# CHAPTER ONE

# 1.INTRODUCTION

## 1.1 Backgrounds:

The production of new safe and functional material, obtained by clean environmentally sound process is a new goal of industry. Animal feed and fertilizers are traditional uses of by-products. These by-products may be turned into functional components (flavor, pigment, antioxidants, anti-microbe preservatives, stabilizer and thickening agents), depending on different factors. Of these is the extraction technology applied, which must be highly efficient, mild, safe, clean and sustainable. Thus with an increasing interest in new oil sources, the seeds of wild plants including the triable pulses receive more attention. (Janardhanan and Vadivel, 1994).

Human beings from time immemorial have been using plants for a multitude of purposes. Oils have always been an integral part of human foods, being essential for health. Industrially, they play an important role in the development of different areas of chemical products, pharmaceutical, cosmetics, paints and most importantly, food (Atef, 2010). Oils are naturally occurring esters of long straight-chain carboxylic acids. They belong to the saponifiable group (contain an ester groups) of lipids. Lipids are biologically produced materials that are relatively insoluble in water but soluble in polar and non-polar organic solvents. Edible oils are constituted of triacylglycerol molecules, mainly formed by unsaturated (oleic, linoleic, linolenic acids etc.) and saturated fatty acids (myristic, palmitic, stearic acids etc.) esterified to Glycerol units (Andersson *et al.*, 2010).

Kernels have been used as a food either alone or with cereals. Tamarind kernel is used in developing food products such as jelly and marmalades (Bhattacharya *et.al*, 1983). Rao and Subramanian (1984) and Marangoni *et.al* (1988) have attempted to produce protein concentrates or cereals or meals from kernels proteins.

There is a lack of information in the literature about the utilization of the seed kernel in Sudan. The only tribes utilize the tamarind seed kernel are Acholi and Madi, in southern Sudan where it is added to cassava flour used to make porridge (Lawerance and Cirino, 1999). In northern Sudan It was concluded that tamarind kernels, considered as a waste, can be converted into a useful by-product, which can be used as a promising substitute source of pectin (Huda, 2009).

In Sudan the tamarind is found as a wild plant and was only used as a beverage especially in Ramadan month where, the seeds are discarded. the chemical composition of some of the indigenous plants of the Sudan including tamarind was evaluated ,The seed was found to be rich in protein, sugar and potassium, thus can suffices the human needs and should be used as famine food(Abu Zaid, 1999).

The seed comprises the seed coat or testa (20-30%) and the kernel or endosperm (70-75%) (Coronel, 1991; Shankaracharya, 1998). Tamarind seed is the raw material used in the manufacture of tamarind seed kernel powder (TKP), polysaccharide (jellose), adhesive and tannin. The seeds are also used for other purposes and are presently gaining importance as an alternative source of protein, rich in some essential amino acids. Unlike the pulp the seed is a good source of protein and oil. There has been considerable interest amongst chemists, food technologists and nutritionists in the study of the properties of tamarind seeds. From this point of view, the

by-product like tamarind seed can be used as a cheap source of oil to increase the added value of tamarind seeds.

The oil yield, fatty acid composition and the physicochemical and quality characteristics of *Tamarindus indica* Linn seed oils obtained by solvent extraction were determined as well as optimized process conditions. *T. indica* oil was tested for its physical and chemical properties including percentage of fatty acid, kinematic viscosity, saponification value, unsaponifiable matter. The stability of *T. indica* oil during storage at room temperature and during heat treatment was studied (Balaji *et al*., 2014).

On other hand the chemical composition of the leaf oil of *Tamarindus indica* L. was studied by GC/MS. Thirteen components were identified , of which limonene (24,4% ) and benzyl benzoate (40,6% ) were most predominant (Jorge Pino , Julio , Renato and Juan Aguero , 2002).

Most researches, on tamarind, done in Sudan were on the pulp and for medicinal aspects. Khalid *et.al*. (1997) investigated the potential of anti lesihmanial activity of some Sudanese medicinal plants. The results indicated that the methanolic extract of tamarind failed to exhibit any significant anti-leishmanial activity against leishmania at concentration less than 100µg/ml. Mahmoud and Homeida (1994), indicated that a significant reduction was observed in the AUC plasma concentration versus time and Cmax (the peak plasma concentration) of chloroquine as a result of co-administration with each of the three beverages (Tamarind, hibiscus and lemon), also a parallel reduction in the drug anti malarial efficiency was expected. Imbabi and Abu Alfutuh (1992) investigated the molluscicidal activity of tamarind pulp and found that the activity was greater in the sample extracted with methanol than with water, this was referred to the presence of saponins. Tamarindial extracted from tamarind pulp was found

to have fungicidial and bactericidial properties (Imbabi *et.al* 1992). El Sheikh (1987) studied the toxicity of certain Sudanese plants extracts, including tamarind, on the different stages of Schistosoma mansoni. The tamarind extract showed toxic effect at 50ppm concentration for the maracidia and cercaria, while was highly toxic on the adult worm.

Research concerning the chemical, technological and usage of tamarind seed in Sudan are scarce. This research is investigating the proximate analysis of *Tamarindus indica* seeds and Characterization of the seeds oil could be considered as a milestone and guide for further research.

## 1.2 Objectives:

This research was therefore undertaken to investigate the properties and Characterization of the *Tamarindus indica* seeds oil. To achieve these objectives the following steps were attempted:

- Sample preparation of *Tamarindus indica* seeds.
- Proximate analysis of seeds.
- Extraction of oil from seeds by solvent extraction.
- Determination of some physiochemical properties of the extracted oil.
- Identification of the major components of the oil using Gas Chromatography-Mass spectroscopy.

# CHAPTER TWO

# 2. LITERATURE REVIEW

## 2.1 History of Tamarind Culture:

## 2.1 1Origin:

*Tamarindus indica* (L.), a member of the family Leguminosae (Fabaceae), is native to dry Savannas of the tropical Africa (Bhattacharya *et. al*., 1994). In ancient times the tree was introduced to Asia by Arab traders (Duke *et al*., 1981, Morton, 1987) and with its pleasant and acidic tasting fruit, the name tamarind driven from the Arabic name tamar-al- hind which means "date of India" also known as tamarindo (Spanish and Portuguese) and Tamarin, tamarinier, tamarindier (French). The origin of species is still subject to debate, some authorities tracing the origin to Indian sub-continent but most evidences placing its origin within Africa, either central Africa or Ethiopia (Gunassena and Hughes, 2000) and Nigeria across ecological zones (Keay and Onochie, 1964).

Tamarind is an important woody perennial fruity species. It is found throughout the tropics for its beauty, ornamental adaptability to variable climatic and edaphic conditions and fruit production (El Siddig *et.al*, 1999). The international survey of unexploited tropical and sub-tropical perennials revealed that tamarind is cultivated on an orchard basis in Caribbean, Central America, South America, South central Asia and South East Asia (Sedgely and Gardner, 1989).

### 2.1.2 Description of the tree:

The tamarind tree is a medium sized, semi evergreen with short strong trunk with grey scaly bark. The leaves are alternate pinnately compound 7-15cm long with pulvinus at the base of the petiole. There are 10-20 pairs of small leaflets, arranged opposite, entire almost sessile, oblong. The inflorescence is small terminal drooping raceme 5-10 cm long. The flowers are small, scented and attractive with yellow and red colour. The pods are usually curved, flattened and vary considerably in size and shape, they are constricted, indehiscent 1-10 seeded. When ripe the fruits are stiff and brittle. The seeds are obovate, flattened, brown, about 1-15mm long and joined to each other with tough fibre running through brown sticky pulp. Tamarind tree is long lived and attains a large size but the rate of growth after the seedling stage is slow. The tree begins to bear fruits at the age of 13-14 years and continues to yield abundant crops for more than 60 years (TWI, 1976).

### 2.1.3 Distribution of tamarind in Africa:

Tamarind is found in Angola, Benin, Burundi, Cameroon, Central Africa Republic, Ivory Coast, Djibouti, Eritrea, Ethiopia, Ghana, Guinea, Guinea Bissau, Kenya, Liberia, South Africa, Sudan, Tanzania, Malawi, Nigeria, Senegal, SieraLeone, Somalia, Uganda, Zaire, Zambia and Zimbabwe (Fries, 1992). It is worth mentioning that Africa does not produce tamarind on large scale though local people use it widely.

### 2.1.4 Distribution of tamarind in Sudan:

*Tamarindus indica* is a Sudanese tree which spreads into Sahelo-Sudanian zone. It is sometimes planted on account of its dense shade and fruit quality. A number of plant species (e.g., coriander, lupin, roselle,

watermelon, okra and tamarind) grown in Sudan could be classified as a neglected and/or under utilized crop. These species, although important for people, receive little or no attention as far as research and development is concerned. However, some of the crops including tamarind play important roles in the economy of Sudan and contribute considerable shares in the national and international trade (Hamid, 2006). In Sudan the tamarind is cited with baobab on sandy soils and Khors (water source) in short grasses Savannas in Kordofans Darfur, Blue Nile, Bahr El Ghazal (El Amin, 1990).

### 2.1.5 Tamarind varieties:

In some regions of the world there is a type with reddish flesh which is distinguished from the ordinary brown fleshy type and regarded as superior in quality. There are two types which are sweeter than the others; one is the "Makham waan" in Thailand, the other known as "Manila sweet" distributed by the USA department of Agriculture, subtropical Horticulture Research Unit, Miami. In Sudan there are no distinguished varieties (El-Siddig *et.al* ,1999).

### 2.1.6 Uses of tamarind tree:

Tamarind tree is a valuable timber species widely used for manufacturing of tool handles, furniture, charcoal, oil mills, rice ponders and fuel wood. The leaves are an important source of food and herbal medicine. The most valuable part of the tamarind tree is the fruit which contains the pulp, shell, fibre and the seed. The pulp constitutes (30-50%) of the ripe fruit (Purseglove, 1987; Shankarachya, 1998). The shell and the fibre account for 11- 30% and the seed about 25 - 40% of the fruit (Champan, 1984; Shankarachya, 1998). The edible pulp of ripe fruits is used as a flavouring agent in cooking, soups, jams chutneys, sauces, juices and the preparation of

beverages (Siddhuraju *et. al.*, 1995). The pulp is the richest natural source of tartaric acid (8-18%) and is the main acidulate used in the preparation of food in India and many other Asian countries. Other industrial products of tamarind are tamarind juice, tamarind juice concentrate, tamarind pickles and paste (Shankarachya, 1998). Tamarind has an exceedingly wide range of domestic and industrial uses, yet this important tree remains under exploited.

### 2.1.7 Uses of tamarind seeds:

Till 1943, tamarind seed did not find any major food or non food application. Only during the famine periods, the tribes in India mixed the roasted and de-husked tamarind seed powder with the flour of other food grains and used it as a famine food and also as a feed for animals (Mohamedain, 1991). During the Second World War, to meet the scarcity of starch (an edible product) for the Indian textile industry, research was carried out at the Forest Research Institute (FRI) at Dehra Dun (India) to find a substitute for starch for sizing cotton yarn and cloth. As a result Tamarind Kernel Powder (TKP) was found to be a reasonable substitute for starch as sizing material for cotton and jute. This effort created a world wide utilization of tamarind seed which is the major by-product in the tamarind industry. The seed consists of 30% testa and 70% endosperm, the testa contains 40% of water soluble component, 80% of which is tannin and colouring matter. The presence of tannins and other colouring matter in the testa make the whole seed unsuitable for human consumption. Therefore, the testa has to be separated from the kernels by boiling or roasting. Otherwise, such side effects as depression, constipation and gastro-intestinal disorders may result (Anon, 1976).The tamarind endosperm or kernels are grounded to give a cream or a buff coloured powder which yield a water soluble poly-saccharide that is known in the literature by various names such as Tamarind

Seed Polysaccharide, (TSP) as reported by Srivastava and Singh, (1967), tamarind pectin, tamarind polyose, tikernose, tamarind gum, tamarind amyloid (because of its starch-like response to iodine).

The seed is also used in the vegetable and processing industries, tamarind xyloglucan "tamarind gum" is commercially available as a food additive for improving the viscosity and texture of processed foods (Sone and Sota, 1994).Seeds could be used as a cheaper source of protein to alleviate protein malnutrition which is widespread in many developing countries (Siddhuraju *et. al*, 1995).

Seed testa is the residual product in preparation of kernel powder used as dye stuff due to the presence of Leuceanthocyamins and also as a plywood adhesive. The testa contains crude fibre 21.6%, ash 7.4% and the tannins 20-24%. It can be used as a low cost source of antioxidant in lipid containing food.

## 2.1.8 Composition of seed kernel:

Tamarind kernel consisted of 63.32-73.48% polysaccharides, 17% protein, 7% fat, 2% crude fibre and 2.8% mineral matter (Bhattacharya *et. al*., 1994).

## 2.1.9 Chemical composition of tamarind seed:

Morton (1987) indicated that the seed of tamarind contains approximately 63% starch, 14-18% albuminoids and 4.5-6.5% of semi drying oil. Seeds of the tree legume *Tamarindous indica* were evaluated as a potential source of food or food ingredients (Marangoni *et al.*, 1988). Crude protein and nitrogen free extract comprised 15.5% and 59% of the seed respectively. Pentose sugars constitute approximately 20% of the soluble sugars. The crude fats present are 4.5% which contain a relatively large

proportion of unsaturated fatty acids. The macronutrients; calcium, magnesium, potassium and phosphorus are low in comparison to cultivated legumes. Alkaline extraction of the seeds showed that about 70% of the proteins were extractable. The principle sugars of the seeds are mannose, glucose and ribose. Moisture, ash and crude fibre were found as 9.4%, 3.2% and 8% respectively. Leakey (1999) reviewed a published data on the nutritive values of the flesh, kernels and seed oils of seventeen fruit species, including *Tamarindus indica*, which contains high quality of protein and can be added to cereals to fortify them. The kernel can also be used in production of the hydrocolloid (tamarind gum). Proximate analysis of seed kernels shows that 65.1-72.2% is non fibre carbohydrate, 15.4-22.7 is protein, 3.9-7.4% is oil and 0.7-8.2% is crude fibre.

Yusuf *et al*. (2007) evaluated the nutrient content of the whole seed and seed nuts of tamarind, they found that 21.25-22.2% was crude protein, carbohydrates ranged from 8.9-17.1%, crude fibre was 2.33-3.82%, crude lipid was 6.94-11.43% in seed nut and whole seeds, respectively. Moisture content was higher in the seed nuts at about 19.9%. The mineral content of the seed is higher than the seed coat.

The chemical composition of the whole seed of *Tamarindus indica* as investigated by Ishola *et. al*. (1990), Bhattacharya *et. al*., (1993) and Morad *et.al.*, (1978) showed moisture contents range between 9.4-11.3%, proteins 13.3-26.9%, fat/oil 4.5-16.2%, crude fibre 7.4-8.8%, carbohydrate 50-57.1%, total ash 1.6-4.2%, nitrogen free extract 59%, total yield 59%, total sugars 11.3-25.3%, reducing sugars 7.43% and starch 33.1%. Also Ishola *et. al*., (1990), Bhattacharya *et.al*., (1993) and Morad *et. al*., (1978) reported that the kernels of tamarind seed contained 15-20.9% protein, 3.9-8% fat/oil, 2.5-8.2% crude fibre, 65.1-72.2% carbohydrate, 2.4-4.2% total ash and 11.4-

22.7% moisture as well as 20.2 % of tannins in seeds coat (testa). Ibrahim *et.al.*, (1959) in their evaluation of tamarind seed grown in Sudan found seven hydrocarbons in the unsaponifiable matter of seed and the GLC of methylated fatty acids revealed the presence of palmitic, oleic, linoleic and eicosanoic as the major fatty acids of the seeds.

## 2.2 Seed oil:

The seed oil is golden yellow, semi-drying oil, which in some respects resembles groundnut oil. The major fatty acids were palmitic, oleic, linoleic, and eicosanoic. The lipids contained a relatively large proportion of unsaturated fatty acids, with linoleic acid (36-49%) in the highest concentration. Other major fatty acids are oleic acid (15-27%) and palmitic acid (14-20%) (Singh, 1973).

Seeds give an amber coloured oil, free of smell and sweet to taste, which resembles linseed oil (Watt, 1893). As in the pulp, the saponification value is high, because it contains low molecular weight fatty acids. The iodine value of seed lipids is much lower than in pulp lipids suggesting lower unsaturation and probably higher stability of seed oil. Andriamanantena et al. (1983) evaluated the Fatty acid composition of tamarind seed oil: Palmitic (14-20%), Stearic (6-7%), Oleic (15-27%), Linoleic (36-49%), Arachidic (2-4%), Behenic (3-5%) and Lignoceric (3-8%).

Tamarind seeds also contain 6 to 8 percent of oil rich in unsaturated fatty acids. Due to the presence of unsaturated oil the tamarind starch develops yellow colour and rancidity which affects the quality of the starch. Extraction of oil from starch, improves the latter's quality and utility and huge quantities of oil would also be produced. The oil is recovered

from dehulled kernels by extraction after they are flaked or converted into cake by being passed in expellers. The oil is suitable for use in the production of soaps (IS 9587. 1980).

The seed kernel of tamarind contains 10-15% by weight oil with amber colour, free of smell and sweet to taste, which resembles peanut oil, (Allen and Allen, 1981). It could be used for making varnishes, paints and burning oil in lamps (Watt, 1983). The oil is said to be palatable and of culinary quality (Morton, 1987).

The physicochemical properties of the oils were analyzed. The T. indica seeds contain crude oil and fatty acids, i.e. 8% and 2.92% respectively (Balaji *et. al*, 2014).

## 2.3 Oil extraction:

According to (Balaji *et. al*, 2014), study deals with extraction of oil by use of solvent extraction. Four main operating parameters affecting the solid liquid extraction of *T. indica* seeds were optimized based on the maximum oil yield. The optimum conditions for the lab scale solid liquid extraction was obtained at temperature reflux (around 80 °C), extraction time 6h, solid to solvent ratio of 1:6w/v, agitation speed 100rpm and ethanol as a solvent. Ethanol gives better oil yield compared to hexane, chloroform, methanol, isopropanol and petroleum ether. Based on the observations made above, it was concluded that ethanol, a green and safe solvent can be a better alternative to other solvents.

Andriamanantena *et. al*, (1983) extracted the oil with hexane and a mixture of chloroform and methanol; the yield was 6.0-6.4% and 7.4-9.0%, respectively.

## 2.4 Oil characterization:

IS 9507 – 1980 prescribes requirements and methods of sampling and test for tamarind kernel oil which stated that, the oil shall be obtained from clean and sound kernels of tamarind( *Tamarindus indica* ) tree by process of solvent extraction. It shall be clear and free from adulterants, sediments, suspended and other foreign matter, separated water and added coloring matter. Solvent-extracted oil shall be obtained from seed kernels using solvent hexane conforming to IS: 9587-1980. The oil shall also comply with the Following requirements:

| No | CHARACTERISTIC | REQUIREMENT |
|---|---|---|
| 1 | Moisture and insoluble impurities percent by mass, Max | 0.25 - |
| 2 | Refractive index at 40°C | I.460 0 - I.470 0 |
| 3 | Iodine value ( Wijs ) | 100 - 120 |
| 4 | Acid value, Max | 20- |
| 5 | Unsaponifiable matter, percent by mass, Max | 3- |
| 6 | Flash point , “C, Min | 100- |

Balaji Panchal *et.al,* (2014) reported the results of Physical and chemical properties of ethanol extracted oil from T.indica seeds was greenish in colour. The free fatty acid content was 2.92±0.30% . Kinematic viscosity (40 $^{O}$C) was 38.00±0.10 $mm^2$/sec. Specific gravity (25 $^{O}$C) was 0.911±0.40 $g/cm^3$ . Acid value was 0.5±0.02 mgKOH/g. Iodine value was 95.00±0.40 gI/100g . Flash point was 110 $^{O}$C . Cloud point was 2 $^{O}$C . Pour point was -4 $^{O}$C. Saponification value was 186.10±0.30 mgKOH/g.

Unsaponifiable matter was 1.22±0.20 %. Peroxide value was 4.61±0.30 $mgO_2/g$. State at room temperature was Liquid .As well is as determination free fatty acids composition (%) of *T.indica* seed oil by Gas Chromatography-Mass spectrometry (GC-MS) to be found Oleic acid (C18:1) 0.19 %, Linoleic acid (C18:2) 0.41 %, Myristic acid (C14:1) 1 %, Luric acid (C12:0) 0.32 % , Octanoic acid (C8:0) 0.3 % , Palmitic acid (C16:0) 0.13 % , Stearic acid (C18:0) 0.4 % , Lignoceric acid (C24:0) 0.14 % , Arachidic acid (C20:0) 0.06 % , Behenic acid (C22:0) 0.02 %.

## 2.5 Oil uses:

In Bengal, the oil is used for making varnish to paint idols (Rama Rao, 1975; Anon, 1976) and light lamps (Lewis and Neelakantan, 1964 a; Salim *et al.,* 1998). The oil is said to be palatableand of culinary quality (Morton, 1987). In Indonesia, oil extracted from the seed is used as a hair dressing.

Research results indicate that the *T. indica* seed oil can be used as a potential alternative to nutritional food. Terminalia catapa seed oil injection has been used in clinical trials for the treatment of rectal prolapse in children (Balaji *et.al,*2014). After refining, the oil can be used as cooking oil. Also it can be used in the Soap making.

# CHAPTER THREE

# 3. MATERIALS AND METHODS

## 3.1 Chemicals and materials:

### 3.1.1 Chemicals:

All chemicals and reagents used in this study were of analytical grade.

### 3.1.2 Plant materials:

Samples of tamarind were purchased from the local market.

### 3.1.3 Sample preparation:

Immature and damaged seeds of tamarind were removed. The Fresh seeds of *T. indica* were dried in an incubator for 2 days at 40 $^{0}$C, crushed manually and then powdered. The powder was then divided into two portions and kept in plastic containers at room temperature. One part was used for proximate analysis while the other part was reserved for oil extraction.

## 3.2 Proximate analysis:

Determination of moisture, protein, fibre , and ash were carried out in duplicate, while the oil content was in triplicate. All proximate results were expressed as a percentage of the weight of samples analyzed.

### 3.2.1 Moisture content:

The moisture content was determined by moisture analyzer- MAC 50/1/WH at 120ºC for 15 minutes.

### 3.2.2 Protein content:

The nitrogen content was estimated according to pearson (1968) . (0.2 g) of sample was weighted and transfer to a Kjeldahl digestion flask. (1 g) of catalyst mixture (90% anhydrous sodium sulphate and 10% copper sulphate) were added followed by 3 ml of conc. sulphuric acid. The flask was heated gently, cooled and digested into distilling flask with 15 ml of NaOH 40% solution. (50 ml) of boric acid solution and 3 drop of methyl red were placed in receiving flask. The distillation apparatus was connected up with delivery tube dipping below the boric acid solution. The tap was closed and the ammonia distilled into the boric acid solution. The distillate solution was titrated against 0.1 N HCL. The percentage of nitrogen was calculated: 1ml 0.1 N = 0.00014 g N. and then crude protein was calculated using approximate factor: N x 6.25.

### 3.2.2 Crude fibre content:

The crude fibre content was determined according to pearson (1968) by extracting the sample with Hexane in sohxlet apparatus. (5 g) of the extracted sample was dried in air and transferred to 500 ml conical flask. (200 ml) of 0.225 N sulphuric acid was added and brought to boiling point. The content was filtered using Buchner funnel while it was hot, washed with (200 ml) NaOH 0.313 N and boiled gently to boiling point for 30 minutes. Allowed to stand for 1 minute and then filtered through ash less dry weighted filter paper and washed with HCL 1%, hot water until free from acid and alcohol .Then dried at 105 $^{0}$C to constant weight. The paper and content was ignited at 550 $^{0}$C for 1 hour. The weight of the ash was subtracted from the increase of weight in the paper and the difference is reported as fiber

### 3.2.3 Oil content:

Oil was extracted from the whole seeds (kernel and Coat) by a method described by Bajali *et.al* (2014) using solvent extraction, in three batches at laboratory scale. To 100 grams of tamarind seed powder, (600 ml) of Hexane was added based on Soxhlet principles. The extraction was obtained at temperature reflux 80 $^0$C and extraction time 6 hours.

The extracted oil was filtered through gosh crucible No. 3. Then dried on oven at 80 $^0$C and 105 $^0$C for one hour to ensure that all Hexane and water was evaporated respectively. The percentage of oil was determined by difference from pre dried and weighed round bottom flask.

$$\text{Oil content} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100\%$$

### 3.2.4 Ash content:

Determining the total ash was performed according to pearson (1968), by weighted out (5 g) of the sample into platinum dish which had previously been ignited and cooled before weighed. Then the dish and content were ignited, first gently on fire and then on furnace at 550 $^0$C. The dish and content were transferred to desiccators for 10 minutes .then the ash content was calculated as a percentage.

$$\text{Ash content} = \frac{\text{Weight of the ash}}{\text{Weight of sample}} \times 100\%$$

### 3.2.5 Carbohydrates:

The content of carbohydrates was calculated as a percentage by difference.

Carbohydrates = 100 – (Oil % + Ash%+ Moisture%+Protein%+Fibre%).

## 3.3 Physical and chemical properties of *T. Indica* seed oil:

All physical and chemical properties were performed due to the Indian standard (Is 548.Part 1.1964) in duplicate.

### 3.3.1Physical properties:

### 3.3.1.1 Moisture content:

The moisture and any other material contained in the oil or fat which is volatile under the conditions of the prescribed test.

(5 g) of the oil was weighted accurately into moisture dish which had been dried previously. Cooled in the desiccators and then weighed. The dish was place in the air-oven for approximately one hour at 105 ± $I^0$C. The dish was removed from the oven. Cooled In the desiccators to room temperature and weighed. This procedure was repeated but kept the dish in the oven only for half an hour each time until the difference between the two successive weightings does not exceed one milligram.

$$\text{Moisture and volatile matter content, percent by weight} = \frac{100\,ώ}{W}$$

Where:

ώ = loss in weight in g of the material upon drying, and

W = weight in g of the material taken for the test.

### 3.3.1.2 Refractive Index:

The ratio of the velocity of light in vacuum to the velocity of light in the oil or fat; more generally, it expressed the ratio between the sine of the angle of incidence to the sine of the angle of refraction when a ray of light of a known wave-length passes from air into the oil or fat.

This experiment was performed using refractometer. the sample was already liquid, filtered through goash crucible No. 3 to remove any impurities and the last traces of moisture to make sure that the sample was completely dry. The temperature of the refractometer was adjust to 30.0 ± 0.l °C. The prisms were cleaned and completely dried, then a few drops of the sample was placed on the lower prism. Closed the prisms, tightened firmly with the screw-head, and allowed to stand for one minute. The instrument and light were adjusted to obtain the most possible distinct reading, and the refractive index was determined.

$$\text{Temperature correction } R = R' + K ( T' - T)$$

Where:

R = the reading of the refractometer reduced to specified temperature, T $^{O}$C

R' = the reading at T' $^{o}$C

K = constant, 0·000 385 for oils

T' = the temperature at which the reading R' is taken;

T = the specified temperature (.generally 40'0°0).

### 3.3.1.3 Color:

This method determines the colour of oils by comparison with Lovibond glasses of known colour characteristics. The colour is expressed as the sum total of the yellow and red slides used to match the colour of the oil in a cell of the specified size in the tintometer.

The sample was already liquid, filtered through goash crucible No. 3 to remove any impurities and the last traces of moisture to make sure that the sample was completely dried and free from impurities. The glass cell was cleaned with carbon tetrachloride and allowed to dry. Filled with the clear filtered sample and placed in position in the tintometer, placed along' side of it such red, yellow, blue or neutral Lovibond glass slides or any, combinations of these to match the colour shade of the oil, the colours of the oil and of the combination of the glass slides was observed through an eyepiece.

Report the colour of the oil in terms of Lovibond units as follows:

Colour reading in cell = (a Y + 5 bR )

*Where*

a = the sum total of the various yellow (Y) slides used, and

b = the sum total of the various red (R) slides used.

### 3.3.1.4 Density:

A material's density is defined as its mass per unit volume. It is, essentially, a measurement of how tightly matter is crammed together. The density of water was determined with the help of specific gravity bottle, 25 ml capacity. The pre weighted and dried specific gravity bottle was filled with distilled water up to the mark and weighted accurately on sensitive balance. Then the gravity bottle was cleaned, dried and filled with oil to calculate the density of oil by the same method.

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

$$\text{Density of water} = \frac{\text{weight of water}}{\text{Volume of wate}}$$

$$\text{Density of Oil} = \frac{\text{weight of oil}}{\text{weight of water}} \times \text{density of water}$$

## 3.3.2 Chemical properties:

### 3.3.2.1 Saponification value:

The number of milligrams of potassium hydroxide required to saponify completely one gram of the oil or fat. The sample was already liquid, filtered through goash crucible No. 3 to remove any impurities and the last traces of moisture to make sure that the sample was completely dry and free from impurities. The sample was mixed thoroughly, and weighed accurately by difference about (2·0 g) in a conical flask. (25 ml) of the alcoholic potassium hydroxide solution was added and connected to reflux air condenser to the flask. Heated on a water-bath for one hour, Boiled gently but steadily until the sample was completely saponified as indicated by absence of any oily matter and appearance of clear solution. After the flask and condenser had cooled, One milliliter of phenolphthalein indicator solution was added , and titrated with standard hydrochloric acid. The blank was Prepared and conducted to determination at the same time.

$$\text{Saponification value} = \frac{56.1\ (S - B)\ N}{W}$$

Where:

S = volume in ml of standard hydrochloric acid required for the sample,

B = volume in ml of standard hydrochloric acid required for the blank,

N = normality of the standard hydrochloric acid, and

W= weight in g of the material taken for the test.

### 3.3.2.2 Acid value and free fatty acid:

The number of milligrams of potassium hydroxide required to neutralize the free acid present in one gram of the oil or fat. The oil was mixed thoroughly before weighed. Known quantity of oil was weighed accurately in conical flask, (50 ml) of freshly neutralized hot ethyl alcohol. and about one milliliter of phenolphthalein indicator solution were added. The mixture was boiled for about five minutes and titrated while hot with standard aqueous alkali solution, shaking vigorously during titration.

$$\text{Acid Value} = \frac{56.1 \times V \times N}{W}$$

The acidity of the oil or fat indicated by its acid value is frequently expressed as free fatty acids present in the sample. The calculations in terms of different fatty acids are as follows:

A) Free Fatty Acid in terms of Oleic acid ,% by weight = $\frac{28.2\ VN}{W}$

B) Free Fatty Acid in terms of Lauric acid ,% by weight = $\frac{20.0\ VN}{W}$

C) Free Fatty Acid in terms of ricinoleic acid ,% by weight = $\frac{29.8\ VN}{W}$

D) Free Fatty Acid in terms of palmitic acid ,% by weight = $\frac{25.6\ VN}{W}$

*Where*

**V** = volume in ml of standard potassium hydroxide solution used,

**N** = normality of standard potassium hydroxide solution used, and

**W** = weight in g of the material taken for the test.

### 3.3.2.3 Peroxide value:

Peroxide value was evaluated according to AOCS Official Method (2003). 2.5 g oil samples were weighed into a conical flask and 30 ml of solvent mixture of glacial acetic acid-chloroform in the ratio of 3:2, respectively, were added to the oil samples. Half ml saturated potassium iodide (KI) solution was added to the solution and allowed to stand for 1 min thereafter, 30 ml of distilled water were added and titrated with 0.01 N sodium thiosulfate solution using starch indicator until the yellow color was discharged. A blank was prepared alongside the oil samples.

$$\text{Peroxide value} = \frac{10 \times (V1 - V2)}{M}$$

*Where*

**$V_1$**: volume of $Na_2S_2O_3$ for determination of test sample in ml,

**$V_2$**: volume of $Na_2S_2O_3$ for determination of blank solution in ml and

**M**: mass of test portion in g.

### 3.3.2.4 Gas Chromatography-Mass spectrometry (GC-MS) analysis:

#### 3.3.2.4.1 Sample Preparation (Methylation):

(2 ml) of sample was taken into test tube. (7 ml) of alcholoic NaOH ,that prepared by dissolving (2 g) sodium hydroxide in 100 ml methanol was added.(7 ml) of alcoholic $H_2SO_4$ 1% , that prepared by mixing (I ml) Conc. $H_2SO_4$ + 99 ml methanol was added .Then shacked for 3 minutes and left to overnight .(2 ml) of saturated NaCl was added. Another (2 ml) of hexane was added, shacked for 3 minutes and the hexane layer was collected. (5 µl)

from collected hexane was diluted with (5 ml) diethyl ether and (1 g) of sodium sulphate as drying agent. Filtered through syringe filter 0.45 µm .

### 3.3.2.4.2 Sample injection:

(1 µl) was directly injected to GC.MS-QP2010 Ultra equipped with Rtx-5MS, 30 m length, 0.25mm diameter and 0.25µl thickness. The column temperature was kept at 60 °C for 10 min, with increase at 10 °C per min up to injector temperature 300 °C, split ratio 1:0, the carrier gas (Helium) flow rate 1.5 ml/min. The compounds were identified by the GC-MS intensity of retention time (RT) and by comparison with those present in NIST11S.LIB .The results were expressed as the relative percentage of each individual compound present in sample given by the corresponding RT.

# CHAPTER FOUR

# 4. RESULTS AND DISCUSSION

## 4.1 Proximate composition of tamarind whole seed:

Tamarind seeds were analyzed for their chemical composition. The proximate chemical composition of tamarind sample was conducted to estimate the proximate composition hence; the data presented in table 1 shows the proximate composition of tamarind seed. The results were expressed on dry weight basis.

The moisture content of tamarind whole seed was found to be (5.12 ±0.2034). This value is lower than the value of (19.9%) reported by Yusuf *et.al.*, (2007) , and lower than the range of (9.4-11.3%) found by Ishola *et. al*. (1990), Bhattacharya *et. al.*, (1993) and Morad *et.al*., (1978). According to pearson(1968) classification of oil based on their moisture content, Tamarind seeds oil classified as semi-drying oil.

The protein content of tamarind whole seed was found to be (26.71 ±0.8651) which is higher than the value (21.25 - 22.2%) evaluated by Yusuf et al. (2007), and lies within the range of (13.3-26.9%) investigated by Ishola *et. al*. (1990), Bhattacharya *et. al.*, (1993) and Morad *et.al*., (1978).

The fibre content value shown in table 1 was (2.79 ±0.2103) which lies within the range of 2.33-3.82% found by Yusuf et al. (2007), while is lower than the value (7.4-8.8%) reported by Ishola *et. al*. (1990), Bhattacharya *et. al.*, (1993) and Morad *et.al*., (1978) for whole seed.

The oil content value shown in table 1 was (3.26 ±0.0550) which is lower than the value of (4.5-16.2%) estimated by Ishola *et. al*. (1990), Bhattacharya *et. al.*, (1993) and Morad *et.al*., (1978) for whole seed.

The ash content of tamarind whole seed was found to be (3.53 ±0.0130), which lies within the range of (1.6-4.2%) reported by Ishola *et. al*. (1990), Bhattacharya *et. al*., (1993) and Morad *et.al*., (1978).

The carbohydrate content value shown in table 1 was (58.59 ±0.6021), which is higher than the value (8.9-17.1%) estimated by Yusuf et al. (2007) almost agrees with the value (50-57.1%) reported by Ishola *et. al*. (1990), Bhattacharya *et. al*., (1993) and Morad *et.al*., (1978) for whole seed. ,

**Table 1: Proximate chemical composition of the *Tamarindus indica* seeds:**

| Parameters (%) | Result |
|---|---|
| Moisture | 5.12 ±0.2034 |
| Protein | 26.71 ±0.8651 |
| Fibre | 2.79 ±0.2103 |
| Oil | 3.26 ±0.0550 |
| Ash | 3.53 ±0.0130 |
| Carbohydrate (by difference) | 58.59±0.6021 |

- Oil value is a mean of three determinations, while other values are a mean of two determinations.

## 4.2 Physical and chemical properties of *T. Indica* seed oil:

Table 2, present the results of the physicochemical analysis of the oil of *T. indica* was visually greenish in colour, liquid at room temperature (30 $^0$C) and has density of (0.855±0.05) g/cm$^3$ . The moisture content of the oil combatable within IS: 4370-1966 for *T.indica* seeds oil. The highest

saponification value (195.25 ±1.40) mgKOH/g lies within the range 184 – 196 required in the IS: 9587-1980, while higher than the value (186.10±0.30) found by Balaji Panchal *et,al* (2014) and higher than sunflower and corn oils. Such value indicates the average molecular weight of triglycerides in the oil.

The acid value (3.51±.64) mgKOH/g which is higher than the value (0.5±0.02) reported by Balaji Panchal *et , al* (2014) and lower than the value (20) required in the IS: 9587-1980.The acid value calculation lead to(1.78 %) free fatty acid as Oleic acid, (1.26 %) as Lauric acid ,(1.88 %) as Ricinoleic and (1.62 %) as Palmitic acid.

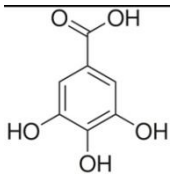
The peroxide value was (17.22±0.22) $mgO_2$/g oil in *T. indica* ,although it is acceptable for crude oil but it is relatively high, this may be interpreted by the presence of tannins in percentage up to 20% in the seed coat which is defined as a type of polyphenols rich with hydroxyl groups as shown in figure 1. Tannins have tendency to interact with aqueous solution of protein and macromolecules to form insoluble precipitates.

## Table 2: Physical and chemical properties of oil from *T.indica* whole seeds:

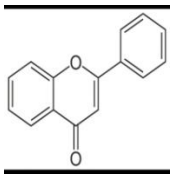
| Parameters | Result |
|---|---|
| Moisture | 0.27 % ±0.01 |
| Refractive index (30 $^0$C) | 1.4606 ±0.0002 |
| Colour | Blue = 3.5 ±0.2<br>Yellow = 7.3 ±0.3<br>Red = 4.3 ±0.3 |
| Density | 0.855 g/cm$^3$ ± 0.001 |

| | |
|---|---|
| Saponification value | 195.25 mgKOH/g ± 1.5 |
| Peroxide value | 17.22 mg$O_2$/g ±0.22 |
| Acid value | 3.5 mgKOH/g ± 0.2 |
| Free fatty acid | as Oleic acid = 1.78 %<br>as Lauric acid =1.26 %<br>as Ricinoleic = 1.88 %<br>as Palmitic acid = 1.62 % |

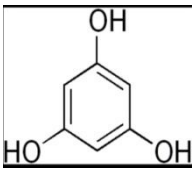
**Figure 1: Tannins three major structure on their monomer form:**




Hydrolysable Tannins




Condensed Tannins




Phloro Tannins

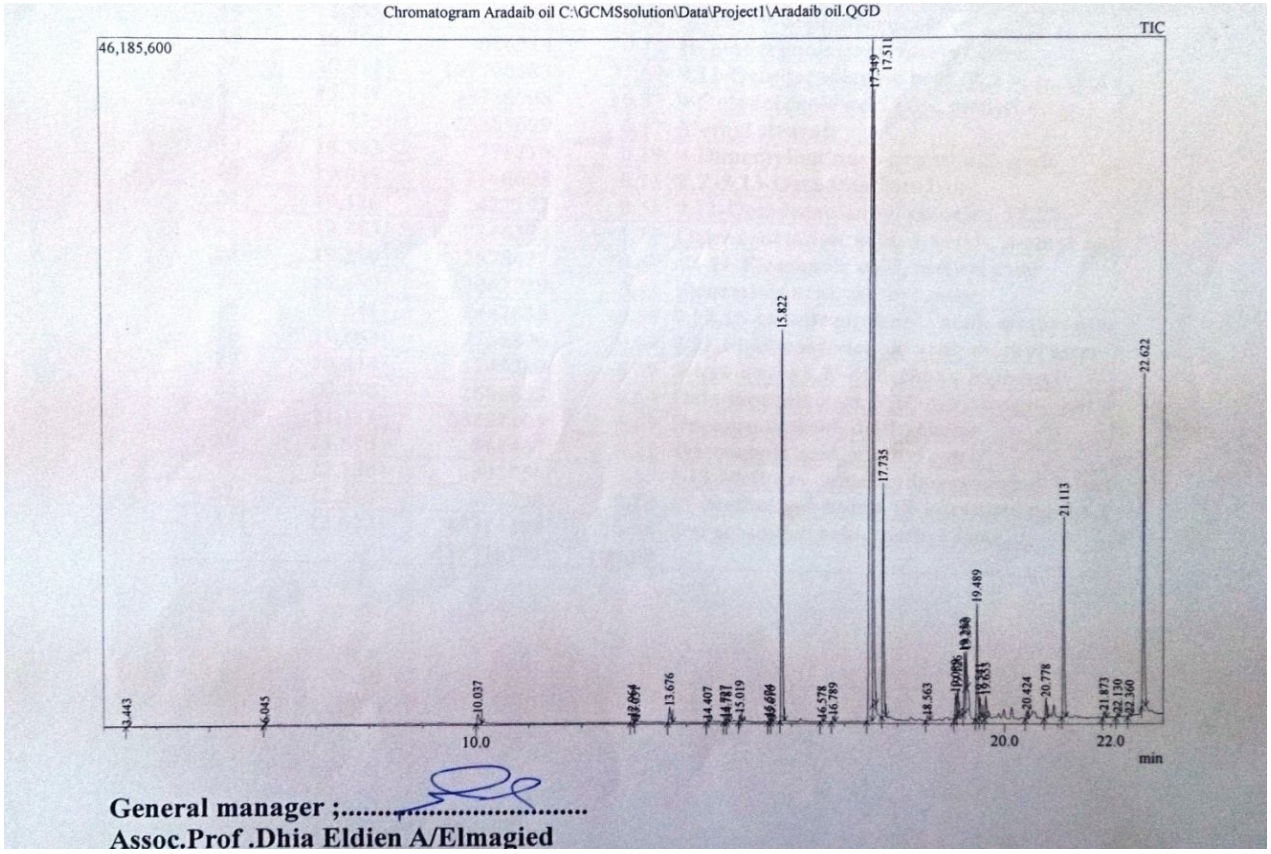
The GC-MS analysis of the *T. indica* seed oil is shown in Figure 2, where thirty three components were identified. The fatty acid composition data presented in Table 3 consist mainly of linoleic acid (37.59 %) followed by oleic acid (16.37 %), palmitic acid (11.72 %), lignoceric acid (10.56 %),stearic acid (6.17 %), behenic acid (5.69 %), arachidic acid (3.11 %) and Octadecanoic (1.70 %), some other free fatty acids were detected as major components ,while myristic acid which appear as main component in fatty acid analysis chromatogram reported by Balaji Panchal *et ,al* (2014) was absent. The detected levels of antinutritional fatty acid, behenic acid in *T. indica* (5.69%) is much higher than the value (0.02%) reported by Balaji

*et,al* (2014).Linoleic and linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance.

The values of all fatty acids in this study were found to be quite different from result that earlier reported by Balaji P. *et ,al* (2014),which found to be Oleic acid (0.19 %), Linoleic acid (0.41 %), Myristic acid (1 %), Luric acid (0.32 %) , Octanoic acid (0.3 %) , Palmitic acid (0.13 %) , Stearic acid (0.4 %) , Lignoceric acid (0.14 %) , Arachidic acid (0.06 %) , Behenic acid 0.02 %. Main while it can be considered to be within the range of Palmitic (14-20%), Stearic (6-7%), Oleic (15-27%), Linoleic (36-49%), Arachidic (2-4%), Behenic (3-5%) and Lignoceric (3-8%) investigated by Andriamanantena *et, al* (1983).

This could be due to the variation in environmental conditions in which the plants were grown. The levels of fatty acids were known to vary largely with season and geographical location. The variation in the fatty acid composition and their percentages could be due to the fact that the plant seeds are from different ecological origin. The variation in the composition and oil yield observed in this study could be related to several factors for example changes in temperature, extraction and environmental effect. The composition of the fatty acids (FA) in the plant fruit seed oils studied showed presence of various components which may be of nutritive value since they contain appreciable quantity of essential FA (EFA), which are long-chain polyunsaturated FA (PFA) derived from linolenic, linoleic and oleic acids ,that play important role in human life .

## Figure 2: GC-MS chromatogram of Tarmindus indica seed oil:



## Table 3: Fatty acid composition (%) of *T.indica* seed oil:

| Fatty acid | Determined values (%) |
|---|---|
| linoleic acid | 37.59 |
| Oleic acid | 16.37 |
| palmitic acid | 11.72 |
| lignoceric acid | 10.56 |
| Stearic acid | 6.17 |
| Behenic acid | 5.69 |
| Arachidic acid | 3.11 |
| Octadecanoic | 1.70 |
| Eicosenoic acid | 0.69 |
| Propenoic acid | 0.67 |
| Octadecanoic acid | 0.64 |
| Docosatrienoic acid | 0.64 |
| Linoleic acid | 0.58 |
| Margaric acid | 0.14 |

## Conclusions:

The present study deals with the general composition of the whole seed of tamarind and the extracted oil by use of solvent extraction. The followings are the conclusions drawn out from this study:

- The proximate composition of tamarind whole seed indicated that the carbohydrates are the dominant fraction (58. 59%) followed by protein (26.71%).
- The tamarind seed sample was extracted by hexane, temperature reflux 80 $^{0}$C, extraction time 6 hours and solid to solvent ratio of 1:6w/v yielded 3.26% of crude oil.
- The physicochemical properties analyzed of the oil show high saponification value (195.25 mgKOH/g) ,which explore their potential usage in the soap making and high peroxide value (17.22 mg$O_2$/g).
- The GC-MS study identified thirty three components, of which linoleic acid (37.59%) is predominant, followed by Oleic acid (16.37%), palmitic acid (11.72%) and lignoceric acid (10.56%), this indicated that, the stability of oil is high and may be use as anti-oxidant by blending with other vegetable oils.

## Recommendation and suggestion for further studies:

The results obtained in this study showed clearly that tamarind seeds can be used as a source for the extraction of oil that can be used in many industry applications. Further research is therefore required to extent the knowledge in this area of research. The following are suggested for further work in this field:-

- Other simple and economic methods of extraction, which can be effectively applied on wide area of industry.
- Further research to evaluate effects of *T. indica* treatments on the biological activities and anti-oxidant principles of extracted oil is recommended to explore their potential uses for pharmaceutical applications.
- To encourage the research of other possible part of tamarind that contains appreciated percentage of oil, such as pulp and leaf.
- Isolation of Essential fatty acid component in the oil that has a great nutrition value.
- By- product at factory scale can be used as animal feed (contain considerable percentage of protein).