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Isolation, Characterization and antimicrobial activity of Tamarind (*Tamarindus indica*) seed oil

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Abstract

The aim of this study was the isolation and characterization of tamarind seed oil. Tamarind samples were obtained from local market. Ash, moisture, protein and Mineral contents of seeds were measured using different analytical techniques. The fruit was tested for vitamin (C) contents. Tamarind oil was extracted by n-hexane using soxhlet apparatus and its chemical composition was investigated by GC/MS spectroscopy. Some physical and chemical properties of the extracted oil were also measured. They include density, acid value, peroxide value, saponification value and ester value and finally its antimicrobial activity was carried out .The obtained results showed that the percentage ash content for tamarind seed was 2.47%,the moisture content was 8.38% and protein as 20.78%. The inductively coupled plasma analysis showed that, tamarind seeds were rich in some minerals of macro and micro level including potassium(K), calcium(Ca), magnesium(Mg), phosphorous(P), copper(Cu), manganese (Mn) and zinc(Zn).The hazardous elements showed very low concentrations in tamarind seeds. Aluminium and silicon as undesired elements showed considerable concentrations in tamarind seeds. Titanium (Ti) ,strontium (Sr) and lead (Pb) were showed notable concentrations. The Tamarind fruit showed high content of vitamin (C). The GC-MS analysis of the oil gave considerable constituents of essential fatty acids including linoleic acid (44.956%), Oleic acid (23.98%), palmatic acid (11.984%), stearic acid (4.476%) and arachidic acid (3.032%). The antimicrobial activity of n-hexane extract of the seeds was evaluated against five gram-positive and gram-negative and it showed activity against bacteria *Escherichia coli* and fungi *Candida ablicans*.

Keywords: *Tamarindus indica*, seed oil, antimicrobial activity, GC-MS, fabacea family

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1. Introduction

Tamarindus indica a member of the family leguinosae fabacea 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 of 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).

1.1. Distribution in Sudan:

Tamarindus indica is a Sudanese tree which spreads into Sahelo-Sudanian zone. It is sometimes planted on account of it is dense shade and fruit quality. A number of plant species (e.g., coriander, lupine, Roselle, watermelon, okra and tamarind) grown in Sudan could be classified as a neglected and or underutilized crop. These species, although important for people, receive little or no attention as for as research and development is convened. However, some of the crops including tamarind play important role in the economy of Sudan and contribute considerable share 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 ElGhazal (El Amin, 1990).

1.2. Chemical composition of tamarind seed:

Morton (1987) indicated that the seed of tamarind contains approximately 63% starch, 14-18% albuminiods and 4.5-6.5% of semi drying oil. Seeds of the tree legume *Tamarindus 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. The crude fats present are 4.5% which contain a relatively large proportion of unsaturated fatty acids. The principle sugars of the seeds are mannose glucose and ribose. Moisture, ash, and crude fiber were found as 9.4%, 3.2%, and 8% respectively. 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, crude fiber was 2.33-3.82% moisture content was higher in 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 Bhattacharya et al., (1993) and Morad et al., (1978) showed moisture content range between 9.4-11.3%, proteins 13.3-26.9% fat/oil 4.5-16.2% crude fiber 7.4-8.8%, total ash1.6-4.2%.

Ibrahim et al., (1959) in their evaluation of tamarind seed grown in Sudan found seven hydrocarbon in the unsaponifiable matter of seed and GLC of methylated fatty acids revealed the presence of Palmatic, Oleic, Linoleic, and eicoasnoic as the major fatty acids of the seeds.

1.3. Seed oil:

The seed oil is golden yellow, semi-drying oil, which in some respects resembles groundnut oil. The major fatty acids were palmatic, oleic, linoleci, and eicosanoic. The lipids contained a relatively large proportion of unsaturated fatty acid, with linoleic acid (36-49%) in the highest concentration. Other major fatty acids are oleic acid (15-27%) and palmatic acid (12-20%) Singh, 1973). The physicochemical properties of the oils were analyzed. The *T. indica* seeds contain crude oil and fatty acid .i.e.8% and 2.92% respectively (Balaji *et. al,* 2014). Most research, on tamarind done in Sudan was on the pulp and for medicinal aspects. Khalid *et, al*,. (2007) investigated the potential of anti lesihmanail activity of some Sudanese medicinal plants. The results indicated that the methanolic extract of tamarind failed to

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exhibit any significant anti-leshimania activity against leishmania at concentration less than 100mg\ml. Mohmoud and Homeida (1994), indicated that a significant reduction was observed in the AUC plasma concentration versus time and C max (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. Imbibe 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 fungicidal and bactericidal properties (Imbabi *et. al*, 1992). 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 seed oil could be considered as milestone and guide for further research.

2. Materials and Methods

2.1. Collection and preparation of samples:

Five newly harvested fruit samples of different sizes were collected from shop for tamarind juice .The hard outer shells of the fruits were broken and the dark brown seeds were separated.

2.2 Chemicals:

- Potassium hydroxide(KOH) -99.9%- BDH chemicals ltd poole-England.
- Sodium hydroxide(NaOH)-99.6%. Lab tech chemicals.
- Sulfuric acid- 99.5%- d=1.84 g/cm^3- ALPHA CHEMIKA – India.
- Ethanol(CH_3CH_2OH)- 96%- African Modern Distillation for ethanol-Sudan.
- Chloroform- 99.8% - Lab tech chemicals- India.
- Glacial acetic acid - 99%- d= 1.040 g/cm^3- S D fine chem limited – India.
- Hydrochloric acid (HCl)- 35%- d= 1.200 g/cm^3- ALPHA CHEMIKA – India.
- Sodium thiosulphate-98%- S D fine chem limited – India.
- Phenolphthalein indicator - S D fine chem limited – India.
- Starch- Chadwell health ESSEX – ENGLAND.
- Potassium iodide (KI)-66%- S D fine chem limited – India.
- Normal hexane-97%-d= 0.6606g/ml- Chevron philipes chemical company.
- Nitric acid (HNO_3)- 99.9%- d= 1.5129 g/cm^3 ALPHA CHEMIKA – India.
- Sodium chloride (NaCl)- . 99.9%- Lab tech chemicals.
-distill water

2.3. Instruments.

Soxhelt extraction system (Duran UK), Sensitive balance (GH252) UK., Rotatory evaporator (Buchi Switzerland), Moistur analyzer (Dsh-50 -10Auto), Inductivity Coupled Plasma (ICP – OES725 ES) (Vista-MPX-CCD), Electric muffle furnace 575(TAPP T211 om-39, Gas chromatography-Mass spectrometer (GC/Mass) QP2010-Ultra'Simadzu Company-Japan.

2.4. Methods:

2.4.1. Oil content:

100 grams of Tamarind seeds were crushed to a coarse powder using mortar and pestle. The coarsely powdered sample was successively extracted by hexane using soxhlet extractor .Extraction was carried out for six hours till the colour of solvent at the last siphoning time turned colorless. Solvent was then evaporated under reduced pressure using rotary evaporator. The extracted oil was left in open beaker at room temperature for complete evaporation of the solvent. The yield percentage was calculated as follow:

Oil content= (Weight of oil obtained / weight of plant sample) X100%

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2.4.2. Protein content:

(1g) of sample was weighted and transferred to a Kjeldahl digestion flask.(1g) of catalyst mixture (90% anhydrous sodium sulphate and 10% copper sulphate) were added followed by 3 ml conc. Sulphuric acid, the flask was heated gently, cooled and digested into distilling flask with 15 ml of NaOH 40%soluation. (50ml) of boric acid solution and 3 drop of methyl red were placed in receiving flask. The distillation apparatus was connected up with delivery tub dipping below the boric acid solution. The distillate solution was titrated against o.1NHCL. The percentage of nitrogen was calculated: 1ml o.1 N=0.00014g N. and then crude protein was calculated using approximate factor Nx6.25

2.4.3. Moisture content

About 2 grams of crushed seeds were analyzed by using Moisture analyzer device, the experiment was repeated three times and the average weights were recorded.

2.4.4. Ash content

Determination the total ash was preformed according to Pearson (1968), by weighted out (5g) of the sample into platinum dish which had previously been ignited and cooled before weighted. 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 1 0 minutes. Then the ash content was calculated as a percentage.

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

2.4.5. Oil Density

The density of water was determined with help of specific gravity bottle, 5 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 filed with oil to calculate the density of oil by the same method.

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

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

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

2.4.6. Chemical properties

i. Acid value:

One gram of oil was weighed and dissolved with 50ml of ethanol in a conical flask and about one milliliter of phenolphthalein indicator was added. The mixture was boiled for about five minutes and titrated to pin point end point with 0.1 N potassium hydroxide solutions. Acid value as calculated:

$$\text{Acid value} = \frac{56.1 \times V \times C}{W}$$

Where:

56.1: equivalent weight of KOH
V: the volume in ml of standard volumetric KOH solution used
C: is the exact concentration on KOH solution used (0.1N)
W: is the weight of oil (1g)

The calculations in terms of different fatty acids are as follows:

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

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

C) Free fatty acid in term of ricinoieic acid, % by weight =$\frac{29.8VN}{W}$

D) Free fatty acid in term of palmtic acid, % by weight =$\frac{25.6VN}{W}$

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ii. Saponification value:

2 g of oil were weighed into a 250-ml flask, 25 ml of the ethanolic of potassium hydroxide solution were added, and connected to reflux air condenser to the flask. Heated on a water-bath for one hour, boiled gently until the sample was completely saponified as indicated by absence of any oily matter and appearance of clean 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.

$$\text{Saponification value} = \frac{28.05\ (V2-V1)}{W}$$

V1: is the volume of titrant used in oil titration
V2: is the volume of the titrant used in blank titration
W: is the weight of the oil

iii. Determination of Peroxide value

2.5 g oil samples were weighed in a 250 ml conical flask and 30 ml of solvent mixture (2:3) of chloroform and glacial acetic acid were added to the flask content was well Shaken, then Half ml of 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 0.01N sodium thiosulfate solution using starch indictor until the yellow color was discharge. A blank was prepared alongside the oil samples.

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

Where:

V1: is the volume of titrant used in oil titration
V2: is the volume of the titrant used in blank titration
W: is the weight of the oil

iv. Determination of Ester value

Ester value was obtained by substracting the acid value from saponification value.

v. Determination of Vitamin C content

50 mls of the saturated tamarind juice were prepared by dissolving excess grams of tamarind in 50 ml of distilled water, the solution was filtered and transferred by pipette into 250 ml volumetric flask the solution was diluted to the mark with distilled water.10 ml of the solution was transferred into titration flask and 20ml of iodine were added and 1ml of starch. The solution was titrated against thiosulphate (0.05N) solution the experiment was repeated three times until constant value was reached

$$\text{Vitamin C} = \frac{B-S}{W} \times 0.0891 \times 10$$

Where:

B= is the volume of the titrant used in blank titration.
S= is the volume of titrant used in oil titration.
W= weight of sample.

vi. Determination of minerals (ICP Analysis)

0.5g/50 ml of sample was burned for five hours by using furnace and then 5 ml of hydrogen peroxide and 5ml of nitric acid and 5 ml of hydrochloric acid were added. A prepared solution containing analyte elements is aspirated into the plasma generated by inductively coupled plasma source; the optimized elements produced characteristic emission spectral lines, which are separated by simultaneous optical spectrometer. The intensity of spectral line of an element is proportional to its concentration.

2.4.7. Preparation of bacterial suspensions:

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37° C for 24 hours. The bacterial growth was harvested and

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washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.4.8. Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

2.4.9. Testing of antibacterial susceptibility

i. Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

2.4.10. Gas Chromatography - Mass spectrometry (GC-MS) analysis:

i. Sample preparation (Methylation):

2ml of sample was taken into test tube. (7 ml) of alcoholic sodium hydroxide that prepared by dissolving (2g) sodium hydroxide in 100 ml methanol was added.(7ml) of alcoholic sulphuric acid(1%) that prepared by mixing (1ml) Conc. sulphuric acid+99 ml methanol was added. Then shacked for 3 minutes and left to overnight. (2ml) of saturated NaCl was added. Another (2ml) of hexane was added, shacked for 3 minutes and the hexane layer was collected.(5ml) from collected hexane was diluted with (5ml) diethyl ether and (1g) of sodium sulphate as drying agent. Filtered through syringe filter 0.45um.

ii. Sample injection:

(1u1) was directly injected to GC.MS-QP2010 Ultra equipped with Rtx-5 MS, 30 m length, o.25mm diameter and 0.25ml thickness. The column temperature was kept at 60c for 10 min, with increase at 10c per min up to injector temperature 300c, split ratio 1:0, the carrier gas (Helium0flow rate 1.5 ml/min. the compound were identified by the GC-MS intensity of retention time (RT) and by comparison with those present in NISTIIS.LIB. the results were expressed as the relative percentage of each individual compound present in sample given by the corresponding RT.

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3. Results and Discussion

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 composition of tamarind seed. The results were expressed on dry weight basis.

Table 1: Proximate chemical composition of *Tamarindus indica* seed:

Test	Result
Oil	3.19
Moisture	8.38
Protein	26.71
Ash	3.53

The oil content value shown in table (1)was (3.19) which is lower than the value of *Bhattacharya et. al*., (1993) and Morad *et.al*., (1978) for the whole seed.

The moisture content of tamarind whole seed was found to be (8, 38). 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 Bhattacharya *et. al*., (1993) and Morad *et .al.,* (1978). According to Pearson (1968) classification of oil based on their moisture content, Tamarind seeds oil classification as semi-drying oil The protein content of tamarind whole seed was found to be (23.71) 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 Bhattacharya *et.al.,* (1993) and Morad *et, al.,* (1978). The ash content of tamarind whole seed was found to be (3.53), which lies within the range of 91.6-4.2%) reported by Ishole *et.al.,* (1990), Bhattacharya *et.al.,* (1993) and Morad *et.al.,* (1978).

Table (2) present the results of the physicochemical analysis of oil of *T. indica* was visually green with yellow in colour, liquid at room temperature (30 ^{0}c) and has density of (0.855)gcm^{3}.

Table 2: physical and chemical properties of oil from *T. indica* seeds:

Test	Result
Color	Red =4.3 Yellow =7.3 Blue =3.5
Density	0.855g/cm^{3}
Saponification value	196.6mgKOH/g
Peroxide value	17.2mgO_2/g
Acid value	3.9mgKOH/g
Ester value	192.7mg/g
Free fatty acid	as Oleic acid = 1.9% as Lauric acid =1.4% as Ricinolic acid = 2.08% as palmitic acid =1.79%
Vitamin C	4.32mg/100g

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The saponification value (196.6) mg KOH/g is a higher than the value (186.10) found by Balaji Panchal *et. al.,* (2014) such value indicates the average molecular weight of triglycerides in the oil. High saponification value in tamarind oil suggests that, it could be used for production of soap. The acid value (3.9) mg KOH/g which is higher than the value (0.5) reported by Balaji Panchal *et.al.,* (2014) the acid value calculation lead to (1.97%)free fatty acid as Oleic acid, (1.4%) as Lauric acid, (2.08%) as Ricinoleic and (1.79%) as Palmitic acid .

The peroxide value was (17.2) mgO_2/g oil in *T. indica*, although it is acceptable for crude oil but it is relatively high.

Mineral contents of *T. indica* seeds:

The results obtained by using inductively coupled plasma (ICP) analysis, it was showed that the percentage of heavy metals was as follow: Cadmium <0.0005 ppm for the seeds. The concentration of trace elements in the seed were; Aluminum (239.1ppm), Barium (<0.0006 ppm), Cobalt (0.0400ppm), Copper (6.250ppm), Iron (138.5ppm), Manganese (4.930ppm), Sodium (7.080ppm), Vanadium (3.550 ppm), and Zinc (22.21ppm).

Table 3-1 Macronutrients contents of *T. indica* seeds:

Elements	Concentration in seeds (ppm)
Na	7.080
K	4557
Ca	2017
Mg	1755
P	1399

Table 3-2 Macronutrients contents of *T. indica* seeds:

Elements	Concentration in seeds (ppm)
Co	0.0400
Cu	6.250
Fe	138.5
Mn	4.930
Mo	0.3600
Ni	1.280
Zn	22.21

Table 3-3 Toxic and hazardous elements in *T.indica* seeds:

Elements	Concentration in seeds (ppm)
Al	239.1
Ba	<0.0006
Cd	<0.0005
Cr	<0.0006
Li	<0.0007
Pb	<0.0013
Si	315.1
Sr	<0.0006
Ti	20.35
V	3.550

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The minerals content of the tamarind seed were characterized with high concentration of Sodium, calcium and magnesium [Na (168.4ppm), Ca (161.1ppm) and Mg (196.0ppm)] as shown in tables (3-1)(3-2)(3-3) above. The GC-MS analysis of the *T. indica* seed oil was shown in figure 1, where seventeen components were identified. The fatty acid composition data presented in table 4 consist mainly of linoleic acid (44.956%) followed by oleic acid (23.98%), palmitic acid (11.948%), stearic acid (4.476%), arachidic acid (3.032%), octadecanoic (2.097%), myristic acid(0.1056%), palmitioleic acid (0.058%), margaric acid (0.051%) some other free fatty acids were detected as major component, while myristic acid which appear as main component in fatty acid analysis chromatogram reported by Balaji Panchal *et, al* (2014). The detected levels of antinational fatty acid, behenic acid in *T. indica* (0.00%) is lower than the value (0.02%) reported by Balaji *et,al* (2014). Linoleic and linolenic acids are the most important essential fatty acids requited for growth, physiological functions and maintenance. The values of some of the fatty acids were found to be different from that the earlier reported by Balaji *et,al* (2014), which found to be oleic acid (0.19%), Linoleic acid (0.41%), Myristic acid (1%), Luric acid(0.32egra%), Octanoicacid (0.3%), Palmitic acid |(013%), Stearic acid (0.4%), Lignoceric acid (0.14%), Arachidic acid (0.06%), Behenic acid ().02%). 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 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 fatty acid ,which are long-chain polyunsaturated fatty acid derived from linolenic, linoleic and oleic acids, that play important role in human life. These fatty acids play an important role in modulating human metabolism and reduce cholesterol levels; this suggests that tamarind oil may be useful as cooking oil. Tamarind fruit contain 4.32mg/100ml of vitamin C content that are essential for skin care.

Table 4: Fatty acid composition (%) of *T. indica* seed oil:

Fatty acid	Determined values (%)
Linoleic acid	44.956
Oleic acid	
palmitic acid	11.948
stearic acid	4.476
arachidic acid	3.032
Octadecanoic	2.097
myristic acid	0.1056
palmitioleic acid	0.058
margaric acid	0.051
Docosanoic acid	1.988
Tetracosanoic acid	3.590

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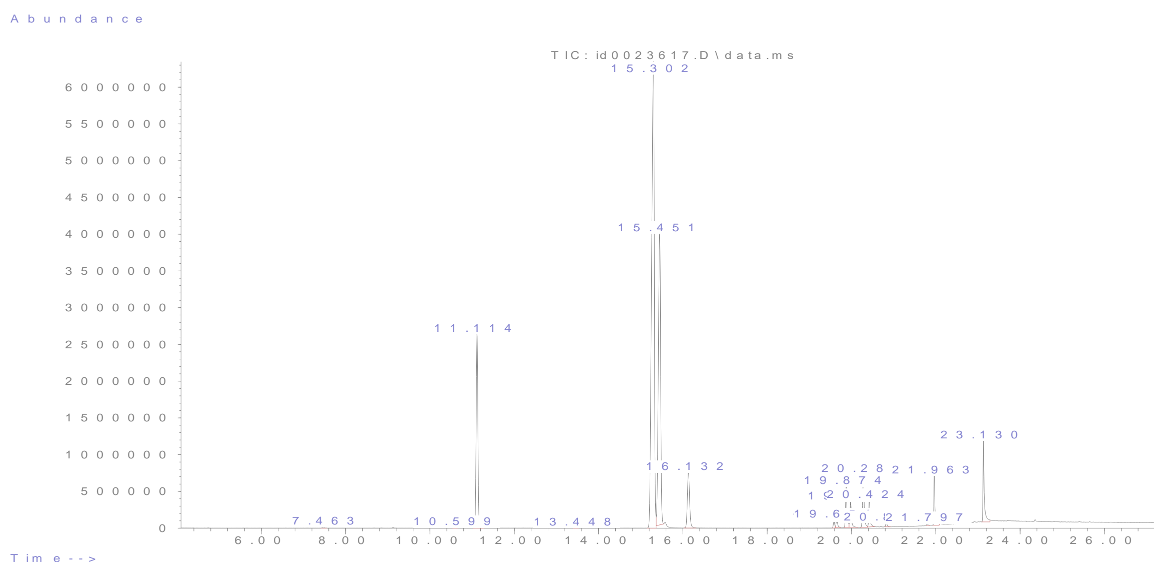

Figure 1.The GC-MS analysis of the *T. indica* seed oil

3.4 Anti-microbial activity

The n- hexane extract of the shade seeds of *Tamarindus indica*. Are tested for the Anti-microbial activity. After the incubation the diameter of the resultant growth inhibition zones was measured, averaged and the results are summarized in table (4).

Table (4) antimicrobial activity of the n-hexane extract:

Bacteria and fungi	Zone of inhibition (diameter, mm)
Bacillus subtillis	-
Staphylococcus aureus	
Escherichia coli	13
Pseudomonas aeruginosa	-
Candida albicans	14

The normal Hexane extract showed activity against the bacteria *Escherichia coli* and fungi *Candida albicans* See fig (5) (6).

1-14 low activity
14-18 Medium activity
18 high activity

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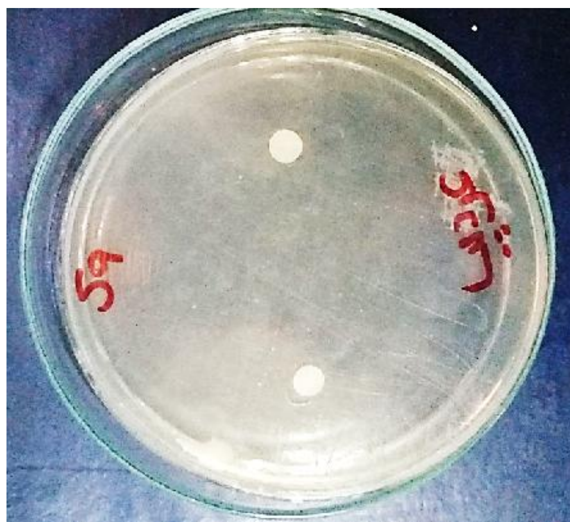

Figure 2. Inhibition zone of n-hexane extract of the seed *Tamarindus indica* against *Staphylococcus aureus.*

Figure 3. Inhibition zone of n-hexane extract of the seed *Tamarindus indica* against *candida albicans*.

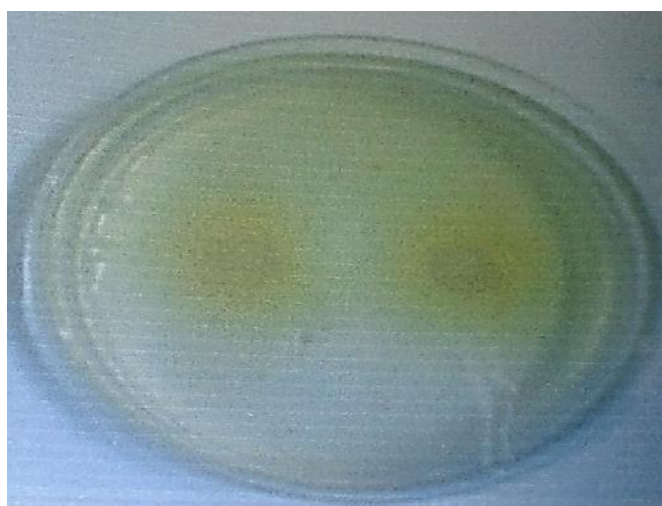

Figure 4. Inhibition zone of n-hexane extract of the seed *Tamarindus indica* against *Escherichia coli*

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4. Conclusion

The tamarind seed sample was extracted by n-hexane, temperature reflux 80 , extraction 6 hours and solid to solvent ratio of 1:6w/v yielded 3.19% of oil. Tamarind oil has good quality when compared with edible oils and also have potential for medicinal uses. The physiochemical properties analyzed of the oil show high saponification value (196.6mgKOH/g), and high peroxide value (17.2mgO_2/g).The GC-MS study identified seventeen components, of which linolice acid (44.956), is predominant, followed by Oleic acid (23.98), palmitic acid(11.984), and stearic acid (4.476), this indicate that , the stability of the oil is high, the obtained results showed that tamarind fruit and seeds have considerable contents of essential minerals that are, important for human health. Tamarind fruit contain high content of vitamin C.

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