CHARACTERISATION OF OIL EXTRACTED FROM *PARINARI CURATELLIFOLIA* (HACHA) NUTS

A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZIMBABWE IN PARTIALFULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY

DATE: .................................................
DECLARATION

I, Tinashe Keith Ndaba, declare that this work is entirely the product of my own findings and has never been presented for any academic award. Any reference to previously published work has been clearly indicated.

Sign: ............................................ Date: ..................................................

STUDENT

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SUPERVISOR

Department of Chemistry, University of Zimbabwe

Sign: ............................................ Date: ..................................................

CHAIRPERSON

Department of Chemistry, University of Zimbabwe
DEDICATION

To my wife Forgiveness and children Anoshamisa and Anouyaish
ACKNOWLEDGEMENTS

I thank God for seeing me through this Masters Programme through the cooperation of the following people: Dr J. Kugara for developing the idea behind the research work and supervising the project. Dr L. Nhamo for supervision. Mr Wakandigara, Dr Makhubalo, Ms Jiri, Professor Zaranyika and Mr S. Kahwai for the foundation of my studies through the courses they taught. The content helped in carrying out the practical work among other things in this research. I would also like to thank the laboratory Technicians for their support in carrying out the practicals. They were always there to assist when required. My gratitude also goes to the rest of the Chemistry Department at the University of Zimbabwe for their support and my fellow students for all the interactions and advice. Lastly, I would like to thank my friends and relatives who supported me financially and my beloved wife and children for their support and encouragement throughout my studies.
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LIST OF ACRONYMS

AOCS  America Oil Chemists Society

AV    Acid value

FAME  Fatty acid methyl ester

FFA   Free fatty acid

IV    Iodine Value

MUFA  Monounsaturated fatty acid

PV    Peroxide value

PUFA  Polyunsaturated fatty acid

SV    Saponification Value

TFA   Trans fatty acids
PHOTOGRAPHS

A to U by T.K.Ndaba

All other photos used in this document have their sources stated.
ABSTRACT

Several oils have been characterised by many researchers as a way of unravelling their usefulness. Chinweubwa Arinze Jude, 2013 extracted oil from ground *Nicotiana tabacum* seeds by soxhlet. The percentage oil content was 36.75%. The oil was characterised in terms of iodine value, saponification value, acid value, free fatty acid content and specific gravity. The oil content of the seeds showed that processing of the oil is economical. The physiochemical properties showed that the oil was highly unsaturated and had semi-drying properties. The semi-drying nature of the oil and its saponification value made it fit for the production of hair shampoo and shoe polish.

In this research, dry parinari curatellifolia seeds were crushed using a stone to obtain nuts from the kernel. 40 grams of the nuts were weighed and oil was extracted from them using soxhlet apparatus. 350 ml hexane was used as a solvent. The solvent was evaporated using a rotary evaporator to remain with the oil. The oil content was calculated based on the remaining residue and the value obtained was 36.75%. The oil was characterised by determining the acid value (AV), peroxide value (PV), iodine value (IV), saponification value (SV) and the fatty acids present using Gas chromatography. AV range was 0.477-0.532. It is an indicator of the proportion of free fatty acids in the oil (It seldom exceed 0.5 for most oils). PV range was 1.69-1.9. It gives the initial evidence of rancidity of oil. The range of values shows that parinari curatellifolia oil is less susceptible to primary oxidation. IV ranged from 70.5-81.54 showing that the oil contains fatty acids with few double bonds. The results are in agreement with fatty acids confirmed to be present in parinari curatellifolia oil by Oladimeji AO *et al* (2011). The fatty acids are oleic acid, gondoic acid and linoleic acid. The first two are monounsaturated and the third one is an essential fatty acid. The oil is quite edible and preferred for good health due to the presence of monounsaturated fatty acids and essential fatty acids. It can also be employed in the cosmetics industries for beauty soaps, hair and skin oil products.
CHAPTER 1: INTRODUCTION

1.1. Background.

Fats and oils are abundant lipids in nature (David W. Ball et al.). They are triglycerides containing three fatty acid units joined to glycerol to form esters. Fats are solid at 25°C whilst oils are liquids at this temperature. These differences in melting points reflect differences in the degree of unsaturation and number of carbon atoms in the constituent fatty acids (David W. Ball et al.).

Fatty acids are carboxylic acids with chain lengths from 6 – 24 carbon atoms. Most naturally occurring fatty acids have an even number of carbon atoms, from 4 to 28 (IUPAC Compendium of Chemical Terminology, 1997). They are classified according to the extent of saturation. Unsaturated fatty acids have one or more double bonds between carbon atoms in the chain while saturated fatty acids have no double bonds in the carbon chain. Vegetable oils are the main source of fatty acid, which consist mainly of triglycerides (95% - 98%), the remaining (5% - 2%) consists of complex mixtures of compounds in a wide range of chemical classes (E. Sikorska, A. Romaniuk, et al., 2004). The proportion of saturated and unsaturated fatty acids has an important role on the properties of vegetable oils. Degradation of vegetable oil is essentially caused by the unsaturated fatty acid oxidation which involves complex reactions that generate mainly hydroperoxides but also volatile compounds through a three-phase process: initiation, propagation and termination (M. Laguerre et al., 2007).

Some of the fatty acids are essential to the human body because the body is incapable of making them. Humans do not have the ability to introduce double bonds in fatty acids beyond carbons 9 and 10, as counted from the carboxylic acid side (Bolsover, Stephen R. et al., 2004). Examples of these essential fatty acids are linoleic acid and linolenic acid.
1.2. Importance of plant based oils

Edible oils from plant sources are important in foods and in various other industries. They are key components of the diet and provide characteristic flavours and textures to foods (D. Rudan-Tasic and C. Klofutar, 1999). Nuts oils, seed oil and oils of fruit and vegetables are receiving much interest due to their high concentration of bioactive lipid components, such as polyunsaturated fatty acids and phytosterols, which have shown various health benefits. Fats and oils are extensively used in the food and also in cosmetics, pharmaceuticals, oleochemicals and other industries (Maria Cristina Straccia, Francesco Siano et al., 2012). Polyunsaturated fats and oils are, more desirable not only indietary terms but also in general application for paint formulation (I.C. Eromosele, C.O. Eromosele et al., 1993). They are very good sources of vitamin E. Fat-soluble vitamins A and D sometimes are added to foods which contain fat because they are good carriers and are widely consumed (Fediol). Tocopherols are important minute constituents of most vegetable fats. They serve as antioxidants to prevent rancidity and as sources of the essential nutrient vitamin. Coloured materials called Carotenoids occur naturally in fats and oils. Their levels are reduced during the normal processing of oils to give them acceptable colour, flavour, and stability (Fediol). This means cold expressed edible oils are rich in nutritional value than their refined products despite the short shelf life. With the prevalence of coronary heart diseases in most communities (even the developing countries in which such diseases have been uncommon in the past five decades), knowing the type of fats one is consuming is critical. Most food staffs prepared in fast foods outlets and restaurants are fried foods. In the interest of business mileage, hydrogenated vegetable oils are used despite their negative health effects. Animal meats, butter, whole milk, and some tropical plant oils, like palm and coconut, are the main sources of saturated fats. Saturated fats raise the level of bad cholesterol with potential health consequences. They should, therefore, be consumed in small quantities as part of a balanced diet. Monounsaturated fats are the most preferred type of fat in the diet because they help to decrease the bad cholesterol in the blood and help to increase the good cholesterol. Good sources of monounsaturated fat are canola oil, olive oil, peanut oil, and most
nuts. Olive oil has the highest percentage of monounsaturated fats of any edible oil (Fediol). Risks of obesity, diabetes, hypertension, and cardiovascular disease, along with their ensuing complications often go hand-in-hand and are strongly linked to lifestyle, especially diet choices (US Department of Health and Human Services). Double bonds present in the naturally occurring unsaturated fats are in the Cis form. Trans fatty acids are associated with health problems and heart diseases. Three main origins for trans fatty acids are bacterial transformation of unsaturated fatty acids in the rumen of ruminant animals, industrial hydrogenation and deodorisation and during heating and frying of oils at very high temperature (Fediol). This implies that choosing virgin vegetable oils in preference to other oils has got the effect of reducing the risk of cardiovascular diseases. Oils from plants are also used to make soaps, skin products, candles, perfumes and other cosmetic products (Dawodu, 2009; Ferris et al., 2001)

1.3. Underutilisation of plant resources

In human history, many plant species have been regularly used for food, fibres, industrial, cultural and medicinal purposes (S. Padulosi and E. Frison; 1999). Some cultivated species are in use today around the world. The rest of the vast majority of the remaining agro-biodiversity have been replaced or fallen into disuse, while others have remained important in their centres of origin or secondary centres of diversity, but largely ignored by commerce and science (S. Padulosi and E. Frison; 1999; Global forum on agricultural research-GFAR). There are many potential species for domestication that have commercial potential in local, regional and international markets. Little or no formal research has been carried out on many of these wild species to assess potential for genetic improvement, reproductive biology or suitability for cultivation. (R.R.B. Leakey and Z. Tchoundjeu; 2001; Diversification of tree crops: Domestication of companion crops for poverty reduction and environmental services; Expl Agric. (2001), volume 37, pp. 279±296).
The situation highlighted by R.R.B. Leakey and Z. Tchoundjeu back then still persists despite research work done by several Scientists to date. This work includes: Regeneration of threatened indigenous fruit species in Uganda by Richard Mawula (2009); Properties of Parinari curatellifolia (hacha or chakata) fruits from different parts of Harare, Zimbabwe by C. Benhura *et al* (2013) and many more published papers. Their work has been influential in keeping interest in the underutilised resources in the world.

In several countries a realisation of the importance of wild edible fruits and vegetables has led to research work aimed at alleviating poverty in less privileged communities. In India, bio-prospecting of wild edibles for rural development in the central Himalayan Mountains has been done. 13 wild fruit species with potential for exploitation and 1 semidomesticated species with good potential for exploitation were selected and examined closely in terms of economic potential. A variety of value-added edible products such as jam, jelly, juice, and squash were made to generate income from these wild fruits for poor rural people. This was demonstrated locally to encourage people to engage in small-scale village industries (Rakesh K. Maikhuri; Kottapalli S. Rao; Krishna G. Saxena; 2004; Mountain Research and Development Vol 24 No 2 May 2004: 110–113).

Another similar research has been done on Cultural significance of medicinal plant families and species among Quechua farmers in Apillapampa, Bolivia. 341 medicinal plant species were identified during guided fieldtrips and transect sampling. Data on medicinal use was obtained from fifteen local Quechua participants, eight of them being traditional healers (Evert Thomas, Ina Vandebrrok, Sabino Sanca, and Patrick Van Damme, 2008; Journal of Ethnopharmacology 122 (2009) 60–67). Such work supported with laboratory research can open up hidden treasures dormant in languishing societies in the world. In addition to documenting the findings, investigations can be made into commercialising these plant species, reproducing them on a large scale for those which can be domesticated and identifying possible markets for the products. Since different plant species thrive in differing climates, nothing can hinder the exportation of products from such scientifically proven
species to other countries where they are not found. A dual benefit is realised in the sense that apart from monetary gains (a great factor in poverty alleviation) there is preservation of the plant species from extinction and a natural sink into oblivion.

1.4. Threatened plant species

With the prevailing volatile environment in the world at large, important natural resources remain under threat. A study carried out in Singapore showed that catastrophic extinctions followed deforestation. Substantial rates of documented and inferred extinctions were confirmed. Forest reserves comprising about 0.25% of Singapore's area now harbour over 50% of the residual native biodiversity. Extrapolations of the observed and inferred local extinction data imply that the current unprecedented rate of habitat destruction in Southeast Asia will result in the loss of 13–42% of regional populations over the next century, half of which will represent global species extinctions (Barry W. Brook, Navjot S. Sodhi and Peter K. L. Ng; 2003; Nature 424, 420-426 (24 July 2003)).

Another study carried on in 2009 by Richard Mawula: Regeneration of threatened indigenous fruit species in Uganda showed that the mobola plum tree faces gradual extinction if deliberate efforts are not put in place to monitor the use of this important resource. Debarking of the trees for medicinal herbs harvesting (as shown by the photographs below) leaves the tree dying from lack of water.

Source: CBM Master’s Thesis No.67-Mawula R. / Regeneration of threatened indigenous fruit species in Uganda
In Zimbabwe, there is evidence that with the growth of organized agriculture, land clearing and deforestation resulting from increasing fuel-wood demands, several species of wild fruit and vegetable plants are rapidly disappearing from the rural dietary. In a survey carried out in Masvingo province by Kaeser-Hancock and Gomez, (1985) it was found that communal farmers recognized the names of certain wild fruit trees but no longer consumed the fruit due to the disappearance of these species from the natural vegetation (Manel I. Gomez, 1988). With the prevalence of tobacco growing in Zimbabwe, the miombo trees face a giant threat of extinction owing to their excellent charcoal. Most farmers cannot afford coal or coke for tobacco curing and heavily rely on wood. Considering that it is a slow growing tree, extinction can be far ahead of aforestation.

In view of this, Manel I. Gomez rightly puts it: It is important, therefore, that efforts be made to investigate and document the lesser-known food resources of plant and animal origin and to conserve those with promising and proven potential as food, to improve the yield and quality of these foods through documenting, selecting and breeding, and to expand utilization through appropriate technologies. It is equally important to preserve the traditional knowledge which up to today has been an oral heritage in a more durable form for propagation through more systematic and widespread channels such as extension and training (Manel I. Gomez, 1988).

Considering the two major factors highlighted above which are, underutilisation of plant resources and the threats of extinction of some important plants, it is imperative to carry out a study on *Parinari curatellifolia*. The tree happens to be found in abundance in Zimbabwe. This fruit has proven to be of great value during the times of drought in Zimbabwe. For example, food shortages caused by economic problems and drought experienced in the year 2008 in Zimbabwe led people of the rural communities to consume *Parinari curatellifolia* fruit. The fruit saved many families from starvation and hunger as the food shortage was severe during the stated period (C. Benhura *et al*; 2013).
1.5. Problem statement

In Uganda the mobola plum tree (muhacha) is viewed as a key source of household income in rural areas (Mawula R. 2009). Locally it has been a chief food supply in times of drought for both man and other animals. However, there is less knowledge on the value (both nutritional and medicinal) of the oil obtained from the seeds (nuts) of the fruit. Furthermore, there is no substantial evidence of investigations done in Zimbabwe to ascertain the nutritional value of the entire nut though it is a known fact that it is highly nutritious. According to one literature source, although analyses have yet to be done, it seems probable that the nut has considerable nutritional value (Lost crops of Africa: Volume III: Fruits, 2008). Given the abundance of the miombo trees in Zimbabwe a break-in into the importance of the tree can lead to a breakthrough in many less privileged societies where this tree is found. This in-turn has got a ripple effect of conservation of this important tree as a result of impartation of knowledge otherwise hidden to many at the present moment.

1.6. Objectives

1.6.1. Overall objective

The overall objective of this investigation is to add on the knowledge base of indigenous wild species of Zimbabwe and the world through determining the nutritional, medicinal, economic, social and environmental importance of the oil extracted from the nuts of miombo (hacha) fruits.

1.6.2. Specific objectives

- To determine the oil content of parinari curatellifolia nuts found in Zimbabwe as a percentage of the entire nut.
- To characterise the oil extracted from the nuts.
- To relate the findings from the investigation to nutritional, medicinal, economic, social and environmental importance in Zimbabwe.
CHAPTER 2: LITERATURE REVIEW

Mobola plum (*parinari curatellifolia*) plants are widespread in tropical Africa from Senegal to Kenya and southwards to northern South Africa, with the highest concentration in Zimbabwe and the low veld region in South Africa, *Fruitipedia*. It is from the Chrysobalanaceae family (mobola family/coco plum family) of plants. The fruits have an oval shape, with grey scales and skin colour ranging from yellow to reddish brown (Ruffo K, Birme A and B Tengnas; 2002). When ripe, the fruit is edible, rich in vitamin C and can readily be made into a nutritious syrup or more commonly porridge. The fruits are occasionally utilized for the brewing of alcoholic beverages. The seeds, which bear oil, may be eaten raw in the form of nuts whilst leaf and bark extracts may be used as a remedy for symptoms of pneumonia or to treat ailments of the eye or ear. The bark and leaf extracts can also be used for tanning purposes, for example the tanning of leather, (Maharaj, V. and Glen, H. F, 2008). Although the tree grows and matures slowly it is so highly valued such that it could have a future as a plantation crop. The combination of a tasty fruit and an easily stored nut provides a double advantage for domestication. Species of parinari are easily reproduced via root suckers. These root cuttings provide the key to propagating good specimens. Through them, quality plantings could be quickly and easily established across much of Africa, clustered perhaps in villages, or scattered alongside roads in the valleys and tracks on hillsides. (*Lost crops of Africa: Volume III: Fruits*, 2008)

The species can regenerate through vegetative propagation though at times they are rarely found regenerating sexually, (*Mawula R. 2009*).

Parinari curatellifolia trees produce abundant nectar and pollen, which makes them popular with honey farmers. It produces good charcoal. The wood has a pink-brown colour, with a featureless grain scored by many narrow pores, which show up well on the flat or tangential surfaces. It is hard and heavy (720 kg/m³). It is borer proof and, despite its less durability if left exposed to weather, it has been used fairly extensively for rafters, beams, and poles, benches, building mortars, railway sleepers, canoes and mine timber. However, it contains silica crystals that make it difficult to work,
as they speedily blunt saw blades and other tools (Lost crops of Africa: Volume III: Fruits, 2008). Dyestuff is extracted from the bark and used in tanning. Leaves are also used for dyeing materials. Medically hot fomentations of the bark are used in the treatment of pneumonia. A leaf decoction is either drunk or used in a bath to remedy fever. Crushed or pulped leaves are used for dressing fractures or dislocations, and for wounds, sores and cuts. After stripping, the twigs can be used as a toothbrush. Parinari curatellifolia is a neat, compact shady tree for the average garden. The high content of cellulose limits the use of the oil cake but it can be used as manure. It is a good tree to grow in orchards or in home gardens, (Orwa C. et al, 2009). Locally (in Zimbabwe) research work has been done on the plant of interest in this proposed investigation, *parinari curatellifolia*. The properties of the fruits from different parts of Harare have been investigated. The parameters evaluated include among others, mass, proportion of pulp, skin and stone in the fruit, moisture, mineral ash and minerals namely magnesium, iron, manganese, copper and phosphorous (C. Benhura et al; 2013). Further research work has been done by the same authors on assessment of the Colour of *Parinari Curatellifolia* fruit using an Image Processing Computer Software Package (C. Benhura et al; 2013). More work is still being done on the products which can be made from this fruit.

A study of the toxicity, anti-diabetic and cardiovascular effects of hydro-ethanolic extract of *Parinari curatellifolia* seed was carried out by S. O. Ogbonnia et al, 2011. The results confirmed that the extract had good hypoglycaemic activity and beneficial effects on cardiovascular risk factors. The study also showed that the doses investigated did not provoke toxic effects to the animals’ heart and liver.

S.E. Atawodi 1 et al, 2013 carried out a research on the antioxidant and hepatoprotective effects of *Parinari curatellifolia* Planch ex Benth root methanolic extract. Methanolic extracts of the roots of
Parinari curatellifolia were confirmed to have significant antioxidant and hepatoprotective effects on acute and chronic liver injuries.

The curative effect of Parinari curatellifolia leaf extract on epiglottitis was investigated by Eze, Henry Tochukwu et al, 2013. Air dried leaves of Parinari curatellifolia were extracted using the soxhlet extractor. The extract of the plant was found to be rich in phytochemicals of medicinal importance such as alkaloids, tannins, saponins, flavonoids, steroids, and cardiac glycosides. An extract of acetic acid had the highest antimicrobial activity with zones of inhibition.

The Phytochemical screening of the ethanolic extracts of the leaves and the root bark done by Halilu, M.E et al, 2010 revealed the presence of terpenoids, saponins, flavonoids, cardiac glycosides, and tannins. Alkaloids were found to be present in leaves only, while anthraquinones were found to be present only in the root bark of the tree. Analysis for elements indicated the presence of the following mineral elements in both the leaves and the root bark in varying concentrations: K, Na, Ca, Al, Cu, Ni, Zn, Mn, Co, Cr, Pb and Cd. The plant is reported to have been used in the treatment of a number of diseases. This may be due to the presence of the phytochemicals detected (Halilu, M.E et al, 2010).

The aqueous and organic solvent extract of the stem of Parinari curatellifolia were screened for phytochemical and antibacterial activity by I. J. Peni et al, 2010. The bacterial activity of the extracts was carried out against a number of bacteria which include Pseudomonas aeruginosa, Salmonella typhi, Klebsella spp, Bacillus Subtilis and Staphylococcus aureus. Phytochemical constituents present in the extract included saponins, balsams, carbohydrate, alkaloids tannins, cardiac glycosides, flavonoids, digitalis glycosides, phenol, terpenes and steroids. The results clearly showed that different solvent extracts of the same plant may have different pharmacological properties, (I. J. Peni et al, 2010).

In another research, antimicrobial activity of ethanol and aqueous extracts of Parinari curatellifolia stem were tested against five dental carries causing bacteria and three fungi strains by agar diffusion
method. The crude extracts showed a broad spectrum of antibacterial activity inhibiting the five strains of bacteria and the three fungi from multiplying. The extracts’ effects were more pronounced against *Aspergillus flavus*, followed by *Streptococcus mutans* and *Staphylococcus aureus* respectively. *S. mutans* and *S. aureus* were the only microorganisms that showed zone of inhibition in all the different concentrations of the ethanol extract of *Parinari curatelifolia*. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoid, anthraquinones, saponins, tannins, cardiac glycosides, steroids, terpenoids, phlobatansins and carbohydrates, (E.O. Oshomoh and M. Idu, 2012)

Given the extensive research work already done on this plant species, there is no doubt that this plant has got a great potential of transforming the medical field at the same time providing breakthroughs for several developing countries where this plant is found. Nevertheless research work done has not been exhaustive. The oil contained in the seeds of the fruit has not been characterised. As already discussed in the previous sections, plant oil characterisation is critical if the full potential of the oil is to be exploited.

Several oils have been characterised by many researchers as a way of unravelling their usefulness. Chinweubwa Arinze Jude, 2013 extracted oil from ground *Nicotiana tabacum* seeds by soxhlet. The percentage oil content was 36.75%. The oil was characterised in terms of iodine value, saponification value, acid value, free fatty acid content and specific gravity. The oil content of the seeds showed that processing of the oil is economical (Ajiwe et al 1995). The physiochemical properties showed that the oil was highly unsaturated and had semi-drying properties. The semi-drying nature of the oil and its saponification value made it fit for the production of hair shampoo and shoe polish (Ajiwe et al 1994)

E. Chivandi et al (2009) investigated the Lipid content and fatty acid profile of the fruit seed of *Diospyros mespiliformis*. The results of the research showed that the low oil yield from
D. mespiliformis seeds limits its potential for commercial exploitation in spite of it being a source of both linoleic acid and alpha linoleic acid.

Y.X. Xu et al discovered that the oil contents of hazelnuts were in the range of 51.4% to 75.1% of dried weight of the kernel. The oils from the hazelnuts investigated in the research were unique in that only two fatty acids (oleic and linoleic) accounted for 90% of their fatty acid content. High linoleic and oleic acids contents and low linolenic acid content improved the oils thermo oxidative stability, while low levels of saturated acids (palmitic and stearic) enhanced their properties in cold environments.

A lot of research work on analytical methods for characterization of plant oil has already been done by several chemists. This leaves no unsurmountable barriers for any institute of research to carry out work on this plant species. Some methods which have been tried and found useful are:

- Using the chromatographic and mass spectrometric methods for the characterization of essential oils and extracts from *Lippia* (Verbenaceae) aromatic plants, (Elena E. Stashenko et al 2013).

- Characterization of vegetable oils by electrospray ionization mass spectrometry fingerprinting method. (Catharino RR et al, 2005).

- Characterization of plant oils on a monolithic silica column using high-performance liquid chromatography, atmospheric pressure chemical ionization and mass spectrometry, (A Jakab and E Forgacs, 2002).

- Characterization of fatty acids composition in vegetable oils using chemometrics and gas chromatography, (Dong-Sun Lee et al, 1998).
CHAPTER 3: MATERIALS AND METHODS

3.1. Sample collection and pre-treatment

Two sets of seeds were used in this investigation. For the first set, 5 kilograms of dry seeds with a hard outer shell were handpicked from the ground below the trees at Harare High school. These were from fruits of past seasons since there had no trace of pulp on them. The collected seeds were stored in a cardboard box at room temperature. The second set consisted of 3 kilograms of dry seeds from fruits collected at Amby, Waterfalls and Arcadia. The fruits were collected fresh in 2013 and the pulp removed for other experimental work. The remaining seeds were sun-dried and stored in a polythene sack. The hard shells of the seeds were crushed and the nuts collected into a plastic bottle.

A- To the left are dried parinari curatellifolia seeds from fresh fruits collected at Amby and Acadia and to the right are dry seeds collected picked from the ground under mobola plum trees at Harare High school. Photographs B and C show crushed seeds with the two nuts inside the hard shell. D shows Parinari curatellifolia nuts.

These were taken to the laboratory for oil extraction.
3.2. Oil extraction

Nuts from the relatively fresh seeds were placed into a motor and crushed using a pestle.

The nuts changed into a paste like butter. During the crushing oil could be seen oozing out from the nuts. 40 grams of the sample was used to extract oil using Soxhlet apparatus. Hexane was used as a solvent for the extraction process.
The initial extraction process was hampered by the compactness of the butter. To cab this, the second sample of nuts from dry kernels was shredded using a sharp blade into small fragments (See photograph K below).

K - Shredded parinari curatellifolia nuts in an evaporating dish. L - Shredded parinari curatellifolia nuts in soxhlet apparatus.

A 40 gram sample was placed into the Soxhlet apparatus as shown above using hexane as the solvent. The process was quite efficient with several flashes of the solvent being realised per 5 minutes intervals. Oil extracted with this method was labelled sample 2. Sample 1 was the label for the oil obtained using the former method of crushing in a motor.

3.3. Evaporation of solvent

The solvent used in the extraction process (hexane) was evaporated from the oil extract using a Rotary evaporator shown in the photograph below.

Photograph M shows evaporation of hexane solvent from the extracted oil using a rotary evaporator.
The extracted oil samples were kept in glass bottles in a dark cupboard for further laboratory work.

A third sample was obtained from the butter of the crushed nuts by decanting. The oil was floating on top of the butter. This sample was regarded as a cold pressed sample since no solvent and heating was involved. The colour of the oil is golden yellow, common to most oils. Below are photographs of the oils.

![Photographs N, O and P show bottles containing oil extracted from parinari curatellifolia nuts.](image)

### 3.4. Determination of oil content of nuts

The oil content of the seeds was calculated on the basis of mass of sample extracted and mass of the remaining dry residue (free from traces of solvent used for extraction) as below.

\[
\text{Percentage of oil (Sample 2) in seeds} = \frac{(40-25.3) \times 100}{40} \quad \text{(Equation 1)}
\]

### 3.5. Determination of Peroxide Value (PV)

2.6 grams of sample 1 and 2.92 grams of sample 2 were weighed into 250 ml glass stoppered Erlenmeyer flasks. 30 ml of acetic acid - chloroform solution was added into each flask using a measuring cylinder. The contents of the flask were swirled until the sample completely dissolved. Into each flask 0.5ml of saturated potassium Iodide was added and the flasks stoppered. After swirling the contents for about one minute, 30ml of deionized water were added immediately, the flask stoppered and shaken vigorously to liberate the iodine from the chloroform layer. The solution
was titrated using 0.1N Sodium thiosulphate to a pale yellow colour and 1ml of starch solution added. The titration was continued until the blue gray color disappeared in the aqueous (upper layer). The Peroxide value was calculated using the formula below:

\[
\text{Peroxide value} = \frac{(S-B) \times N \text{ thiosulphate} \times 1000}{\text{Weight of sample}}
\]  
(Equation 2)

Where S = titration of sample; B= titration of blank and N normality of sodium thiosulphate.

3.6. Determination of Acid Value (AV)

2.111 grams of sample 1 and 2.35 grams of sample 2 were weighed into 250-mL conical flask and 50 mL of ethanol-ether solution added into each flask. Contents were gently shaken until the sample dissolved. A few drops of phenolphthalein indicator were added into each flask and the solution titrated with sodium hydroxide titrant until a pink colouration was observed. The volume of sodium hydroxide titrant used for each sample was noted and used for calculating the acid value using the formula below.

\[
\text{Acid value} = \frac{\text{Volume NaOH} \times 5.61}{\text{Weight of oil}}
\]  
(Equation 3)

3.7. Determination of Saponification value (SV)

2.51 grams of sample 1 and 2.43 grams of sample 2 were weighed into round bottomed flasks. 25ml of 0.5N alcoholic KOH was added into each flask using a bulb pipette and mixed well. Another flask containing only 25ml of KOH was set up as a blank. The apparatus were attached to reflux condensers and placed in boiling water in a bath for one hour as shown by the photos below.
Photographs Q and R show a water bath and oil samples after refluxing to determine the Saponification value.

After one hour the flasks were cooled to room temperature and phenolphthalein indicator was added to all the flasks. The contents were titrated with 0.5N HCl until the pink colouration of phenolphthalein disappeared marking the end point of the reaction.

The difference between the blank and test reading which gives the number of millilitres of 0.5N KOH required to saponify mass of oil weighed was used to find the Saponification value. The saponification value (number) is equal to milligrams of KOH consumed by 1 gram of oil. It can be calculated using the formula below:

\[
\text{Saponification value} = \frac{\text{Normality of KOH} \times \text{Equivalents of KOH} \times \text{Volume of KOH}}{\text{Mass of oil sample}}
\]  

(Equation 4)

3.8. Determination of Iodine Value (IV)

0.270 grams of sample 1 and 0.249 grams of sample 2 were weighed into clean dry iodine flasks. A third flask containing no oil was used as a blank. 25ml of Chloramine T reagent was pipetted into each of the flasks. The flasks were stoppered mixed well by shaking and then kept at room temperature in a dark cupboard. After 20-25 minutes, 5ml of 10% potassium iodide solution was added to each flask using a 10ml measuring cylinder. Again the flasks were stoppered and shaken. After shaking, each flask was titrated separately using 0.1M sodium thiosulphate solution with shaking until a pale yellow colour was observed. Starch solution indicator was added and the
titration was continued to the end point when the greyish blue colouration disappeared. The iodine value was calculated from the formula shown below:

\[ \text{Iodine Value} = \frac{(V_1 - V_2) \times 12.69 \times 1000 \times M}{W} \]  

(Equation 5)

Where, \( V_1 \) and \( V_2 \) are volumes in ml of sodium thiosulphate solution of molarity \( M \) consumed by a known volume of Chloramine T reagent (25ml) without and with \( W \) mg of the oil.

3.9. Determination of Fatty Acid methyl Esters (FAME) using Gas Chromatography

3.9.1 Derivatization of fatty acids in the oil to FAMEs

Since fatty acids are not volatile by nature, they were derivatised into volatile FAMEs for analysis by Gas Chromatography. Four oil samples were weighed into 20ml screw cap test tubes as shown by the Table 1 below.

<table>
<thead>
<tr>
<th>SAMPLE IDENTITY</th>
<th>MASS (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (olive oil)</td>
<td>0.6302</td>
</tr>
<tr>
<td>Cold pressed sample</td>
<td>0.5838</td>
</tr>
<tr>
<td>Sample 1</td>
<td>0.4599</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.6275</td>
</tr>
</tbody>
</table>

After weighing, 2ml of n-hexane was added to each test tube to dissolve the oil samples followed by 1ml of 2M methanolic KOH. The mixture was vigorously shaken for 30 seconds followed by boiling for 2 minutes in a water bath at 70\(^{\circ}\)C. After boiling, the tubes were allowed to cool and 1.2ml of 1M HCl was added into each test tube. The contents were gently stirred. The tubes were placed in a beaker to allow phase separation. 1ml of n-hexane was added to each tube after phase separation.
Photographs S, T and U show test tubes in which the samples for gas chromatography were derivatized.

The upper phase containing the FAMEs was taken for Gas Chromatography analysis.

3.9.2 Gas Chromatography analysis for FAMEs

The Instrument used for the analysis is a Varian Star 1 with a Flame Ionisation Detector (FID). The column was a Carbowax packed column and the carrier gas used was nitrogen. The run time for each sample was set at 33.332 minutes. 1 μl was drawn from the sample using a syringe and injected into the instrument.
3.10. Flowchart of procedures

![Flowchart of procedures and results expected](image)

**Figure 3.1**: Flowchart of procedures and results expected
CHAPTER 4: RESULTS

4.1. Results

Table 4.1: Results

<table>
<thead>
<tr>
<th>RESULT NUMBER</th>
<th>PARAMETER</th>
<th>VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>1</td>
<td>Oil content</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>Peroxide Value (PV)</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>Acid Value (AV)</td>
<td>0.532</td>
</tr>
<tr>
<td>4</td>
<td>Saponification Value (SV)</td>
<td>149.7</td>
</tr>
<tr>
<td>5</td>
<td>Iodine Value (IV)</td>
<td>70.50</td>
</tr>
<tr>
<td><strong>6</strong></td>
<td>Fatty Acid composition</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Extraction efficiency was reduced because of the compactness of sample hence oil content was not calculated.

**See chromatograms below.

4.2. Chromatograms from Gas chromatography

Due to challenges of getting a suitable capillary column, a packed Carbowax column was made use of. The column failed to achieve separation of the FAMEs and the only peak identified was the solvent peak. The results are shown in the Chromatograms below.
Chromatogram obtained from the analysis of standard sample.

Figure 4.1
Peak information for the chromatogram obtained from the analysis of standard sample.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Peak Name</th>
<th>Result (A)</th>
<th>Ret. Time (min)</th>
<th>Offset (min)</th>
<th>Area (counts)</th>
<th>Slope 1/2</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>67.2490</td>
<td>1.582</td>
<td>0.000</td>
<td>2201065</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.9366</td>
<td>1.791</td>
<td>0.000</td>
<td>21688</td>
<td>0.000</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1.7054</td>
<td>13.735</td>
<td>0.000</td>
<td>40636</td>
<td>0.000</td>
<td>0.0</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total Unidentified Counts: 2263291 counts
Detected Peaks: 3
Identified Peaks: 0
Rejected Peaks: 0
Amount Standard: N/A
Multiplier: 1.000000
Divisor: 1.000000
Baseline Offset: -1 microVolts
Noise (used): 20 microVolts - monitored before this run
Manual injection

Figure 4.2
Chromatogram obtained from the analysis of one of the oil samples.

Figure 4.3
Peak information for the chromatogram obtained from the analysis of one of the oil samples.

Figure 4.4
CHAPTER 5: DISCUSSION

5.1. Oil content of nuts

High oil content in plant seeds implies that exploiting them for oil would be economical (Ikhuoria et al., 2008). The oil content of the nuts as shown in Table 4.1 Result number 1 is 36.75%. This is quite significant as it compares well with oil content of some common sources of edible oil. The table below shows the oil content of common edible oil sources.

Table 5.1(a) Oil content of common edible oil sources

<table>
<thead>
<tr>
<th>SEED</th>
<th>TOTAL FAT CONTENT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>corn</td>
<td>4</td>
</tr>
<tr>
<td>grape</td>
<td>20</td>
</tr>
<tr>
<td>olive</td>
<td>20</td>
</tr>
<tr>
<td>rape (canola)</td>
<td>30</td>
</tr>
<tr>
<td>coconut</td>
<td>35</td>
</tr>
<tr>
<td>flax (linseed)</td>
<td>35</td>
</tr>
<tr>
<td>palm kernel</td>
<td>35</td>
</tr>
<tr>
<td>cottonseed</td>
<td>40</td>
</tr>
<tr>
<td>cashew</td>
<td>42</td>
</tr>
<tr>
<td>pumpkin</td>
<td>47</td>
</tr>
<tr>
<td>sunflower</td>
<td>47</td>
</tr>
<tr>
<td>peanut (groundnut)</td>
<td>48</td>
</tr>
<tr>
<td>sesame</td>
<td>49</td>
</tr>
<tr>
<td>walnut</td>
<td>60</td>
</tr>
<tr>
<td>macadamia</td>
<td>72</td>
</tr>
</tbody>
</table>


Table 5.1(b) Comparison table

<table>
<thead>
<tr>
<th>SEED</th>
<th>TOTAL FAT CONTENT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parinari curatellifolia</td>
<td>36.75</td>
</tr>
</tbody>
</table>
Result 1 (oil content) in Table 4.1 corresponds well with that in literature. According to one source, seed kernel has high oil content (37.75%). The oil is commonly used for paints, varnishes, printing and engraving inks (Planch. ex Benth). The oil is also used for cooking. Literature has limited uses of the oil indicating that it is underutilised. Other parameters investigated (i.e. IV, AV, PV and SV) show that this oil is high quality oil which can be used for cosmetics, medical formulations and can be recommended as one of the safest oils health wise.

The animal feed industry productively utilizes the by-products associated with the refining of oilseeds into valuable food materials. Doing this contributes value to the value-chain segment, which produces the by-product. In some instances, the additional economic value contributed through utilization of the by-product may be a deciding factor in the economic viability of the value chain segment (Nick Bajjalieh, FAO-Food and Agriculture organization of the United Nations). Proteins from seeds are a critical contributor to the commercial viability of the animal production industries, as they exist today. Conversely, the animal production industries’ use of proteins from oil seeds returns considerable value to the oilseed production and processing industries (FAO). Hence processing of Parinari curatellifolia nuts to obtain the valuable oil has the bonus of very useful oil extraction residue not only as an animal feed but human consumption too. The extraction processes employed in this research indicated that it is possible to obtain cold pressed oil from the nuts. This ensures that high quality oil (free from toxins) for human consumption can be obtained leaving a residue which can be used in preparing porridge, vegetarian mince and many possible products. Simple home oil press machines are available for household preparations and industrial scale sized equipment is also available (PerryVidex equipment among other suppliers). However, a viable way of obtaining the kernels from the hard shell housing must be devised. In this research work, manual crushing and seed separation was employed which can serve for a small scale oil production in the home. For large scale production, designs of less expensive and more efficient equipment are needed.
5.2 Peroxide Value (PV)

The values for the peroxide values for sample 1 and sample 2 areas shown in Table 4.1 were 1.9 and 1.69 respectively. Sample 1 was analysed 49 days after extraction. The sample was stored in a dark cupboard during the 49 days. Sample 2 was analysed a day after extraction.

The peroxide value (PV) is used for determining the peroxide oxygen especially hydro-peroxides. It is expressed in milliequivalents of active oxygen per 1 kilogram of fat. (Source: Swiss Handbook of Foods, chapter 7, research method 5.2). The peroxide value is used for identifying the beginning of oxidative change in fats and oils, during which the oxygen molecule penetrates the fat molecule in the form of a peroxide group. Primary oxidation processes in oil mainly form hydro-peroxides. In general, the smaller the peroxide value, the better the quality of the oil (www.plantanfood.com). However peroxide value decreases as secondary oxidation products appear. This is illustrated by Figure 5.1 below.

![Figure 5.1: Relationship between peroxide value, acid value and Totox value with time](image)

**Figure 5.1:** Relationship between peroxide value, acid value and Totox value with time
The graph shows oxidation of oil with time as measured by the peroxide value (PV), anisidine value and Totox value. Since the peroxide value can decrease over time acid value and Totox value calculations are needed to appreciate the whole oxidation process. Determining peroxide value is a good way to quantify the amount of primary oxidation products in fresh oils. Oils with high levels of peroxides may remain odourless if secondary oxidation has not begun. If oxidation has advanced, the peroxide value may be relatively low but the oil will be rancid. Most customers prefer a peroxide value of less than 10 in marine oils, but peroxide value may need to be as low as 2, depending on customer preference (www.plantanfood.com).

The values obtained for both samples are approximately 2 indicating that the oil has good quality. It is less susceptible to primary oxidation. Despite the fact that sample 1 (PV=1.9) was analysed after 49 days, the oil had not gone rancid since no bad odours were detected. It still had the freshness it had just after extraction. However the difference with the value for sample 2 (PV=1.69) which was analysed a day after extraction indicate that primary oxidation had taken place but to a very small extent. Oxidation takes place at different rates depending on factors such as temperature, light, availability of oxygen, and the presence of moisture and metals such as iron (www.plantanfood.com). This means that proper packaging and storage of the oil can prolong its shelf life.

The type of oil also influences the rate of oxidation. Marine oils are highly susceptible to oxidation due to the large number of polyunsaturated fatty acids (PUFAs) they contain. These unsaturated fatty acids have reactive double bonds between their carbon atoms, whilst saturated fats have no double bonds so they oxidise more slowly (www.plantanfood.com). Therefore the low peroxide value for parinari curatellifolia oil suggests that it does not contain polyunsaturated fatty acids. Since it is a liquid at room temperature, it most likely contains fatty acids with fewer double bonds. A possibility of the presence of monounsaturated fatty acids (MUFAs) cannot be dismissed. Common sources of
MUFAs are olive oil, avocados and nuts. Research shows that diets with healthy amounts of monounsaturated fats have several amazing health benefits, including:

- A decrease risk for breast cancer. Studies on women in Sweden found that those with diets higher in monounsaturated fats (as opposed to polyunsaturated fats) resulted in less frequent incidence of breast cancer (Mark Wolk, Alicia).

- Reduced bad cholesterol levels. The American Heart Association encourages the consumption of MUFAs to improve blood lipid profile.


- Studies have confirmed that switching to monounsaturated fat from diets with Trans fats and polyunsaturated fats results in weight loss.

- Less severe pain and stiffness in sufferers of rheumatoid arthritis. Diet plays a role in reducing the pain and stiffness of those who already have rheumatoid arthritis.

- Reduced fat of the belly. A study published by the American Diabetes Association revealed that diets with monounsaturated fat could improve the loss of belly fat better than high carbohydrate diets (Manzella, Deborah, 2008).

Therefore basing on the values of the peroxide value for the samples analysed in this project, the oil has got health benefits. A research done by Oladimeji AO et al (2011) confirmed the presence of two monounsaturated fatty acids in the oil which are oleic acid and Gondoic acid. Though FAMEs could not be determined in this research, information from literature is in agreement with the results for peroxide value obtained for the samples.
5.3. Acid value (AV)

The acid values for samples 1 and 2 were 0.532 and 0.477 respectively as recorded in Table 4.1 Result number 3. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and it gives a measure of the free fatty acids (FFA) present in the fat or oil. The normal acid value for most oil samples lies within 0.5. If any titratable acid other than a fatty acid is present in the sample, an error would have occurred. A high acid value indicates a rancid oil or fat stored under improper conditions (www.classle.net). The table below shows the acid value of some common oils.

Table 5.2: Acid values of some common oils

<table>
<thead>
<tr>
<th>OIL NAME</th>
<th>ACID VALUE (AV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canola</td>
<td>0.071</td>
</tr>
<tr>
<td>Soya</td>
<td>0.60</td>
</tr>
<tr>
<td>Maize</td>
<td>0.223</td>
</tr>
<tr>
<td>Virgin olive oil</td>
<td>6.6</td>
</tr>
<tr>
<td>Used frying oil</td>
<td>31</td>
</tr>
</tbody>
</table>

Source: www.
The values obtained for the samples are quite acceptable. The high value for sample 1 can be attributed to hydrolysis since it was analysed 49 days after extraction. An increase in the amount of free fatty acids in a sample of oil or fat indicates hydrolysis of triglycerides (ChemPrime). Such reactions occur due to the action of lipase enzyme and it is an indicator of inadequate processing and storage conditions like high temperature and relative humidity and tissue damage. The source of the enzyme can be the tissue from which the oil or fat was extracted or it can be a contaminant from other cells which include microorganisms (ChemPrime). Therefore the shelf life of the oil under investigation can be increased by proper storage. The slight increase in acidity over 49 days (by 0.055) also indicates the stability of the oil.

5.4. Saponification value (SV)

The Saponification values for the two samples were 149.7 (sample 1) and 135.1 (sample 2) (See Table 4.1 Result number 4). The saponification value gives a measure of the average molecular weight of the triglyceride in a sample. The smaller the saponification value the larger the average molecular weight of the triglyceride present. Saponification value is inversely proportional to the average molecular weight of fatty acids. Dividing 56100 by the saponification value gives an approximate measure of the average molecular weight of the acyl chain. This is known as the saponification equivalent. Sample 1 and 2 had average molecular weights of 374.7 amu and 415.2 amu (atomic mass units) respectively. Olive oil has got a saponification value range of 184-196. The average value is 190 which give an average molecular weight of 295.3 for the oil. This is a low value compared to the values of parinari curatellifolia obtained in this research. The reason is that olive oil has got a high percentage of short chain fatty acids than parinari curatellifolia oil. The tables below show a comparison of the two oils in terms of percentage fatty acid composition and chain length.
Table 5.3: Olive oil fatty acid composition

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Chain length</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>16 C atoms</td>
<td>14.4</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>18 C atoms</td>
<td>2.43</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>18 C atoms</td>
<td>60-85</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18 C atoms</td>
<td>9-14</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>18 C atoms</td>
<td>1</td>
</tr>
</tbody>
</table>

Source: [www.sacredearth](http://www.sacredearth)

Table 5.4: Parinari curatellifolia oil fatty acid composition.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Chain length</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>18 C atoms</td>
<td>5.66</td>
</tr>
<tr>
<td>Gondoic acid</td>
<td>20 C atoms</td>
<td>2.37</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18 C atoms</td>
<td>91.97</td>
</tr>
</tbody>
</table>

Source: Oladimeji AO et al. (2011)

Therefore the saponification values obtained confirm the presence of the fatty acids identified by Oladimeji AO et al. (2011).

5.5. Iodine value (IV)

The Iodine values for the two samples were 70.5 (sample 1) and 81.54 (sample 2). Iodine values are used to determine the amount of unsaturation in fatty acids. This unsaturation is in the form of double bonds, which react with iodine containing compounds. The higher the iodine value, the more C=C bonds are present in the fat (Thomas, Alfred, 2002). Saturated oils, fats, and waxes do not react with iodine. Therefore their iodine value is zero (www.britannica.com). An oil or fat with a high iodine value is more reactive, less stable, softer, and more susceptible to oxidation and staling. Drying oils used in paint and varnish industries have relatively high iodine values (about 190). Semidrying oils like soybean oil have intermediate iodine values (about 130). Non-drying oils, such as olive oil have relatively low iodine values (about 80) (www.britannica.com). A drying oil is one that hardens to form a tough solid film after a period of exposure to air. The oil hardens as a result.
of chemical reactions in which the components crosslink by the action of oxygen (Wikipedia). The following table (Table 5.5) shows iodine value ranges for some common fats and oils.

**Table 5.5: Iodine values for some common fats and oils**

<table>
<thead>
<tr>
<th>FAT</th>
<th>IODINE NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut oil</td>
<td>7 – 10</td>
</tr>
<tr>
<td>Palm kernel oil</td>
<td>16 – 19</td>
</tr>
<tr>
<td>Cocoa butter</td>
<td>35 – 40</td>
</tr>
<tr>
<td>Jojoba oil</td>
<td>80</td>
</tr>
<tr>
<td>Palm oil</td>
<td>44 – 51</td>
</tr>
<tr>
<td>Olive oil</td>
<td>80 – 88</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>84 – 105</td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td>100 – 117</td>
</tr>
<tr>
<td>Corn oil</td>
<td>109 – 133</td>
</tr>
<tr>
<td>Wheat germ oil</td>
<td>115 – 134</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>120 – 136</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>125 – 144</td>
</tr>
<tr>
<td>Linseed oil</td>
<td>136 – 178</td>
</tr>
</tbody>
</table>

If the iodine number is below 70, it will be a fat and if the value exceeds 70 it is oil. The values obtained 70.5 and 81.54 tally well with the physical state of the oil. This shows that it does not contain polyunsaturated fatty acids. According to a research by Oladimeji AO et al (2011) oil from parinari curatellifolia seeds was found to contain fatty acids with the structures shown below. The corresponding percentages of each fatty acid in the oil are given.

**Oleic acid (5.66%)**

\[ \text{CH}_3(\text{CH}_2)_7\text{CH} = \text{CH} (\text{CH}_2)_7\text{COOH} \]

**Gondoic acid (2.37%)**

\[ \text{CH}_3(\text{CH}_2)_7\text{CH} = \text{CH} (\text{CH}_2)_9\text{COOH} \]

**Linoleic acid (91.97%)**

\[ \text{CH}_3(\text{CH}_2)_4\text{CH} = \text{CHCH}_2\text{CH} = \text{CH}(\text{CH}_2)_7\text{COOH} \]

The structures show that the numbers of double bonds are few. The most abundant fatty acid has only two double bonds. There is excellent agreement with the results obtained. This means parinari curatellifolia oil is less susceptible to auto-oxidation or getting rancid. The peroxide values obtained for the samples (averaging 2) are in good agreement with the structures of the fatty acids confirmed to be present in the oil.

**5.6. Fatty Acid methyl Esters (FAME)**

In this research the fatty acids were converted to fatty acid methyl esters (FAMEs) and the samples were run on a Varian Star 1 with a Flame Ionisation Detector (FID). As can be seen from the chromatograms in the Appendix, no separation was achieved because of unavailability of a suitable column. A packed carbowax column was used. The only peak detected is the solvent peak. One of the runs indicates that a peak occurred at 13.735 minutes though it is not visible in the chromatogram. The work was also hampered by unavailability of a standard for comparison. Olive oil
and some results of its analysis from literature were employed. However the column did not yield any separation.

Despite the challenge on determination of fatty acids, the results for the other parameters determined in this research are in excellent agreement with what other researchers have discovered. Oladimeji AO et al (2011) carried out a research entitled ‘Proximate analysis and fatty-acid profiles of mobola plum seed’. The researchers discovered that the oil contains a high level of unsaturated fatty acids and hence could be a good source of edible oil for human consumption. The table below shows the fatty acid composition of the oil according to their research and the corresponding percentages.

**Table 5.6: Fatty acid composition of parinari curatellifolia oil**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Chain length</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>C18:1</td>
<td>5.66%</td>
</tr>
<tr>
<td>( CH_3(CH_2)_7CH=CH(CH_2)_7COOH )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gondoic acid</td>
<td>C20:1</td>
<td>2.37%</td>
</tr>
<tr>
<td>( CH_3(CH_2)_9CH=CH(CH_2)_9COOH )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>C18:2</td>
<td>91.97%</td>
</tr>
<tr>
<td>( CH_3(CH_2)_18CH=CHCH_2CH=CH(CH_2)_2COOH )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Oladimeji AO et al (2011)

As previously stated the fatty acids identified show excellent agreement with the acid value peroxide value, saponification value and iodine value determined in the current research. This led the author
of this work to tentatively accept the fatty acids identified as the ones present in the oil under research.

The importance of monounsaturated fatty acids in the diet has already been highlighted. Of the three identified fatty acids (according to Oladimeji AO et al., 2011), two of them are monounsaturated and constitute a total of 8.03% of the fatty acid content. This means that the oil has got great significance in nutrition and health sectors.

Secondly, the third fatty acid identified, linoleic acid is an essential fatty acid. It belongs to one of the two families of essential fatty acids (omega-3 and omega-6), which means that the human body cannot synthesize it from other food components (Burr G.O, 1930). It is an essential fatty acid that must be consumed for good health. A study showed that diet only deficient in linoleate (the salt form of the acid) causes mild skin scaling, hair loss (Cunnane S, Anderson M, 1997) and poor wound healing in rats (Ruthig DJ & Meckling-Gill 1999). These revelations from research work are indicators of the significance of the fatty acids in the body. Both linolenic acid and linoleic acid are important components of cell membranes and are precursors to many other substances in the body such as those involved with regulating blood pressure and inflammatory responses. There is overwhelming support for omega-3 fatty acids in protecting against fatal heart disease and it is known that they have anti-inflammatory effects, which may be important in many diseases. There is also increasing interest in the role of omega-3 fatty acids in the prevention of diabetes and certain types of cancer (Lunn J and Theobald H, 2006). Essential fatty acids have been shown to block tumour formation in animals, as well as stopping the growth of human breast cancer cells. They help in the development and function of the brain and nervous system, and they assist in regulating proper thyroid and adrenal activity. They play a role in thinning blood, which can prevent blood clots that result in heart attacks and stroke. They also possess natural anti-inflammatory properties that relieve symptoms of both arthritis and other autoimmune system diseases (www.fitday.com).
Essential fatty acids regulate immune responses, blood pressure and liver function, as well as help with breaking down cholesterol and blood clotting. They enhance good looks as a diet low in these fatty acids has been shown to create skin problems, including eczema, dandruff, split nails and brittle hair (www.fitday.com).

Considering that linoleic acid is the most abundant fatty acid in parinari curatellifolia, it gives the oil great significance medicinally, health wise, and even economically. Its properties and benefits stated above show that it can be used in the cosmetics industries for various products. A substitution of the commonly used highly processed oils which have negative effects on health with parinari curatellifolia oil will result in great prevention of ailments and preservation of health.
CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

In overall the research showed that parinari curatellifolia nuts contain oil with essential and monounsaturated fatty acids and that the plant is one of the most important species which is underutilised in Zimbabwe.

Specifically, the research revealed that:

- Parinari curatellifolia nuts have got significant amounts of edible oil (36.75% of the nut) which can be exploited giving benefits to the country.
- The oil contains monounsaturated and essential fatty acids and is less susceptible to primary oxidation.
- The oil can be employed in food and cosmetics industries in the making of hair conditioning oils and special bath soaps.

6.2. Recommendations

From the findings of the research undertaken, the author recommend that:

- Equipment for crushing the hard seeds be designed to exploit the oil resource in parinari curatellifolia nuts so as to improve the social and economic status of the nation of Zimbabwe.
- Further research work be done on how the oil can be used in medicine or foods to aid good health.
- Further research of other parameters using better instruments like High Performance liquid Chromatography and Gas Chromatography-Mass spectrometry for finer characterisation of the oil.
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