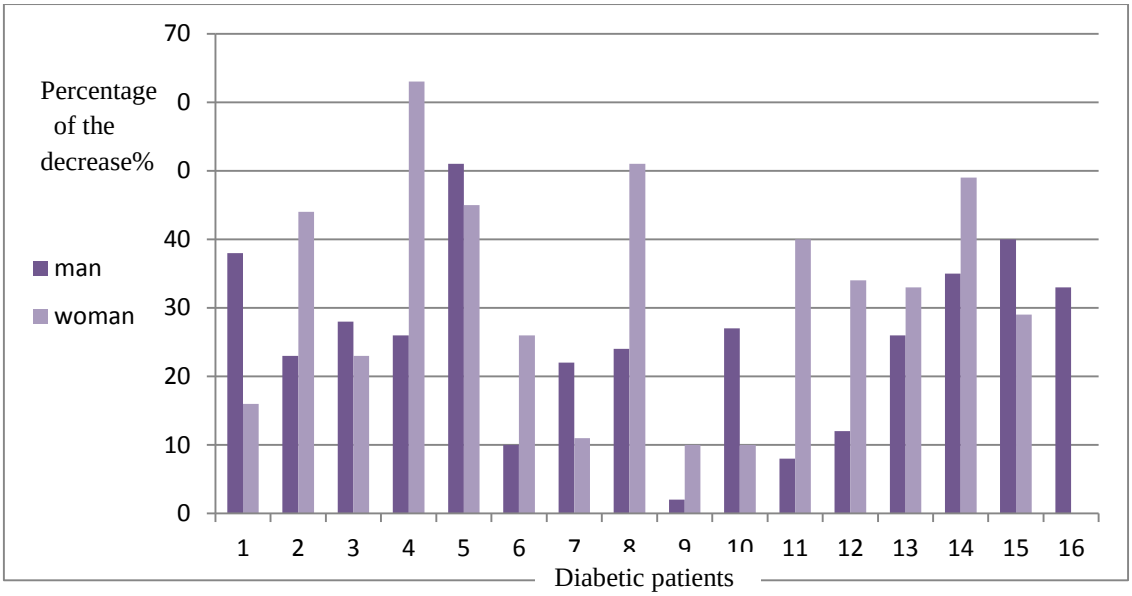

Fig 19: Percentage of the decrease for the men compared to the women (after taking 0.5g).

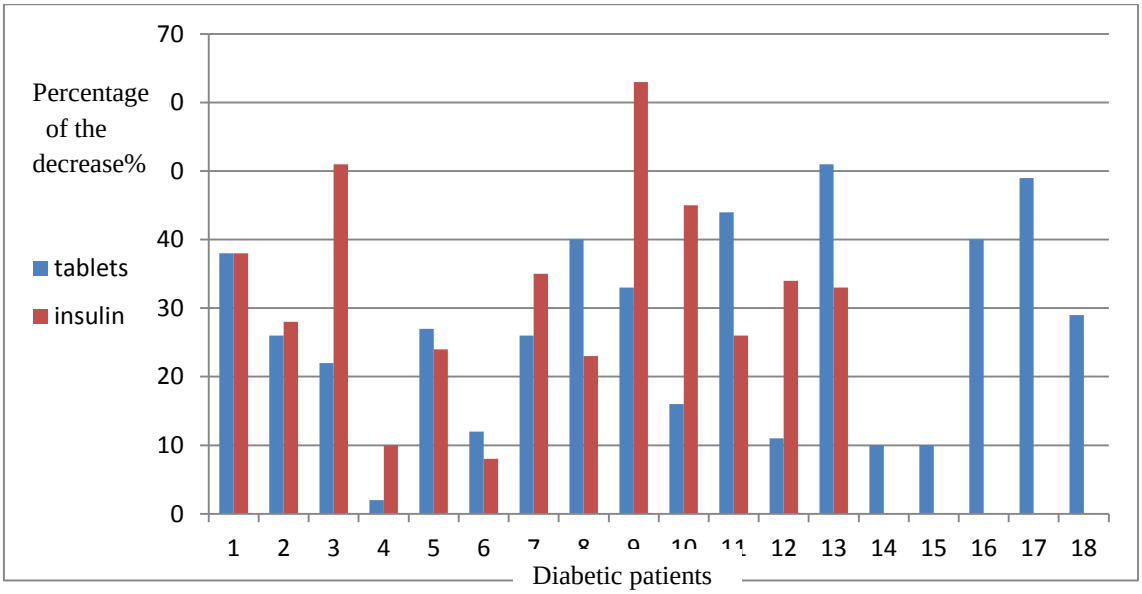

Fig 20: Percentage of the decrease for patients who medicated with tablets compared to patients who medicated with insulin(after taking 0.5g).

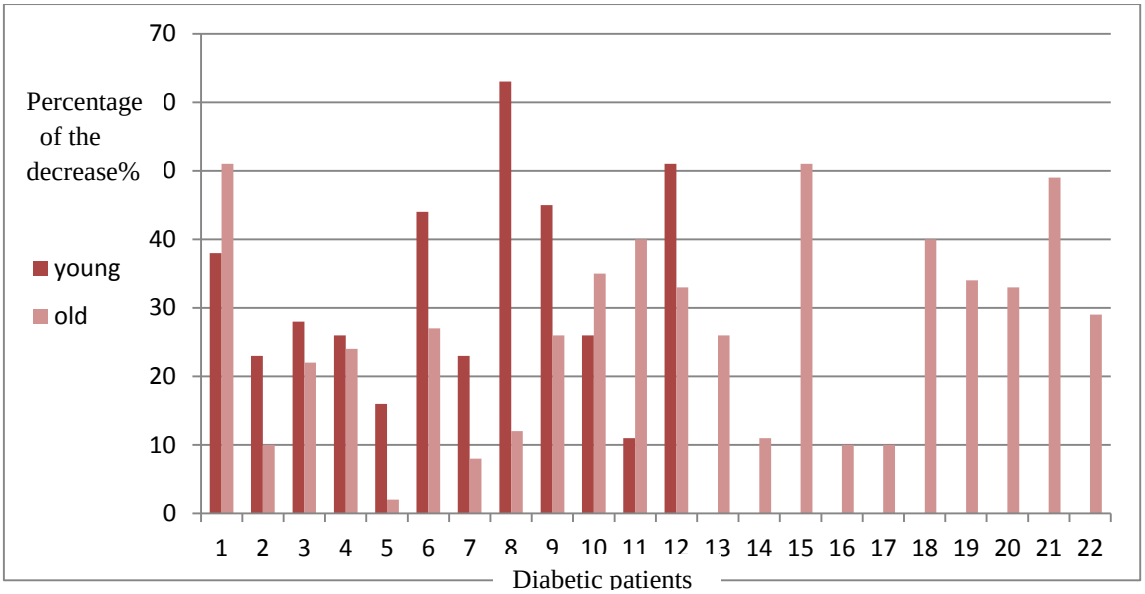

Fig 21: Percentage of the decrease for young diabetic patients compared to old (after taking 0.5g).

The results showed decrease on blood glucose for large number of diabetic patients, but there are three diabetic patients no decrease on their blood glucose. The high percentage of the decrease (51%) is observe in young diabetic patients who medicated with tablets, and low percentage of the decrease (0%) is observe in young and old diabetic patients who medicated with tablets. The high percentage of the decrease for the men is (50%), and low percentage of the decrease is (2%). For the women the high percentage of the decrease is (51%), and low percentage of the decrease is (0%), so percentage of the decrease for the women is lowest than percentage of the decrease for the men (Table 10).

Table (10) Measurement of blood sugar levels two hours after breakfast for all patients before and after taking (0.25g) *M. oleifera* leaves powder:

Sex	Age	Drugs	Blood sugar before taking (mg/dl)	Blood sugar after taking (mg/dl)	Percentage of decrease%
Man	45	tablets	203	100	50
Man	48	tablets	226	196	13
Man	55	insulin	172	168	2
Man	55	tablets	270	180	33
Man	56	tablets	241	193	20
Man	57	tablets	239	172	28
Man	58	insulin	200	183	9
Man	60	tablets	217	183	16
Man	60	tablets	222	217	2
Man	60	insulin	258	241	7
Man	62	tablets	369	300	19
Man	65	tablets	152	132	13
Woman	27	tablets	137	140	0
Woman	40	tablets	283	140	51
Woman	43	tablets	210	120	43
Woman	47	tablets	196	100	49
Woman	48	tablets	234	225	3
Woman	50	insulin	253	200	21
Woman	50	insulin	326	183	44
Woman	50	insulin	207	110	47
Woman	53	tablets	178	178	0
Woman	57	tablets	300	222	26
Woman	60	insulin	246	217	12
Woman	60	insulin	300	203	32
Woman	70	insulin	300	160	47

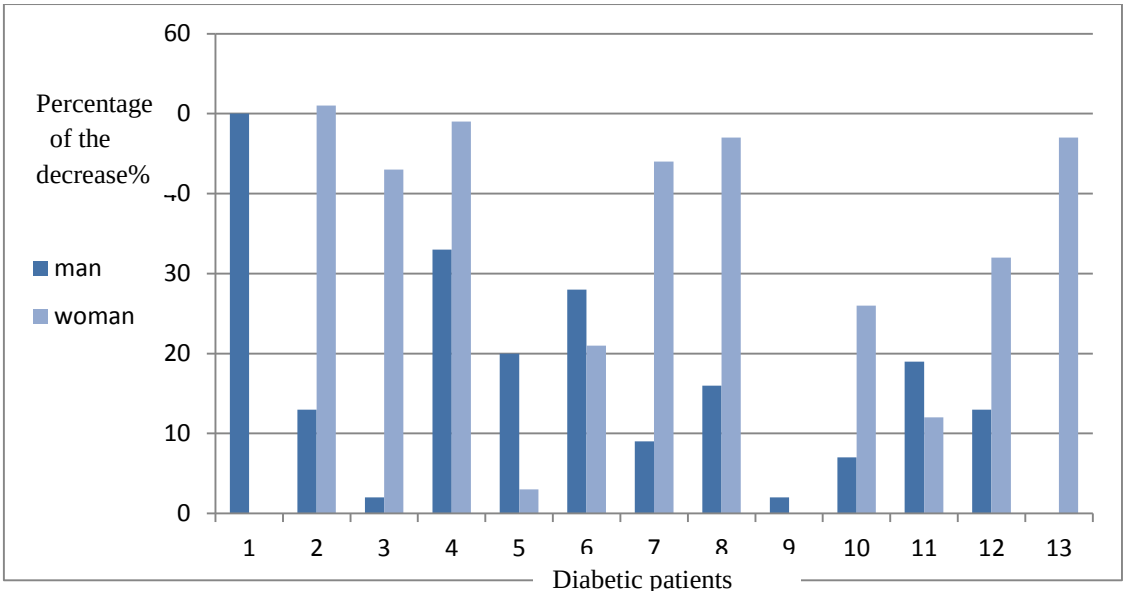

Fig 22: Percentage of the decrease for the men compared to the women (after taking 0.25g).

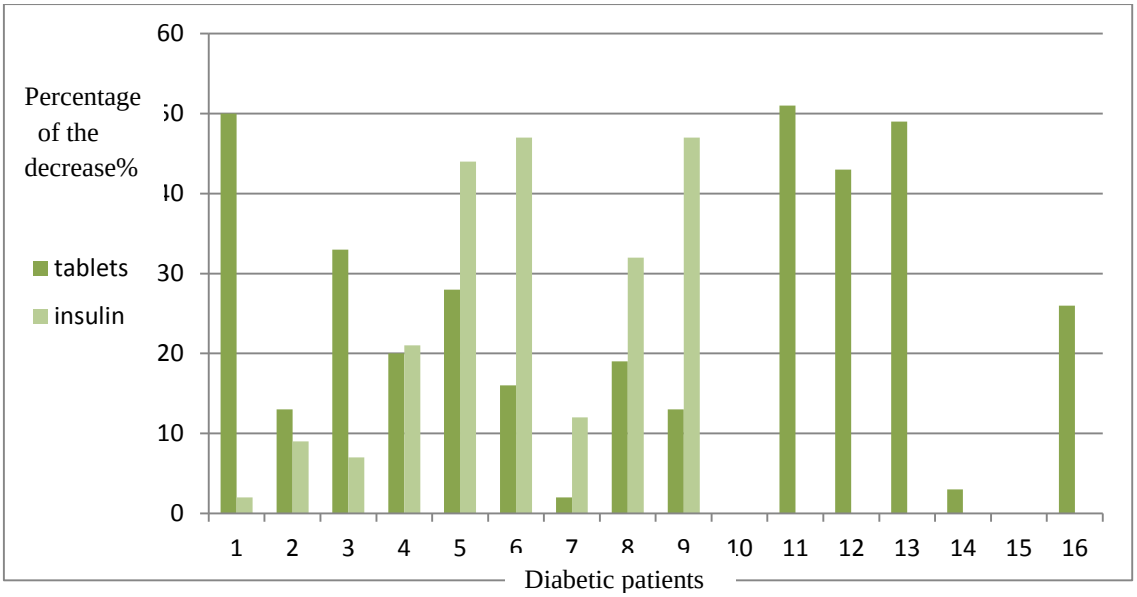

Fig 23: Percentage of the decrease for patients who medicated with tablets compared to patients who medicated with insulin(after taking 0.25g).

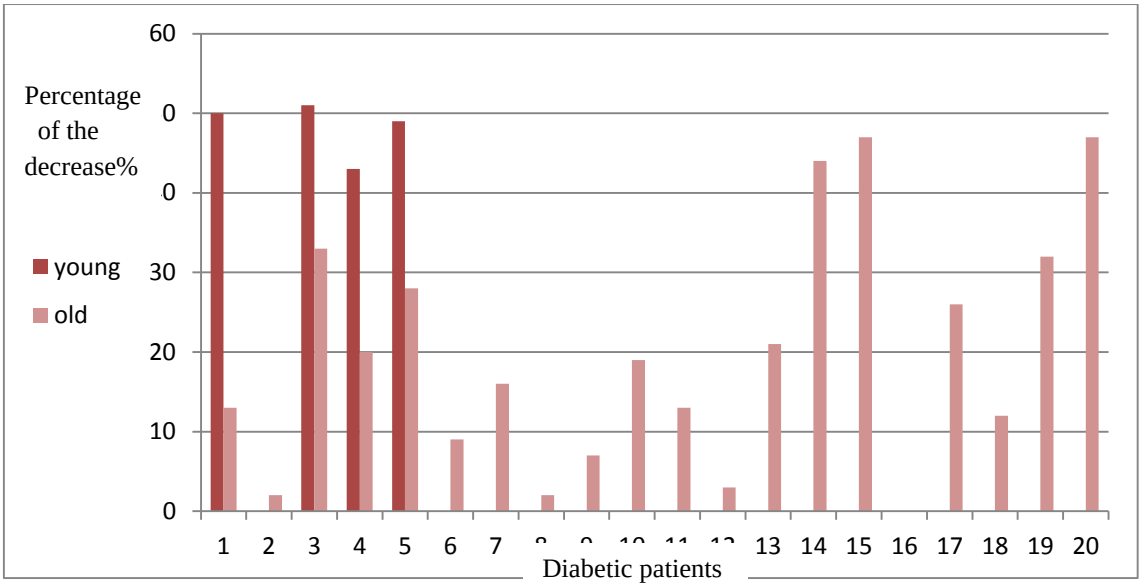

Fig 24: Percentage of the decrease for young diabetic patients compared to old (after taking 0.25g).

The results showed the high percentage of the decrease on blood glucose for men when taking 1.00g is (54%), when taking 0.5g is (51%) and when taking 0.25g is (50%), so the percentage of the decrease on blood glucose increased when dose increased. And the high percentage of the decrease on blood glucose for women when taking 1.00g is (52%), when taking 0.5g is (63%) and when taking 0.25g is (51%), . The percentage of the decrease on blood glucose increased when taking 0.5g, so the suitable dose to consume to reduce blood glucose of diabetic patients is 0.5g (tables 8, 9and10).

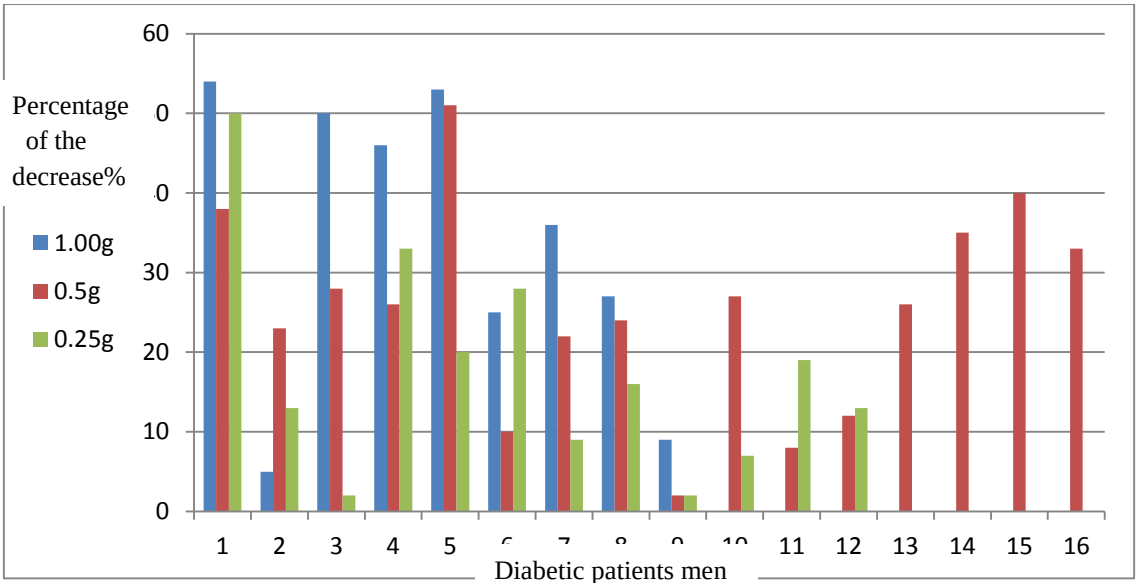

Fig 25: Percentage of the decrease for men when taking 1.00, 0.5 and 0.25g.

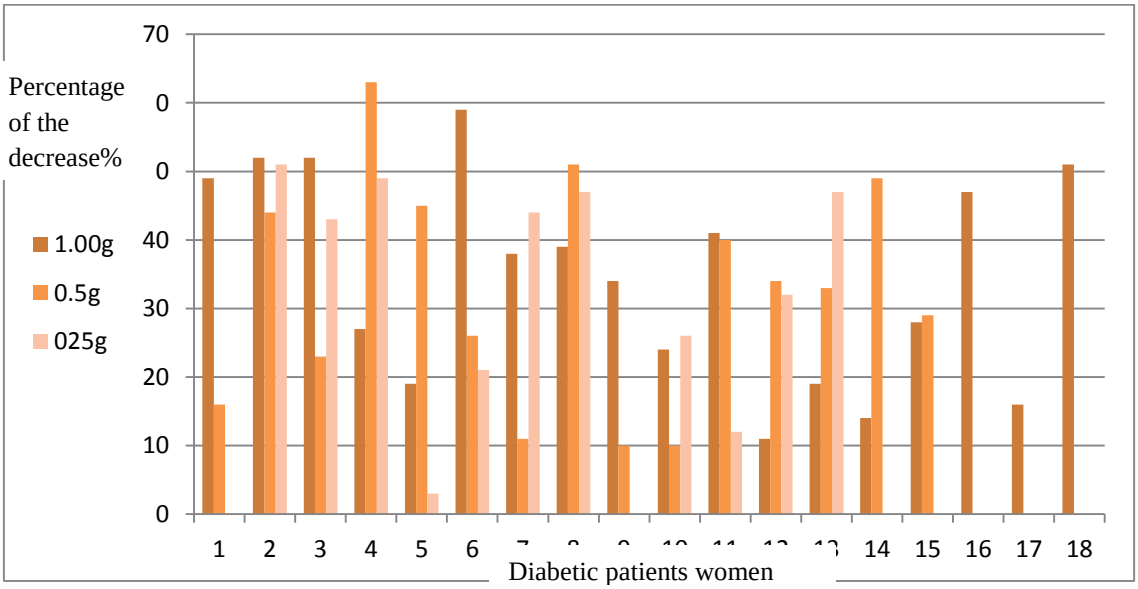

Fig 26: Percentage of the decrease for women when taking 1.00, 0.5 and 0.25g.

3.4 Statistical Analysis for change on blood sugar levels:

The following statistical tools were used in this study for analysis and interpretation of data (Rangaswamy, 1995): Independent T-Test. Statistical analysis compared rate of decreased on blood sugar levels for male against female, young against old, type of drugs and quantity of doses, before and after taking *M. oleifera* leaves powder.

3.4.1 Effect of *M. Oleifera* leaves powder on blood sugar for diabetic patients:

Results indicating *M. oleifera* leaves powder effect on blood sugar. These values showed significant decrease on blood sugar ($p<0.001$). The mean of glucose for diabetes patients before taking of *M. oleifera* (242.58mg/dl ± 6.91), and after taking (170.12mg/d l ± 4.91) (Table 11).

Table (11) Effect of *M. Oleifera* leaves powder on blood sugar in diabetic patients (Mean ± SE):

	Mean of glucose
Before	242.58mg/dl ± (6.91)
After	170.12mg/dl ± (4.91)
P-value	0.000

3.4.2 Effect of *M. Oleifera* leaves powder on blood sugar according to age:

The mean values of blood sugar showed significantly lowering on blood sugar levels ($p<0.001$) after uses *M. oleifera* leaves powder for two groups of young and old diabetic patients. The effect of *M. oleifera* for young patients the mean values (before taking 240.82mg/dl ± 13.92 and after taking 154.11mg/dl ± 8.89) showed significant decrease on blood sugar levels

($p<0.05$) compared to the old patients (before taking 243.46mg/dl ± 7.78 and after taking 178.12mg/dl ± 5.63) (Table 12).

Table (12) Effect of *M. Oleifera* on blood sugar according to age (Mean ± SE):

	Young	Old	P. value
Before	240.82mg/dl ± (13.92)	243.46mg/dl ± (7.78)	0.858
After	154.11mg/dl ± (8.89)	178.12mg/dl ± (5.63)	0.020
P.value	0.000	0.000	

Age range: 20-45(young) , > 45(old)

3.4.3 Effect of *M. oleifera* leaves powder on blood sugar according to sex:

The effect of *M. oleifera* in blood sugar according to sex is presented in table (13). The mean values showed significantly lowering in both sex ($p<0.001$). The results showed no significant difference on blood sugar levels after taking *M. oleifera* leaves powder in male compared to female.

Table (13) Effect of *M. Oleifera* on blood sugar according to sex (Mean ± SE):

	Male	Female	P. value
Before	227.59mg/dl ± (11.51)	249.68mg/dl ± (8.51)	0.137
After	167.81mg/dl ± (9.07)	171.21mg/dl ± (5.87)	0.749
P.value	0.000	0.000	

3.4.4 Effect of *M. Oleifera* leaves powder on blood sugar according to medication:

The mean values showed high significant effect of *M. oleifera* on blood sugar levels ($p<0.001$) in two groups of patients. The mean values of blood sugar levels for patients who taking tablets (229.00mg/dl ± 7.85) was reduce to (167.96mg/dl ± 5.71), and for patients who taking insulin (262.56mg/dl ± 11.92) was reduce to (173.29mg/dl ± 8.85), but no significant difference on rate of decrease on blood sugar for patients who taking tablets compared to patients who taking insulin(Table14).

Table (14) Effect of *M. Oleifera* on blood sugar according to medication (Mean ± SE):

	Insulin	Tablets	P. value
Before	262.56mg/dl ± (11.92)	229.00mg/dl ± (7.85)	0.016
After	173.29mg/dl ± (8.85)	167.96mg/dl ± (5.71)	0.597
P. value	0.000	0.000	

3.4.5 Effect of *M. Oleifera* leaves powder on blood sugar according to the difference of doses:

The results showed decreased on blood sugar levels after used *M. oleifera* powder leaves for patients who taking 0.25, 0.50 and 1.00g. The mean values for all doses showed high significant effect of *M. oleifera* on blood sugar levels ($p<0.001$).The mean values for patients who taking 1.00g showed high decreased on blood sugar levels (before taking *M. oleifera* 252.85mg/dl ± 11.70 , and after taking 162.37mg/dl ± 8.13) compared to patients who taking 0.5 and 0.25g, but no significant difference in the mean values of blood sugar

levels for three groups of patients after used *M. oleifera* leaves powder (Table 15).

Table(15) Effect of *M. Oleifera* on blood sugar according to the difference of doses (Mean ± SE):

	0.25g	0.50g	1.00	P value
Before	237.96mg/dl ± (11.49)	237.52mg/dl ± (12.58)	252.85mg/dl ± (11.70)	0.598
After	180.46mg/dl ± (9.90)	168.19mg/dl ± (7.60)	162.37mg/dl ± (8.13)	0.332
P value	0.000	0.000	0.000	

The results showed a high significant decrease in serum glucose for all groups of diabetic patient**s (**$p<0.001$) after consumed *M. oleifera* leaves powder for a month. This may be due to the higher dietary fiber content present in the *M. oleifera* leaves powder-supplemented. The above studies pointed out that antioxidants like carotenoids, vitamins C and E, and flavonoids had an important role in reducing the blood glucose of the diabetic patients. (Kumar and Mandapaka, 2013) concluded when *M. oleifera* powder administered with the food, serum glucose levels were decreased. It was observed that the percentage decrease in serum glucose levels was 8.9 % (134.33-122.33 mg per 100 ml of serum). The glucose -lowering action of the *M. oleifera* leaves powder was found to be significant ($P < 0.05$) in serum, the study validates scientifically the widely claimed use of *M. oleifera* as an ethno medicine to treat diabetes mellitus. (Dangi *et al*., 2002., Ghasi *et al*., 2000) reported the leaves of *M. oleifera* to be used as a hypo cholesterolemic agent, and hypoglycemic agent. Results in present study

were observed in above studies. The results showed no significant difference on blood sugar levels after used *M. oleifera* leaves powder in male compared to female. This agreed with (Ghiridhari *et al*., 2011) indicated that did not show any deviation between the male and female of the feeding groups both male and female volunteers, blood glucose level had reduced after the administration of *M. oleifera* leaves tablet it concluded that *M. oleifera* leaves tablet have a significant impact on anti diabetic property of the selected patients. Results showed a significantly reduced glucose in both groups of patients, so the effect of *M. oleifera* leaves on blood sugar did not effected with age and this similar to Kumari, 2010 studied hopoglycemic effect of *M. oleifera* leaves (8 g/day) dietary consumption in a 40-day period in type 2 diabetic patients 30-60 years of age with no medication was studied and showed a significantly reduced glucose response as compared to the patients not given *M. oleifera* leaves. The results showed high significant decrease on blood sugar for all groups of diabetic patients when they taken difference doses of *M. oleifera* leaves powder. According to Dandona *et al*., (1996) observed inverse relation between vitamin C and vitamin E and hemoglobin glycation, this content in 1.00g and 0.5g highest than 0.25g, so patients who taking 1.00g and 0.5g showed high decrease on blood sugar levels (before taking 1.00g *M. oleifera* 252.85mg/dl ± 11.70, and after taking 162.37mg/dl ± 8.13), (before taking 0.5g *M. oleifera* 237.52mg/dl ± 12.58, and after taking 168.19mg/dl ± 7.60) compared to patients who taking 0.25g (before taking *M. oleifera* 237.96mg/dl ± 11.49, and after taking 180.46mg/dl ± 9.90). This is similar to Keenoy *et al.*, 1999 presented the effect of supplementation of flavonoid based antioxidant medication to 28 diabetic patients while following a standardized 1.800 - 2.000 calorie diet. Results showed decrease in initial and final values of glycated hemoglobin was significant in experimental group, whereas in control group the decline was non-significant. The above study stated that supplementation of

antioxidants and flavonoids control the glycation process, so decrease in glycated hemoglobin was seen in experimental group. Similar observation was found in present study, so group 2 and 3 showed highest decrease on blood sugar of patients, because they taking highest doses from *M. oleifera* leaves powder which contains highest level from antioxidants and flavonoids which responsible for decrease blood sugar levels. Group 2 showed highest percentage of decrease(63%), so the suitable dose to consume it is 0.5g. The results showed no significant difference in rate of decrease on blood sugar for patients who taking tablets compared to patients who taking insulin. Thus, the study demonstrates that *M. oleifera* possesses a hypolipidaemic effect, and it's useful to take beside drugs for diabetic patients who their blood sugar not lowering by drugs.

3.5 Side effect of taking *M. oleifera* leaves powder on kidney and liver (creatinine, urea and AST, ALT enzymes) for diabetic patients:

The results showed no difference between measurement of creatinine, urea and AST, ALT enzymes before and after used *M. oleifera* leaves powder (Tables 16, 17, 18 and 19).

Table(16) Measurement of creatinine for all patients before and after taking *M. oleifera* leaves powder for a month:

Number of patients	Creatinine before (mg/dl)	Creatinine after (mg/dl)
1	0.8	0.8
2	0.8	0.9
3	0.6	0.8
4	0.9	1.1
5	0.8	0.6
6	0.7	0.6
7	0.9	0.8
8	0.9	1.1
9	0.5	0.8
10	0.8	0.8
11	0.6	0.7
12	1.1	0.6
13	0.8	0.9
14	0.8	0.9
15	0.7	0.7
16	1.1	1.1
17	0.8	0.5
18	0.6	0.9
19	0.5	0.8
20	0.9	0.8
21	0.4	0.8
22	0.6	0.9
23	0.9	0.8
24	0.8	0.9
25	0.6	0.6
26	0.7	0.9
27	0.9	1.2
28	0.8	1.1
29	0.9	1.1
30	0.6	0.8

Table (17) Measurement of urea for all patients before and after taking *M. oleifera* leaves powder for a month:

Number of patients	Urea before(mg/dl)	Urea after (mg/dl)
1	23	25
2	31	31
3	15	22
4	29	25
5	30	33
6	28	24
7	15	16
8	18	21
9	24	31
10	23	22
11	18	25
12	16	17
13	26	30
14	38	33
15	40	32
16	15	15
17	19	23
18	30	34
19	26	29
20	17	22
21	19	21
22	30	26
23	24	26
24	21	20
25	29	29
26	33	38
27	36	36
28	18	15
29	23	25
30	30	29

Table (18) Measurement of AST for all patients before and after taking *M. oleifera* leaves powder for a month:

Number of patients	AST before(mg/dl)	AST after (mg/dl)
1	33	36
2	39	41
3	29	28
4	28	38
5	39	42
6	32	32
7	20	26
8	30	31
9	30	36
10	26	26
11	31	29
12	42	46
13	39	43
14	28	31
15	55	55
16	39	39
17	19	24
18	30	35
19	22	27
20	25	28
21	30	38
22	26	20
23	40	48
24	30	26
25	18	20
26	29	42
27	38	39
28	45	39
29	29	30
30	30	40

Table (19) Measurement of ALT for all patients before and after taking *M. oleifera* leaves powder for a month:

Number of patients	ALT before (mg/dl)	ALT after (mg/dl)
1	39	39
2	42	43
3	28	28
4	36	31
5	44	44
6	48	51
7	39	40
8	29	36
9	26	28
10	34	40
11	35	38
12	55	59
13	34	34
14	29	38
15	58	58
16	28	29
17	29	35
18	44	49
19	30	36
20	21	29
21	39	34
22	31	39
23	46	51
24	27	27
25	33	30
26	46	48
27	31	32
28	36	45
29	44	48
30	40	42

3.6 Statistical Analysis for side effect:

The following statistical tools were used in this study for analysis and interpretation of data (Rangaswamy, 1995): Independent T-Test. Statistical analysis compared results of measurement of creatinine, urea and AST, ALT enzymes, before and after taking *M. oleifera* leaves powder.

3.6.1 Effect of *M. oleifera* leaves powder on creatinine for diabetic patients:

Table (20) showed no statistically significant difference in the mean values of creatinine before and after taking *Moringa oleifera* leaves powder ($p > 0.001$).

Table (20) The effect of *M. oleifera* leaves powder on creatinine for diabetic patients (Mean ± SE):

	Mean ± SE
Creatinine before taking *M. oleifera*	0.760 ± (0.030)
Creatinine after taking *M. oleifera*	0.843 ± (0.032)
P-Value	0.065

3.6.2 Effect of *M. oleifera* leaves powder on urea for diabetic patients:

The mean values (Table 21) showed no significant effect of *M. oleifera* in urea levels ($p > 0.001$).

Table (21) The effect of *M. oleifera* leaves powder on urea for diabetic patients (Mean ± SE):

	Mean ± SE
Urea before taking *M. oleifera*	24.80 ± (1.29)
Urea after taking *M. oleifera*	25.97 ± (1.12)
P-Value	0.497

3.6.3 Effect of *M. oleifera* leaves powder on (AST) for diabetic patients:

The results showed no statistically significant difference in the mean values of (AST) before and after taken *M. oleifera* leaves powder (p> 0.001) (Table 22).

Table (22) The effect of *M. oleifera* leaves powder on (AST) for diabetic patients (Mean ± SE):

	Mean ± SE
AST before taking *M. oleifera*	31.70 ± (1.48)
AST after taking *M. oleifera*	34.53 ± (1.53)
P-Value	0.189

3.6.4 Effect of *M. oleifera* leaves powder on (ALT) for diabetic patients:

The results indicated no statistically significant effect of *M. oleifera* leaves powder on (ALT) for diabetic patients (p> 0.001) presented in Table (23).

Table (23) The effect of *M. oleifera* leaves powder on (ALT) for diabetic patients (Mean ± SE):

	Mean ± SE
ALT before taking *M. oleifera*	36.70 ± (1.60)
ALT after taking *M. oleifera*	39.63 ± (1.59)
P-Value	0.198

Results of this study indicating no statistically significant effect for *M. oleifera* leaves powder on kidney and liver (creatinine, urea and AST, ALT enzymes) ($p > 0.001$). Severe diabetic disorders have been found in arsenic-intoxicated humans (Longnecker *et al*., 2001), (Tseng *et al*., 2002). Arsenic, a naturally occurring toxicant, is present in food, soil and water(Chowdhury *et al*., 1995), (Abernathy *et al*., 1995), (Mukherjee *et al*., 2005). Kidney dysfunction is one of the major health effects of the long term arsenic exposure, and elevated levels of serum urea have been reported to be associated with renal dysfunction and excessive protein catabolism(Wang *et al*.,2009), (Tuot *et al*., 2011). Food supplementation of *M. oleifera* leaves significantly ($P<0.05$) protected arsenic-induced elevation of serum urea levels (Sheikh *et al*., 2014). Arsenic administration substantially increased serum AST and ALT activities (Islam *et al*., 2011*). M. oleifera* leaves as a food supplementation significantly reduced arsenic-induced elevation of AST and ALT activities. Sheikh *et al*., 2014, results indicated that *M. oleifera* leaves had a protective effect on arsenic-induced liver injury. Results of this study agreed with (Suleiman *et al*., 2017) results, they evaluate the effect of *M. oleifera* against Salmonella typhimurium infected changes in liver and kidney function parameters in albino rats. Albino rats were divided into four groups of five rats each (groups A-D). Groups B, C and D were exposed to 0.1ml (3.0x104 CFU) of Salmonella typhimurium. Six hours post- infection, Groups B and C were treated with 50 and 100mg/kg of *M. oleifera* aqueous leaves extract respectively and continued for 21 days. Group D was not treated while group A was neither infected nor treated. Serum alanine transaminase (ALT), aspartate transaminase (AST), urea and creatinine were estimated using standard methods. Results showed S. typhimurium infection significantly ($p<0.05$) increased the levels of ALT and AST, urea and creatinine when compared with control levels (Group A). However, *Moringa oleifera* supplementation was associated with significant

($p<0.05$) decrease in the levels of ALT, AST, urea and creatinine. The study showed S. typhimurium infection induced changes in liver and kidney function parameters and also revealed possible amelioratory effects to these changes after *M. oleifera* supplementation. Due to anti-inflammatory properties, *M. oleifera* has been used in ancient systems of medicine such as Ayurveda to prevent or treat stomach ulcers, liver disease, kidney damage, fungal or yeast infections (such as candida), digestive complaints, and infections (Levy and CHHC., 2018). Pari and Kumar, 2002 showed that an ethanol extract of *Moringa oleifera* leaves protected rats against the hepatotoxicity of various antitubercular drugs including isoniazid, rifampicin, and pyrazinamide. The extract decreased drug-induced levels of AST, ALT, ALP, and bilirubin, and inhibited drug-induced lipid peroxidation in the liver. Das *et al*. 2012 have shown that in mice fed with a high-fat diet, an aqueous extract of *Moringa oleifera* leaves protects against liver damage as demonstrated by reductions in tissue histopathology and serum activities of marker enzymes aspartate aminotransferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP) as well as reduced lipid peroxidation and increases in reduced glutathione. From present study this plant has the potential to help reverse multiple major problems and provide for many unmet human needs. Several studies using experimental rodent models have shown that *M. oleifera* leaves extracts can protect the liver from chemically induced damage(Ndong *et al*., 2007a., Buraimoh, 2011). Eisenbach *et al*., 2007., Patlolla and Tchounwou, 2005 showed Co-administration of *M. oleifera* leaves as a food supplementation significantly reduced arsenic-induced elevation of AST and ALT activities. These results indicated that *M. oleifera* leaves had a protective effect on arsenic-induced liver injury. This study agreed with over 1,300 studies, articles and reports focused on *M. oleifera* benefits, and this plant's healing abilities that are important in parts of the world that are especially susceptible

to disease outbreak and nutritional deficiencies. Fresh leaves of *M. oleifera* containing a safe potential natural antioxidant such as total phenolics antioxidant, vitamin and minerals, so leaves are used as an herbal medicine in treating a wide variety of diseases. The results suggested that *M. oleifera* leaves could be useful therapeutically in future to reduce blood glucose level for diabetic patients , and no side effect in kidney and liver enzymes.

Conclusion

The results showed *M. oleifera* leaves powder containing essential mineral element, so that *M. oleifera* become known as one of the most impressive herbal supplements to hit the holistic health market. On the other hand the study indicated taking of *M. oleifera* leaves powder help control blood sugar levels and allow cells to take up or release glucose (sugar) as needed, this gives *M. oleifera* natural anti diabetic and hormone-balancing properties. The results indicated the suitable dose to consume beside drugs to reduce blood glucose of diabetic patients whom their blood glucose not lowering by drugs is 0.5g (percentage of the decrease on blood glucose is 63%). The results showed no side effect on liver enzymes and kidney for diabetic patients after taking leaves powder. The study concluded *M. oleifera* leaves has potential for nutritional and medicinal to human needs.

Recommendation

-To detect and isolate the component in *Moringa oleifera* leaves powder which responsible to reduce blood sugar for diabetic patients.

-To investigate acting of *Moringa oleifera* leaves powder as a hypocholesterolemic agent.

-To study other benefits of *Moringa oleifera* because its contains natural antibacterial, antifungal and antiviral compounds that protect the skin from various forms of infections.

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