Some Physicochemical Characteristics and Antibacterial Activity of Local Jatropha (Jatropha curcas L.) Seeds oil

Ahmed Siddig Suliman Fadlalseed

B.Sc. (Hon.) in Agriculture (Agricultural Biotechnology) Faculty of Agriculture, University of Khartoum (2009)

A Dissertation

Submitted to University of Gezira in Partial Fullfilment of the Requirements for the Award of the Degree of Master of Science in

Biosciences and Biotechnology (Biotechnology)

Center of Biosciences and Biotechnology

Faculty of Engineering and Technology
August, 2014

Some Physicochemical Characteristics and Antibacterial Activity of Local Jatropha \textit{curcas} L.) Seeds oil

Ahmed Siddig Suliman Fadlalseed

Supervision Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Yasir Mohamed Abdelrahim</td>
<td>Main Supervisor</td>
<td></td>
</tr>
<tr>
<td>Dr. Mai Mohammed Osman Diab</td>
<td>Co-Supervisor</td>
<td></td>
</tr>
</tbody>
</table>

Date: August, 2014
Some Physicochemical Characteristics and Antibacterial Activity of Local Jatropha (*Jatropha curcas* L.) Seeds oil

Ahmed Siddig Suliman Fadlalseed

Examination Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Yasir Mohamed Abdelrahim</td>
<td>Chairperson</td>
<td></td>
</tr>
<tr>
<td>Dr. Asaad Adam Abbas</td>
<td>External Examiner</td>
<td></td>
</tr>
<tr>
<td>Dr. Mutaman Ali Kehail</td>
<td>Internal Examiner</td>
<td></td>
</tr>
</tbody>
</table>

Date of Examination: 12, August, 2014
Dedication

To my Father

To my Mother

To my Brothers

To my Sisters

To my Friends

Dedicate this work
Acknowledgement

Thanks first to God who gave me the ability to complete this work, and thanks to Dr. Yasir Mohamed Abdelrahim who gave did not spare even sees this work the light. And thanks also to Dr. Mai Mohammed Osman Diab, Mutaman Ali Kehail, also not forget to thank the family of the Center of Biosciences and Biotechnology, Faculty of Engineering and Technology, University of the Gezira, and the family of the Department of Botany and Biotechnology, Faculty of Agriculture, University of Khartoum and the family of the Department of Food Science, Faculty of Engineering and Technology, University of the Gezira, and I thank all those who helped me.
Some Physicochemical Characteristics and Antibacterial Activity of Local Jatropha (*Jatropha curcas* L.) Seeds Oil

Ahmed Siddig Ahmed Fadlalseed

Abstract

*Jatropha curcas* plant is a multipurpose plant that considered as potential source of biofuel because its seed kernels contain a high amount of oil (58-60%). Besides oils, *Jatropha* seeds also contain high protein and anti-microbial factors. The present work aimed to study the physicochemical characteristics and the antibacterial activity of *J. curcas* seeds oil. *Jatropha* oil was obtained by mechanical extract, whereas bacterial strains (two gram-positive bacteria: *Bacillus cereus* and *Staphylococcus aureus*, and two gram-negative bacteria: *Escherichia coli* and *Salmonella typhi*) were isolated from pathogenic specimens. Both oil sample and bacterial strains were prepared in the Laboratory of Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum. Disc diffusion method for antimicrobial susceptibility testing was also prepared. Some physical (refractive index, density and viscosity) and chemical (free fatty acids, acid, peroxide and saponification values and iodine number) tests were run for *Jatropha* oil in the Laboratory of Food Analysis, Faculty of Engineering and Technology, University of Gezira. The results of this study revealed that, most of the physicochemical characteristics of *Jatropha* oil were out of the standards to be suitable source of biodiesel. All strains of bacteria were affected by the concentration of *Jatropha* oil: hexane 50%, however just *Staph. aureus* and *E. coli* were affected by concentration of 25%, but the antibacterial activity of *Jatropha* oil was less than antibiotic (positive control), while the concentrations of 75% and 100% did not affected any of the bacterial strains, and this was probably due to high density of the oil which must penetrate through the bacterial membrane. The research recommend to optimize a methods for studying of the physicochemical characteristics of *Jatropha* oil to compare it with the biodiesel standards, and also to detect the active ingredients suitable as an antibacterial for wider range of bacterial strains.
بعض الخصائص الفيزيوكيميائية والنشاط المضاد للبكتريا لزيت بذور نبات الجاتروفا المحلي

أحمد صديق سليمان فضيل السيد

ملخص الدراسة

نبات الجاتروفا هو نبات متعدد المهام ويعتبر كمصدر فعال للوقود الحيوي بسبب إحتواء البذور على كمية كبيرة من الزيت (58 – 60%). ويعتبر الزيت، تحتوي بذور الجاتروفا أيضاً على بروتينات عالية وعوامل مضادة للميكروبات. هدف هذا العمل إلى دراسة الخصائص الفيزيوكيميائية والنشاط المضاد للبكتريا لزيت بذور نبات الجاتروفا. حضر زيت الجاتروفا بالاستخلاص الميكانيكي، في حين أن السلالات البكتيرية (إثنين من البكتريا موجبة جرام: البكتريا الاستافيلوكوكس، والاثنين من البكتريا سالبة جرام: البكتريا الاشريكية الولونية والسالمونيلا) قد تم عزلها من عينات مريضة. تم تحضير كلا من عينات الزيت وسلالات البكتريا في معمل قسم النبات والتقنية البيولوجية الزراعية، كلية الزراعة، جامعة الخرطوم. تم أيضاً تجهيز "طريقة إنتشار القرفص" لاختبار حساسية المضادات الميكروبية. بعض الاختبارات الفيزيائية (معامل الانكسار، الكثافة واللزوم) والفيزيوكيميائية (الأحماض الدهنية الحرة، قيمة الحامض، البيريكسيد والتصبن ورقم اليود) تم إجراؤها على زيت الجاتروفا بمعمل تحليل الأغذية، كلية الهندسة والتكنولوجيا، جامعة الجزيرة. أوضح نتائج هذه الدراسة أن معظم الخصائص الفيزيوكيميائية لزيت الجاتروفا توقع خارج المواصفات ليصبح مصدر مناسب للوقود الحيوي. تأثر كل سلالات البكتريا بتركيز زيت الجاتروفا: الهكسين 50%، في حين أن بكتريا ستيافيلوكوكس والاشريكية الولونية قد تأثرت بالتركيز 25%، ولكن النشاط المضاد للبكتريا لزيت الجاتروفا أقل من نشاط المضاد الحيوي (الشاهد الموجب)، في حين أن تركيز 75% و100% لم تؤثر على أي من سلالات البكتريا. وقد يعني ذلك للكثافة الزيت عالية والتي ينبغي أن تتدفق من خلال الغشاء البكتيري. يوصي البحث بوضع طرق مناسبة لدراسة الخصائص الفيزيوكيميائية لزيت الجاتروفا لمعرفته مع مواصفات الوقود الحيوي، وكذلك يوصي بتحديد المواد الفعالة المناسبة كمضادات بكتيرية لمدى واسع من السلالات البكتيرية.
## CONTENTS

<table>
<thead>
<tr>
<th>Subject</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>iii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>iv</td>
</tr>
<tr>
<td>Abstract</td>
<td>v</td>
</tr>
<tr>
<td>Arabic Abstract</td>
<td>vi</td>
</tr>
<tr>
<td>List of Contents</td>
<td>vii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>x</td>
</tr>
<tr>
<td>List of Images</td>
<td>xi</td>
</tr>
<tr>
<td>List of Plates</td>
<td>xii</td>
</tr>
<tr>
<td><strong>CHAPTER ONE: INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>CHAPTER TWO: LITERATURE REVIEW</strong></td>
<td>3</td>
</tr>
<tr>
<td>2.1. <em>Jatropha curcas</em></td>
<td>3</td>
</tr>
<tr>
<td>2.1.1 Scientific classification</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2. Botanical features</td>
<td>5</td>
</tr>
<tr>
<td>2.1.3. Propagation</td>
<td>5</td>
</tr>
<tr>
<td>2.1.4. Cultivation</td>
<td>6</td>
</tr>
<tr>
<td>2.1.5. Uses</td>
<td>6</td>
</tr>
<tr>
<td>2.1.6. <em>Jatropha</em> seeds</td>
<td>8</td>
</tr>
<tr>
<td>2.1.7. Component of <em>Jatropha curcas</em> seeds</td>
<td>9</td>
</tr>
<tr>
<td>2.1.8. Toxicity</td>
<td>9</td>
</tr>
<tr>
<td>2.2. Hexane</td>
<td>12</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3.3.2. Disc diffusion method</td>
<td>22</td>
</tr>
<tr>
<td>3.3.3 The Physical and Chemical characteristics of <em>Jatropha</em> oil</td>
<td>22</td>
</tr>
<tr>
<td>3.3.3.1 Refraction Index</td>
<td>22</td>
</tr>
<tr>
<td>3.3.3.2 Density</td>
<td>22</td>
</tr>
<tr>
<td>3.3.3.3 Viscosity</td>
<td>22</td>
</tr>
<tr>
<td>3.3.3.4 Free fatty acid</td>
<td>23</td>
</tr>
<tr>
<td>3.3.3.5 Acid value</td>
<td>23</td>
</tr>
<tr>
<td>3.3.3.6 Peroxide value</td>
<td>23</td>
</tr>
<tr>
<td>3.3.3.7 Sapnification Value</td>
<td>24</td>
</tr>
<tr>
<td>3.3.3.8 Iodine Number</td>
<td>25</td>
</tr>
<tr>
<td>3.4. Statistical analysis</td>
<td>25</td>
</tr>
<tr>
<td><strong>CHAPTER FOUR: RESULTS AND DISCUSSION</strong></td>
<td></td>
</tr>
<tr>
<td>4.1 The physical characteristics of <em>Jatropha</em> oil</td>
<td>26</td>
</tr>
<tr>
<td>4.2. The chemical characteristics of <em>Jatropha</em> oil</td>
<td>28</td>
</tr>
<tr>
<td>4.3 The antimicrobial activity of <em>Jatropha</em> oil</td>
<td>32</td>
</tr>
<tr>
<td><strong>CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS</strong></td>
<td></td>
</tr>
<tr>
<td>5.1 Conclusions</td>
<td>37</td>
</tr>
<tr>
<td>5.2. Recommendations</td>
<td>37</td>
</tr>
<tr>
<td><strong>REFERENCES</strong></td>
<td>38</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The physicochemical characteristics of <em>Jatropha</em> oil</td>
<td>11</td>
</tr>
<tr>
<td>4.1</td>
<td>The refractive index, density and viscosity of <em>Jatropha</em> oil</td>
<td>27</td>
</tr>
<tr>
<td>4.2</td>
<td>The chemical characteristics of <em>Jatropha</em> oil</td>
<td>29</td>
</tr>
<tr>
<td>4.3</td>
<td>The mean values inhibition zones (cm) of antimicrobial activities of the varies concentrations of <em>Jatropha</em> oil</td>
<td>33</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Table No</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Jatropha tree</td>
<td>4</td>
</tr>
<tr>
<td>2.2</td>
<td>Jatropha seed</td>
<td>10</td>
</tr>
<tr>
<td>2.3</td>
<td>Jatropha kernel</td>
<td>10</td>
</tr>
<tr>
<td>2.4</td>
<td>Jatropha oil</td>
<td>10</td>
</tr>
</tbody>
</table>
### LIST OF PLATES

<table>
<thead>
<tr>
<th>Plate No</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Plate (4.1) effect of oil conc. (25%) on <em>Staphylococcus</em></td>
<td>34</td>
</tr>
<tr>
<td>4.2</td>
<td>Plate (4.2) effect of oil conc. (25%) on <em>E.coli</em></td>
<td>34</td>
</tr>
<tr>
<td>4.3</td>
<td>Plate (4.3) effect of oil conc. (50%) on <em>Bacillus.</em></td>
<td>35</td>
</tr>
<tr>
<td>4.4</td>
<td>Plate (4.4) effect of oil conc. (50%) on <em>Staphylococcus.</em></td>
<td>35</td>
</tr>
<tr>
<td>4.5</td>
<td>Plate (4.5) effect of oil conc. (50%) on <em>E.coli.</em></td>
<td>36</td>
</tr>
<tr>
<td>4.6</td>
<td>Plate (4.6) effect of oil conc. (50%) on <em>Salmonella.</em></td>
<td>36</td>
</tr>
</tbody>
</table>
CHAPTER ONE
INTRODUCTION

In recent years there has been an increasing interest in the use of natural bioactive compounds, and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources. Infectious diseases caused by bacteria and fungus accounts for high proportion of health problems in the developing countries. Microorganisms have developed resistance to many antibiotics due to indiscriminate use of commercial antimicrobial drugs encouraged scientists to search for new antimicrobial substances from various sources including medicinal plants (Karaman et al., 2003).

Mostly the pharmacological activity of medicinal plants resides in its secondary metabolites which are comparatively smaller molecules in contrast to the primary molecules such as proteins, carbohydrates and lipids. These natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to man and it can help to meet expensive and limited supply of synthetic chemicals. The main advantage of plant products over the synthetic compounds in the treatment of diseases is that it is seen in the eukaryotic system and so it will not have a deleterious effect in higher plants and animals including man (Krishnakumar et al., 1997).

*Jatropha curcas* (Euphorbiaceae family) is a multipurpose plant that has a long history of cultivation in tropical America, Africa, and Asia with many attributes, mainly as potential source of bio-fuel because its seed kernels contain a high amount of oil (58-60%). Besides oils, *Jatropha* seeds also contain high protein, anti-nutritional factors including trypsin inhibitor, lectin, saponin, phytic acid and toxic compounds called phorbol esters (Martinez-Herrera et al., 2006). The natural compounds of *Jatropha* exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint heumatism (Aiyelaagbe et al., 2007). Furthermore, extracts from various parts of *Jatropha curcas*, such as seeds, seed oil, stem barks, roots and leaves have shown anti bactericidal properties (Igbinosa et al., 2009).
The data collected from the study of the physical and chemical properties of the test samples indicated that *Jatropha curcas* are suitable as non-edible vegetable oil feedstock in oleochemical industries (biodiesel, fatty acids, soap, fatty nitrogenous derivatives, surfactants and detergents, etc) (*Azam et al.*, 2005). The oil of this plant is used traditionally for the treatment of sciatica, dropsy, paralysis, rheumatism, dysentery, diarrhea and certain skin diseases (*Igbinosa et al.*, 2009).

**Objective of study**

The present work aimed to study of the physicochemical characteristics and the antibacterial activity of *Jatropha curcas* seeds oil on two gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and two gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*).
CHAPTER TWO
LITERATURE REVIEW

2.1. Jatropha curcas

*Jatropha curcas* is a multipurpose plant which belongs to the *Euphorbiaceae* family. It is thought to be native to Central and South America and widely distributed in Central America, Africa and Asia. Recently, it has been widely planted in Thailand and promoted as a biodiesel plant. Its seed contains 60–66% crude lipid and 30–32% crude protein.

*Jatropha* is a genus of flowering plants in the spurge family, Euphorbiaceae. The name is derived from the Greek words (*Jatros*), meaning "physician," and (*trophe*), meaning "nutrition," hence the common name physic nut. It contains approximately 170 species of succulent plants, shrubs and trees (some are deciduous, like *Jatropha curcas*). Most of these are native to the Americas, with 66 species found in the Old World. Mature plants produce separate male and female flowers. As with many members of the family Euphorbiaceae, *Jatropha* contains compounds that are highly toxic. *Jatropha* tree is resistant to drought and pests, and produces seeds containing 27–40% oil (Fairless, 2007).

2.1.1 Scientific classification

Kingdom : Plantae

Subkingdom : Tracheobionta

Superdivision : Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Rosidae

Order : Euphorbiales

Family: Euphorbiaceae

Genus : Jatropha(Fairless, 2007)
Figure (2.1) Jatropha tree
2.1.2. **Botanical features**

The leaves have significant variability in their morphology. In general, the leaves are green to pale green, alternate to subopposite, and three- to five-lobed with a spiral phyllotaxis. Male and female flowers are produced on the same inflorescence, averaging 20 male flowers to each female flower. The petiole length ranges from 0.24 to 0.90 inches (6.1–23.1 mm). The inflorescence can be formed in the leaf axil. Plants are monoecious and also presents hermaphroditic flowers occasionally (Bioenergy Plantations, 2014).

Fruits are produced in winter, or there may be several crops during the year if soil moisture is good and temperatures are sufficiently high. Most fruit production is concentrated from midsummer to late fall with variations in production peaks where some plants have two or three harvests and some produce continuously through the season. The seeds are mature when the capsule changes from green to yellow. The seeds contain around 20% saturated fatty acids and 80% unsaturated fatty acids, and they yield 25%–40% oil by weight. In addition, the seeds contain other chemical compounds, such as saccharose, raffinose, stachyose, glucose, fructose, galactose, and protein. The oil is largely made up of oleic and linoleic acids. Furthermore, the plant also contains curcasin, arachidic, linoleic, myristic, oleic, palmitic, and stearic acids and curcin. The whole genome was sequenced by Kazusa, Japan in October 2010 (Bioenergy Plantations, 2014).

2.1.3. **Propagation**

*Jatropha curcas* has limited natural vegetative propagation and is usually propagated by seed. Propagation through seed (sexual propagation) leads to a lot of genetic variability in terms of growth, biomass, seed yield and oil content. Low seed viability and the recalcitrant nature of oil seeds also limit seed propagation. However, clonal techniques can help in overcoming these problems that hinder mass propagation of this tree-borne oilseed species. Vegetative propagation has been achieved by stem cuttings, grafting, budding as well as by air layering techniques.

The investigation leads to the recommendation that cuttings should be taken preferably from juvenile plants and treated with 200 mg per liter of IBA (rooting
hormone) to ensure the highest level of rooting in stem cuttings. These vegetative methods have potential for commercial propagation of these plants (Fairless, 2007).

2.1.4. Cultivation

The plant can grow in wastelands and grows on almost any terrain, even on gravelly, sandy and saline soils. It can thrive in poor and stony soils, although new research suggests that the plant's ability to adapt to these poor soils is not as extensive as had been previously stated. Complete germination is achieved within 9 days. Adding manure during the germination has negative effects during that phase, but is favorable if applied after germination is achieved. It can be propagated by cuttings, which yields faster results than multiplication by seeds.

The flowers only develop terminally (at the end of a stem), so a good ramification (plants presenting many branches) produces the greatest amount of fruits. The plants are self-compatible. Another productivity factor is the ratio between female and male flowers within an inflorescence, more female flowers mean more fruits.

While Jatropha curcas starts yielding from 9–12 months time, the best yields are obtained only after 2 – 3 years time. If planted in hedges, the reported productivity of Jatropha is from 0.8 kg. to 1.0 kg. of seed per meter of live fence. The seed production is around 3.5 tons/ hectare (Seed production ranges from about 0.4 tons per hectare in first year to over 5 tons per hectare after 3 years) (Fairless, 2007).

2.1.5. Uses

The stems of Jatropha cuneata are used for basket making by the Seri people in Sonora, Mexico. The stems are roasted, split and soaked through an elaborate process. The reddish dye that is often used is made from the root of another plant species, Krameria grayi. Spicy jatropha (J. integerrima) is cultivated as an ornamental in the tropics for its continuously blooming crimson flowers. Buddha belly plant (J. podagrica) was used to tan leather and produce a red dye in Mexico and the southwestern United States. It is also used as a house plant.

The oil from Jatropha curcas is mainly converted into biodiesel for use in diesel engines. The cake can be used for fish or animal feed (if detoxified), biomass feedstock to power electricity plants, or as biogas or high-quality organic fertilizer. It can also be used as a bio-pesticide and for medicinal purposes.
The added benefits of *Jatropha* begin with the tree itself. *Jatropha* is used as a living fence around agricultural fields in some countries, such as Mali and Haiti. The toxicity of *Jatropha* makes it an attractive choice for a live fence since it deters grazing animals from eating their way through the tree line to the fields. *Jatropha* hedges provide a barrier to wind and soil erosion, increasing the fertility of the fields (Openshaw, 2000).

The young leaves may be safely eaten, steamed or stewed. Cooked with goat meat, they are said to advantageously counteract its smell. Pounded leaves are applied near horses' eyes to repel flies in India. Hydrogen cyanide (HCN) is present in the leaves. The extracts of the plants are dangerous to use but water can easily release it over if not too much extract is applied.

The species is listed as a honey plant. Hydrogen cyanide is present. Nuts can be construed for home cooking fuel in briquette form replacing charcoal zed timber as in Haiti. Sometimes roasted and eaten, although they are purgative. Also they can be burned like candlenuts when strung on grass. HCN is present Little, in South Sudan used as a contraceptive. Interest exists in producing animal feed from the bio-waste once the oil is expressed, as in the case with Haiti, where *Jatropha curcas* grows prolifically and animal feed as sort of supply.

The oil has been used for illumination, soap, candles the adulteration of olive oil, and making Turkey red oil. Turkey red oil, also called sulphonated (or sulfated) castor oil, is the only oil that completely disperses in water. It is made by adding sulfuric acid to pure *Jatropha* oil. It was the first synthetic detergent after ordinary soap, as this allows easy use for making bath oil products. It is used in formulating lubricants, softeners, and dyeing assistants (List and Horhammer, 1979).

Seed extraction is made simple with the use of the Universal Nut Sheller, an appropriate technology designed by the Full Belly Project. The oily seeds are processed into oil, which may be used directly to fuel combustion engines or may be subjected to trans esterification to produce biodiesel. *Jatropha* oil is not suitable for human consumption, as it induces strong vomiting and diarrhea. Roots ashes are used as a salt substitute. HCN and rotenone are present (Birgit, 2006). Bark also used as a fish poison. HCN is present (Levingston and Zamora, 2006). Latex Strongly inhibits the watermelon mosaic virus. Sap sometimes used for marking.
Mexicans grow the shrub as a host for the lac insect, which is used in medicine as hepatoprotective and antiobesity drug. Used for erosion control. The decoction of leaves is used against cough and as an antiseptic after birth (Isawami, 1978). Latex has antimicrobial properties against many species. The oil of this plant is used traditionally for the treatment of sciatica dropsy, paralysis, rheumatism, dysentery, diarrhea and certain skin diseases.

2.1.6. Jatropha seeds

The seeds of Jatropha contain viscous oil, which can be used for manufacture of candles and soap, in cosmetics industry, as a diesel/paraffin substitute or extender. This latter use has important implications for meeting the demand for rural energy services and also exploring practical substitutes for fossil fuels to counter greenhouse gas accumulation in the atmosphere. These characteristics along with its versatility make it of vital importance to developing countries (Kumar and Sharma, 2008). The seeds contain about 300–350 g/ kg oil, which can be used as a fuel directly or, in its trans-esterified form, as a substitute for diesel. The seed has a hard, black outer shell containing a white kernel. The proportions of shell and kernel range from 350 to 400 g/ kg and from 600 to 650 g/ kg respectively (Kumar and Sharma, 2008).

The seed weighs approximately from 0.53 -0.86 g and its kernel contains 22 -27 % protein and 57 -63 % lipid indicating good nutritional value. The seed contains 60–66% crude lipid and 30–32% crude protein. The seeds can be transported without deterioration and at low cost due to its high specific mass. The seeds contain about 30% oil. Oil may be produced from whole seeds using a screw press. The oil fraction of J. curcas consists of both saturated (14.2% palmitic acid and 7.0% stearic acid) and unsaturated fatty acids (44.7% oleic acid and 32.8% linoleic acid), and little amount of palmitoleic 0.7%, linolenic 0.2, margaric 0.1%, myristic 0.1% (Edem, 2002).

The seed cake left after extraction of oil provides a high amount of protein and all concentrations of essential amino acids except lysine are higher than those of the Food and Agriculture Organization (FAO) reference pattern suggested for pre-school children (Makkar and Becker, 1997). However, major constituents contained in the seed cake are toxic compound and anti-nutritional factors. The main toxic component of the seed cake is phorbol esters and the anti-nutritional factors found in the seed cake are trypsin.
inhibitor, phytic acid, lectin and saponin. Phorbol esters have been classified as the main toxic agents in *J. curcas* seed cake responsible for toxicity (Martínez-Herrera *et al.*, 2006).

The extraction of oil from *Jatropha* seeds is associated with generation of substantial amount of seedcake waste at an average rate of 500 g cake per kg of seeds used (Zanzi *et al.*, 2008). Inspite of its high protein content along with presence of all essential amino acids, except lysine, it cannot be used in feed formulation due to the presence of potential anti-nutritional components like phorbol esters (PE), lectins and trypsin inhibitors. The PEs, have been identified as main toxicants in JSC, which could not be destroyed even by heating at 160 C⁰ for 30 minutes (Makkar *et al.*, 1998).

### 2.1.7. Component of *Jatropha curcas* seeds

*Jatropha curcas* seeds contain 35% oil, 15-17% crude protein, 20% lignin, 15-18% fiber, 5-10% carbohydrates and 5% ash (Dianika, 2009). The physicochemical characteristics of Nigerian oil sample (Belewu *et al.*, 2010) in comparison to the standard range are presented in Table (2.1).

### 2.1.8. Toxicity

Much like other members of the family Euphorbiaceae, *Jatropha* plants contain several toxic compounds, including lectin, saponin, carcinogenic phorbolesters, and a trypsin inhibitor. The seeds of this genus are also a source of the highly poisonous toxalbumin curcin. Despite this, the seeds are occasionally eaten after roasting, which reduces some of the toxicity. Its sap is a skin irritant, and ingesting as few as three untreated seeds can be fatal to humans. In 2005 Western Australia banned *Jatropha gossypiiifolia* as invasive and highly toxic to people and animals (MacIntyre, 2007).

Phorbol esters have been classified as the main toxic agents in *J. curcas* seed cake responsible for toxicity (Martínez-Herrera *et al.*, 2006). The toxicity of phorbol esters limits the use of *J. curcas* seed cake as a human or animal food. The phorbol esters affect humans and animals by causing tumor promotion, cell proliferation, blood platelet activation, lymphocyte mitogenesis, erythema of the skin, prostaglandin production and stimulation of degranulation in neutrophils (Aitken, 1986).
Figure (2.2): Jatropha seed

Figure (2.3): Jatropha kernel

Figure (2.4): Jatropha oil
Table (2.1) The physicochemical characteristics of *Jatropha* oil

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Refraction index</td>
<td>0.895</td>
<td>1.93</td>
<td>1.47</td>
</tr>
<tr>
<td>Density (g/ml)</td>
<td>0.881</td>
<td>0.903</td>
<td>0.917</td>
</tr>
<tr>
<td>Viscosity (at room temperature)</td>
<td>76.18</td>
<td>42.88</td>
<td>52.6</td>
</tr>
<tr>
<td>Free Fatty Acid %</td>
<td>11.94</td>
<td>2.25</td>
<td>2.33</td>
</tr>
<tr>
<td>Acid value (mg/g)</td>
<td>23.87</td>
<td>4.48</td>
<td>1.0-38.2</td>
</tr>
<tr>
<td>Peroxide value (mg/kg)</td>
<td>44</td>
<td>ND</td>
<td>1.93</td>
</tr>
<tr>
<td>Saponification (mg/g)</td>
<td>230</td>
<td>193.55</td>
<td>188-198</td>
</tr>
<tr>
<td>Iodine No. (g/100g)</td>
<td>26.09</td>
<td>103.62</td>
<td>90.8-112.5</td>
</tr>
</tbody>
</table>

ND: not detected
2.2. Hexane

Hexane is an alkane of six carbon atoms, with the chemical formula \( C_6H_{14} \). The term may refer to any of the five structural isomers with that formula, or to a mixture of them. Hexanes are significant constituents of gasoline. They are all colorless liquids at room temperature, odorless when pure, with boiling points between 50 and 70 °C. They are widely used as cheap, relatively safe, largely unreactive, and easily evaporated non-polar solvents (Scott Organic Chemistry, 2007).

2.2.1. Uses

In industry, hexanes are used in the formulation of glues for shoes, leather products, and roofing. They are also used to extract cooking oils (such as canola oil) from seeds, for cleansing and degreasing a variety of items, and in textile manufacturing. A typical laboratory use of hexanes is to extract oil and grease contaminants from water and soil for analysis (Tema, 2003).

In many applications (especially pharmaceutical), the use of \( n \)-hexane is being phased out due to its long term toxicity. It is often replaced by \( n \)-heptane, which will not form the toxic metabolite hexane-2,5-dione.

2.2.3. Toxicity

The acute toxicity of \( n \)-hexane is low, although it is a mild anesthetic. Inhalation of high concentrations produces first a state of mild euphoria, followed by somnolence with headaches and nausea. The toxicity of \( n \)-hexane has been extensively discussed by the Agency for Toxic Substances and Disease Registry (1999).

The long-term toxicity of \( n \)-hexane in humans is well known. Extensive peripheral nervous system failure is known to occur in humans chronically exposed to levels of \( n \)-hexane ranging from 400 to 600 ppm, with occasional exposures up to 2,500 ppm. The initial symptoms are tingling and cramps in the arms and legs, followed by general muscular weakness. In severe cases, atrophy of the skeletal muscles is observed, along with a loss of coordination and problems of vision. Similar symptoms are observed in animal models. They are associated with a degeneration of the peripheral nervous system (and eventually the central nervous system), starting with the distal portions of the longer and wider nerve axons. The toxicity is not due to hexane itself but to one of its metabolites, hexane-2,5-dione. It is believed that this reacts with the amino group of the
side chain of lysine residues in proteins, causing cross-linking and a loss of protein function.

Chronic intoxication from hexane has been observed in recreational solvent abusers, and in workers in the shoe manufacturing, furniture restoration, and automobile manufacturing industries, and recently, plastic recyclers, and assemblers and cleaners of capacitive touch screen devices (Hathaway et al., 2004).

2.2.4. Use in food processing

Hexane has been used to extract oil from grains as well as protein from soy, to such an extent that in 2007, grain processors were responsible for more than two-thirds of hexane emissions in the United States. The report also pointed out that the hexane can persist in the final food product created; in a sample of processed soy, the oil contained 10 ppm, the meal 21 ppm and the grits 14 ppm hexane. The adverse health effects seem specific to n-hexane; they are much reduced or absent for other isomers. Therefore, the food oil extraction industry, which relied heavily on hexane, has been considering switching to other solvents, including isohexane (2-methylpentane) (Peter et al., 1997).

2.3. Antibiotics

In order to be useful in treating human infections, antibiotics must selectively target bacteria for eradication and not the cells of its human host. Indeed, modern antibiotics act either on processes that are unique to bacteria--such as the synthesis of cell walls or folic acid--or on bacterium-specific targets within processes that are common to both bacterium and human cells, including protein or DNA replication. Following are some examples. Most bacteria produce a cell wall that is composed partly of a macromolecule called peptidoglycan, itself made up of amino sugars and short peptides. Human cells do not make or need peptidoglycan. Penicillin, one of the first antibiotics to be used widely, prevents the final cross-linking step, or transpeptidation, in assembly of this macromolecule. The result is a very fragile cell wall that bursts, killing the bacterium. No harm comes to the human host because penicillin does not inhibit any biochemical process that goes on within us (Harry, 2006).

Bacteria can also be selectively eradicated by targeting their metabolic pathways. Sulfonamides, such as sulfamethoxazole, are similar in structure to para-aminobenzoic acid, a compound critical for synthesis of folic acid. All cells require folic acid and it can
diffuse easily into human cells. But the vitamin cannot enter bacterial cells and thus bacteria must make their own. The sulfa drugs such as sulfonamides inhibit a critical enzyme--dihydropteroate synthase--in this process. Once the process is stopped, the bacteria can no longer grow.

Another kind of antibiotic -tetracycline- also inhibits bacterial growth by stopping protein synthesis. Both bacteria and humans carry out protein synthesis on structures called ribosomes. Tetracycline can cross the membranes of bacteria and accumulate in high concentrations in the cytoplasm. Tetracycline then binds to a single site on the ribosome--the 30S (smaller) ribosomal subunit--and blocks a key RNA interaction, which shuts off the lengthening protein chain. In human cells, however, tetracycline does not accumulate in sufficient concentrations to stop protein synthesis.

Similarly, DNA replication must occur in both bacteria and human cells. The process is sufficiently different in each that antibiotics such as ciprofloxacin--a fluoroquinolone notable for its activity against the anthrax bacillus--can specifically target an enzyme called DNA gyrase in bacteria. This enzyme relaxes tightly wound chromosomal DNA, thereby allowing DNA replication to proceed. But this antibiotic does not affect the DNA gyrase of humans and thus, again, bacteria die while the host remains unharmed. Many other compounds can kill both bacterial and human cells. It is the selective action of antibiotics against bacteria that make them useful in the treatment of infections while at the same time allowing the host to live another day (Harry, 2006).

2.4. Bacillus

*Bacillus* is a genus of Gram-positive, rod-shaped (bacillus), bacteria and a member of the phylum Firmicutes. Bacillus species can be obligate aerobes (oxygen reliant), or facultative anaerobes (having the ability to be aerobic or anaerobic). They will test positive for the enzyme catalase when there has been oxygen used or present (Graumann, 2012). Ubiquitous in nature, *Bacillus* includes both free-living (non-parasitic) and parasitic pathogenic species. Under stressful environmental conditions, the bacteria can produce oval endospores that are not true spores but which the bacteria can reduce themselves to and remain in a dormant state for very long periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera of Firmicutes. Many species of *Bacillus* can produce copious
amounts of enzymes which are made use of in different industries. Some Bacillus species can form intracellular inclusions of polyhydroxy alkanoates under certain adverse environmental conditions, as in a lack of elements such as phosphorus, nitrogen, or oxygen combined with an excessive supply of carbon sources. *B. subtilis* has proved a valuable model for research. Other species of *Bacillus* are important pathogens, causing anthrax and food poisoning (Scheffers, 2012).

### 2.4.1. Industrial significance

Many *Bacillus* species are able to secrete large quantities of enzymes. *Bacillus amyloliquefaciens* is the source of a natural antibiotic protein barnase (a ribonuclease), alpha amylase used in starch hydrolysis, the protease subtilisin used with detergents, and the BamH1 restriction enzyme used in DNA research. A portion of the *Bacillus thuringiensis* genome was incorporated into corn crops and cotton. The resulting GMOs are therefore resistant to some insect pests (Xu and Cote, 2003).

### 2.4.2. Clinical significance

Two *Bacillus* species are considered medically significant: *B. anthracis*, which causes anthrax, and *B. cereus*, which causes food poisoning similar to that caused by *Staphylococcus*. A third species, *B. thuringiensis*, is an important insect pathogen, and is sometimes used to control insect pests. The type species is *B. subtilis*, an important model organism. It is also a notable food spoiler, causing ropiness in bread and related food. Some environmental and commercial strains (*B. coagulans*) may play a role in food spoilage of highly acidic, tomato based products. An easy way to isolate *Bacillus* is by placing nonsterile soil in a test tube with water, shaking, placing in melted mannitol salt agar, and incubating at room temperature for at least a day. Colonies are usually large, spreading and irregularly shaped. Under the microscope, the Bacillus cells appear as rods, and a substantial portion usually contain an oval endospore at one end, making it bulge (Glöckner and Rossello-Mora, 2008).

The cell wall of *Bacillus* is a structure on the outside of the cell that forms the second barrier between the bacterium and the environment, and at the same time maintains the rod shape and withstands the pressure generated by the cell's turgor. The cell wall is composed of teichoic and teichuronic acids. *B. subtilis* is the first bacterium for which the role of an actin-like cytoskeleton in cell shape determination and
peptidoglycan synthesis was identified, and for which the entire set of peptidoglycan-synthesizing enzymes was localised. The role of the cytoskeleton in shape generation and maintenance is important (Scheffers, 2012).

2.5. *Staphylococcus*

*Staphylococcus aureus* is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans.

*S. aureus* is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus. The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* produces staphylococcal enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning (Montville and Matthews, 2008). *S. intermedius*, a *Staphylococcus* species which is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning (Le Loir et al., 2003).

2.5.1. Growth and survival characteristics

The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity (aw), pH, the presence of oxygen and composition of the food. These physical growth parameters vary for different *S. aureus* strains (Stewart, 2003).

2.5.2. Symptoms of disease

Staphylococcal food poisoning symptoms generally have a rapid onset, appearing around 3 hours after ingestion (range 1–6 hours). Common symptoms include nausea, vomiting, abdominal cramps and diarrhoea. Individuals may not demonstrate all the symptoms associated with the illness. In severe cases, headache, muscle cramping and transient changes in blood pressure and pulse rate may occur. Recovery is usually between 1–3 days (Stewart, 2003). Fatalities are rare (0.03% for the general public) but are occasionally reported in young children and the elderly (4.4% fatality rate) (Montville and Matthews, 2008). *S. aureus* can cause various non-food related health issues such as skin inflammations (e.g. boils and sty's), mastitis, respiratory infections, wound sepsis and toxic shock syndrome (Stewart, 2003).
2.5.3. Virulence and infectivity

Staphylococcal food poisoning is an intoxication that is caused by the ingestion of food containing pre-formed SE (Argudin et al., 2010). There are several different types of SE; enterotoxin A is most commonly associated with staphylococcal food poisoning. Enterotoxins D, E and H, and to a lesser extent B, G and I, have also been associated with staphylococcal food poisoning (Seo and Bohach, 2007). SEs are produced during the exponential phase of *S. aureus* growth, with the quantity being strain dependent.

2.5.4. Mode of transmission

Staphylococcal food poisoning occurs when food is consumed that contains SE produced by *S. aureus*. Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination via direct contact or through respiratory secretions (Argudin et al., 2010). The incidence of staphylococcal food poisoning is seasonal. Most cases occur in the late summer when temperatures are warm and food is stored improperly (Montville and Matthews, 2008).

Foods associated with outbreaks of staphylococcal food poisoning include meat and meat products, poultry and egg products, milk and dairy products, salads, cream-filled bakery products and sandwich fillings. Foods that require extensive handling during preparation and are kept above refrigeration temperature (4°C) for extended periods after preparation are often involved in staphylococcal food poisoning (Argudin et al., 2010). All people are believed to be susceptible to staphylococcal food poisoning. However, the severity of symptoms may vary depending on the amount of SE consumed in the food and the general health of individuals. The young and elderly are more likely to develop more serious symptoms (FDA, 2012).

2.6. *Escherichia coli*

*Escherichia coli* (commonly abbreviated *E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. The genus belongs in a group of bacteria informally known as coliforms, and is a member of the Enterobacteriaceae family (Brenner et al., 2005).

Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005). The harmless strains are part of the normal
flora of the gut, and can benefit their hosts by producing vitamin K2 (Bentley and Meganathan, 1982). *E. coli* and related bacteria constitute about 0.1% of gut flora, and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination (Eckburg et al., 2005).

### 2.6.1. Biology and biochemistry

*E. coli* is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped, and are about 2.0 micrometers (μm) long and 0.25-1.0 μm in diameter, with a cell volume of 0.6–0.7 μm³ (Kubitschek, 1990). It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen-consuming organisms, such as methanogens or sulphate-reducing bacteria (Madigan and Martinko, 2006). Optimal growth of *E. coli* occurs at 37 °C (98.6 °F) but some laboratory strains can multiply at temperatures of up to 49 °C (Fotadar et al., 2005). *E. coli* normally colonizes an infant's gastrointestinal tract within 40 hours of birth. In the bowel, it adheres to the mucus of the large intestine. It is the primary facultative anaerobe of the human gastrointestinal tract (Todar, 2007).

### 2.6.2. Therapeutic use of nonpathogenic strain

Nonpathogenic Escherichia coli strain Nissle 1917 also known as Mutaflor and *Escherichia coli* O83:K24:H31 (known as Colinfant) (Lodinová-Zádníková et al., 2003) are used as a probiotic agents in medicine, mainly for the treatment of various gastroenterological diseases (Grozdanov et al., 2004) including inflammatory bowel disease (Kamada et al., 2005).

### 2.6.3. Pathogenicity

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia (Todar, 2007).
2.6.4. Role in biotechnology

*E. coli* plays an important role in modern biological engineering and industrial microbiology (Lee, 1996). The work of Stanley Norman Cohen and Herbert Boyer in *E. coli*, using plasmids and restriction enzymes to create recombinant DNA, became a foundation of biotechnology (Russo, 2003). *E. coli* was one of the first organisms to have its genome sequenced; the complete genome of *E. coli* K12 was published by Science in 1997 (Blattner *et al.*, 1997).

2.7. *Salmonella*

Is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with diameters around 0.8 to 1.5 μm, lengths from 2 to 5 μm, and peritrichous flagella, (flagella that are all around the cell body). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes. There are only two species of Salmonella; *Salmonella bongori* and *Salmonella enterica* of which there are around six subspecies and innumerable serovars. *Salmonella* belongs to the same family as *Escherichia*, which has as a species *E. coli*. Most subspecies of *Salmonella* produce hydrogen sulfide (Clark and Barret, 1987), which can readily be detected by growing them on media containing ferrous sulfate, such as is used in the triple sugar iron test (TSI). Most isolates exist in two phases: a motile phase I and a nonmotile phase II. Cultures that are nonmotile upon primary culture may be switched to the motile phase using a Cragie tube. *Salmonella* is found worldwide in both cold-blooded and warm-blooded animals, and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning (Ryan and Ray, 2004).

2.7.1. Sources of infection

- Infected food,
- Poor kitchen hygiene,
- Excretions from sick or infected but apparently healthy people and animals,
- Polluted surface and standing water,
- Unhygienically melt water contains bacteria,
*Salmonella* bacteria can survive for some time without a host; thus, they are frequently found in polluted water, contamination from the excrement of carrier animals being particularly important. The most recent case of *Salmonella* infection had been detected mid-2012 in seven EU countries. Over 400 people had been infected with *Salmonella enterica* serovar Stanley. After several DNA analyses, a report detected turkey production as the source of infection *(EFSA, 2012)*.
CHAPTER THREE
MATERIAL AND METHODS

3.1. Study area

This experiment was conducted in the Laboratory of Department of Plant and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum (January, 2014) and in the Laboratory of Food Analysis, Faculty of Engineering and Technology, University of Gezira (April, 2014).

3.2. Materials

3.2.1. Jatropha oil

*Jatropha* oil was obtained by mechanical extract from Department of Botany and Agricultural Biotechnology, and was diluted by hexane to obtained the varies concentration by taken 2.5 ml oil and added to 7.5 ml hexane to obtained the concentration of 25%, and 5 ml oil to 5 ml hexane to obtained the concentration of 50%, and 7.5 ml oil to 2.5 ml hexane to obtained the concentration of 75% and pure oil and hexane to obtained 100% and 0%, respectively.

3.2.2. Bacteria samples

The samples used of Bacteria involved the pure culture of:

- *Bacillus cereus*.
- *Staphylococcus aureus*.
- *Escherichia coli*.
- *Salmonella typhi*.

Which were isolated from pathogenic specimen in Laboratory of Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum?

3.3. Methods

3.3.1. Preparation of medium

- Nutrient broth:
  
  Nutrient broth was prepared by suspended 3.25 g/250 ml distilled water, used to encourage the growth of bacterial strains in test tube.
• Nutrient agar:
  
  The nutrient agar was prepared by suspended 7 g/250 ml distilled water and the inverted plates were left to dry.

3.3.2. Disc diffusion method

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by (Bauer et al., 1966) to assess the presence of antibacterial activities of the Jatropha oil. Each of paper discs impregnated with the Jatropha oil (25%, 50%, 75%, and 100%), and One positive control which is a paper discs impregnated with tetracycline (500 mg/10 ml) and one negative control by hexane pure. Then paper discs were placed on the surface suitably spaced apart. Plates were incubated for 18 – 24 hours at 37°C depending on the species of the bacteria used in test. After the incubation, the plates were then examined for the presence of zones inhibition of the bacterial growth around impersonated discs which indicated the susceptibility of the organisms to these treatments. The tests were repeated six times to ensure reliability.

The absence of inhibition zones around other discs indicated resistance of the organisms to these treatments. The diameter of the zones inhibition indicated to the degree of the sensitivity of the organisms.

3.3.3 The Physical and Chemical characteristics of Jatropha oil

3.3.3.1 Refraction Index

Determination of the refractive index was done using a refractometer (Appe 60) at a constant temperature of 28 °C. Few drops of the sample was placed on the prism. The prisms was closed and allowed to standing for 1-2 minutes. The instrument was adjusted and lighted to obtain the most distinct reading possible and to determine the refractive index.

3.3.3.2 Density

Determination of the weight of the mass (m) to its volume (V) per ml (Kopecký, F.1999), it was done by using Pycnometer (Appe 60) it was measured at 35 °C.

3.3.3.3 Viscosity

Was measured by Brookfield viscometer (as poise). And since that, the viscosity is affected greatly by both temperature and water content, was measures at 35 °C.
A test sample was taken and adjust the water bath to the required temperature, and waited until the temperature has stabilised. Sample was brought to approximately the required temperature. Then, fall with the sample cup of the viscometer to the prescribed height. Sample cups was put in the thermostated cup holder. Then the spindle was introduced in the product and attach the spindle to the drive and wait until the sample has reached the required temperature. The motor was started and selected the rotational frequency recommended for the sample being studied and waited until a constant reading is displayed (ISO, 1989).

\[ V = F \times R \]

Where:

- \( V \): is the viscosity.
- \( F \): is the factor depending on the rotational frequency / spindle combination.
- \( R \): is the mean value of the two readings displayed by the viscometer.

### 3.3.3.4 Free fatty acid (F.F.A)

About 10 g of sample was transferred to conical flask (250 ml). 50 ml of hexane (solvent) was added and the content was shaken while warming. 1.0 ml of Phenolphthalein was added. The solution was then mixed and titrated with KOH until the faint pink color persists (AOSC, 2000).

\[ \text{F.F.A}\% = \frac{\text{volume of KOH}}{\text{Weight of sample}} \times 2.82 \]

### 3.3.3.5 Acid value

Acid value was calculated from the output of free fatty acid test according to (AOSC, 2000) as follow:

\[ \text{A.V} = \frac{\text{volume of KOH}}{\text{Weight of sample}} \times 5.64 \]

### 3.3.3.6 Peroxide value

About 1 ml of oil was weighed into a clean dry boiling tube and 1 g powdered Potassium Iodide was added while still liquid. 20 ml of the acetic acid - chloroform solution (at ratio 2:1) was added. The tube was placed in the boiling water so that the
liquid boils within 30 seconds. The contents was poured into flask containing 20 ml of potassium iodide solution (5%). 25 ml of distilled water was immediately added by graduated cylinder, and was titrated with 0.002M sodium thiosulfate solution using starch indicator. The starting deep red orange color of the solution was the sign for titration with mixing slowly until the color lightens. When the blue gray color disappears in the aqueous (upper layer) the titration was then stopped (AOSC, 2000). The mls of titrant used to two decimal places were accurately recorded.

\[
\text{Peroxide value} = \frac{(V - V_s) T \times 1000}{M}
\]

Where:
V: Blank titration value
Vs: Sample titration value
T: Molarity of Sodium thiosulfate
M: Mass of sample

**3.3.3.7 Saponification Value**

About 0.1 g of sample was weighed and dissolved in 2 ml of hydrochloric acid and then the contents was transferred to conical flask 250 ml by rinsing the beaker three times with 2 ml of HCL. 25 ml of standard alcohol solution was added to KOH (0.1 M). The flask was tightly fitted with a corked condenser and refluxed on a boiling water path for 30 – 60 minutes. Similarly another flask containing everything except the fat was treated. After refluxing the solution was then let to cool on room temperature and then titrated with HCL and Phenolphthalein indicator (given pink color) (AOSC, 2000).

\[
S.V = \frac{56.1 \times (B - T) \times M}{\text{Wt. of sample}}
\]

Where:
B: value of the blank titration.
T: value of the sample titration.
M: Molarity of HCL.
3.3.3.8 Iodine Number

About 10 g of sample was weighed and placed in 250 ml stopped volumetric flask. 10 ml of Chloroform then 25ml of ICL were added and shacked vigorously. The solution was allowed to stand in the dark for 30 – 45 minutes. 20 ml of KI and 100 water were added. The solution was then mixed and titrated with sodium thiosulphate solution 0.1M using starch solution as an indicator (given blue color) (AOAC, 2000).

\[
\text{Iodine Number} = (B - T) M \times 12.7 \quad \text{Wt. of sample}
\]

Where

B : value of the blank titration.

T : value of the sample titration.

M : Molarity of Na₂S₂O.

3.4. Statistical analysis

The data obtained in this experiment were analyzed using the Excel- program. ANOVA and simple descriptive analysis were used to detect the significant difference.
4.1 The physical characteristics of *Jatropha* oil

The physical characteristics (refractive index, density and viscosity) of *Jatropha* oil sample were presented in table (4.1). The refractive index of *Jatropha* oil sample was 1.473. It was 1.47, 1.93 and 0.895 for the standard value (Bioenergy Plantations, 2014), Malaysian sample (Emil, 2009), and Nigerian sample (Belewu et al., 2010), respectively. The refractive index of the Sudanese sample was similar to that of the standard. Refractive index is the ratio of the speed of light in a vacuum to the speed in the medium. A coefficient shows the extent to which Article electromagnetic waves. Visible light transparency of most materials has coefficients of refraction between 1 to 2. Almost all solids and liquids have a refractive index greater than 1.3 and measured by refractometer (AOAC, 1984).

The density of *Jatropha* oil sample was 0.92 g/ml. The density was 0.917, 0.903 and 0.881 for the standard value (Bioenergy Plantations, 2014), Malaysian sample (Emil, 2009), and Nigerian sample (Belewu et al., 2010), respectively. The density of the Sudanese sample was similar to that of the standard. Volume number density is the number of specified objects per unit volume and measured by pycnometer (AOAC, 1984).

The viscosity of *Jatropha* oil sample was 2.5 poise. The viscosity was 52.6, 42.88 and 76.18 for the standard value (Bioenergy Plantations, 2014), Malaysian sample (Emil, 2009), and Nigerian sample (Belewu et al., 2010), respectively. The viscosity of the Sudanese sample was less than those samples. Absolute viscosity provides a measure of a fluid’s internal resistance to flow. For liquids, viscosity corresponds to the informal notion of "thickness", and it is measured usually by Brookfield viscometer (AOAC, 1984).
Table (4.1): The refractive index, density and viscosity of *Jatropha* oil

<table>
<thead>
<tr>
<th>Parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refraction index</td>
<td>1.473</td>
</tr>
<tr>
<td>Density (g/ml)</td>
<td>0.92</td>
</tr>
<tr>
<td>Viscosity (poise) (at 35 °C)</td>
<td>2.50</td>
</tr>
</tbody>
</table>
4.2. The chemical characteristics of *Jatropha* oil

The chemical characteristics (free fatty acids, acid value, peroxide value, saponification value and iodine number) of *Jatropha* oil sample were presented in Table (4.2). The free fatty acid of *Jatropha* oil was 0.61%. It was 2.33, 2.25 and 11.94 for the standard value (*Bioenergy Plantations, 2014*), Malaysian sample (*Emil, 2009*), and Nigerian sample (*Belewu et al., 2010*), respectively. Fatty acids are usually derived from triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids" (*AOAC, 2000*).

The acid value of *Jatropha* oil was 1.20. It was 1.0 - 38.2, 4.48 and 23.87 for the standard value (*Bioenergy Plantations, 2014*), Malaysian sample (*Emil, 2009*), and Nigerian sample (*Belewu et al., 2010*), respectively. Acid value is the number of mg of KOH or NAOH required to neutralize the free fatty acid Present in one gram of sample (*AOAC, 2000*).

The peroxide value of *Jatropha* oil was 56.50. It was 1.93 and 44 for the standard value (*Bioenergy Plantations, 2014*) and Nigerian sample (*Belewu et al., 2010*), respectively. The peroxide value is determined by measuring the amount of iodine which is formed by the reaction of peroxides (formed in fat or oil) with iodide ion. The acidic conditions (excess acetic acid) prevent formation of hypoiodite which would interfere with the reaction (*AOAC, 2000*).

The saponification value of *Jatropha* oil was 15.15. It was 188 – 198, 193.55 and 230 for the standard value (*Bioenergy Plantations, 2014*), Malaysian sample and Nigerian sample (*Belewu et al., 2010*), respectively. Saponification value is the number of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of the sample (*AOAC, 2000*).

The iodine number of *Jatropha* oil was 4.30. It was 90.8- 111.25, 103.62 and 26.09 for the standard value (*Bioenergy Plantations, 2014*), Malaysian sample and Nigerian sample (*Belewu et al., 2010*), respectively. Iodine value is the weight of iodine absorbed by 100 parts of sample by weight (*AOAC (2000)*).

The acid value of *Jatropha* oil sample, fall within the standard range, whereas, peroxide value, free fatty acids, saponification and iodine number of the Sudanese sample were out of the standard ranges.
Table (4.2): The chemical characteristics of *Jatropha* oil

<table>
<thead>
<tr>
<th>Parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Fatty Acid %</td>
<td>0.61</td>
</tr>
<tr>
<td>Acid value (mg/kg)</td>
<td>1.20</td>
</tr>
<tr>
<td>Peroxide value (mg/kg)</td>
<td>56.50</td>
</tr>
<tr>
<td>Saponification value (mg/kg)</td>
<td>15.15</td>
</tr>
<tr>
<td>Iodine no. (mg/kg)</td>
<td>4.30</td>
</tr>
</tbody>
</table>
Refractive index of a substance measures how the substance affects light passing through it and to an extent tells its degree of purity (Oladele, Oshodi, 2008).

The density of a material is defined as the measured of its mass per unit volume (e.g. in g/ml). Generally, the density of oil decreases with molecular weight, yet increase with unsaturation level (Gunstone, 2004).

Viscosity defined as resistance liquid to flow. Viscosity increased with molecular weight but decreased with increasing unsaturated level and temperature (Nouredini et al, 1992). The viscosity of Jatropha seed oil must be reduced for biodiesel application since the kinematic viscosity of biodiesel were very low compared to vegetable oils. High viscosity of the Jatropha oil seed are not suitable if its use directly as engine fuel (Agarwal and Agarwal, 2007), often results in operational problems such as carbon deposits, oil ring sticking, and thickening and gelling of lubricating oil as a result of contamination by the vegetable oils.

The F.F.A and moisture contents have significant effects on the transesterification of glycerides with alcohol using catalyst (Goodrum, 2002). The high FFA content (>1% w/w) will happen soap formation and the separation of products will be exceedingly difficult, and as a result, it has low yield of biodiesel product (Crabbe et al., 2001).

Peroxide value were high compared to Nigerian and standard. Peroxide value is an indicator of the deterioration of lipids due to oxidation at the double bond of an unsaturated fatty acid which causes rancidity. The high iodine value and oxidative stability show that the seed oil upholds the good qualities for semi-drying oil purposes (Eronmosele et al., 1997).

Standard saponification value of Jatropha oil was 193.55. High saponification value indicated that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries.

The iodine value is a measure of the unsaturation of fats and oils. Higher iodine value indicated that higher unsaturation of fats and oils. The limitation of unsaturated fatty acids, as a biodiesel, is necessary due to the fact that heating higher unsaturated fatty acids results in polymerization of glycerides. This can lead to the formation of deposits or to deterioration of the lubricating. Fuels with this characteristic (e.g Sunflower oil, soybean oil and safflower oil) also likely to produce thick sludges in the sump of the
engine, when fuel seeps down the sides of the cylinder into crankcase. The iodine values of *Jatropha curcas* place them in the semi-drying oil group. High iodine value of *Jatropha* are caused by high content of unsaturation fatty acid such as oleic acid and linoleic acid. The iodine values of *Jatropha* seed oil suggest their use in production of alkyd resin, shoe polish, varnishes etc. (Akintayo, 2004). The peroxide value of *Jatropha* oil showed a low value (as crude seed oil) of 1.93 mg/kg, proving the oxidative stabilities of the seed oil relatively. The high iodine value and oxidative stability shows that the seed oil upholds the good qualities of semidrying oil purposes (Eromosele *et al.*, 1997).

Therefore, the physical and chemical properties of the *Jatropha curcas* oil may be changes during handling and storage. Many researchers have been conducted to characterize these physical and chemical changes in the other vegetable oils (Monyem and Van Gerpen, 2001; Leung *et al.*, 2006).
4.3 The antimicrobial activity of *Jatropha* oil

The antimicrobial activities of the varies concentration of *Jatropha* oil against four bacteria strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*) were assessed by the presence or absence of zones inhibition (Table, 4.3). The concentrations of 50% and 25% (Plate 4.1 – 4.6) have potential antimicrobial activities to both strains of gram positive and gram negative bacteria but less than those of antibiotic. The concentration of 25% showed antimicrobial activities to one gram positive and one gram negative bacteria. Other concentration 75% and 100% showed no activities. The difference between the used concentrations was significant (at P=0.011). Hexane was showed antimicrobial activities. The toxicity of hexane has been extensively discussed by the *Agency for Toxic Substances and Disease Registry* (1999), but its activity was less than antibiotic and lower *Jatropha* oil concentrations.

*Jatropha curcas* is a useful perennial plant. It’s seed oil is used to be ingredient for soap making and biodiesel. Their bioactive compounds are interesting. Crude leaf (Di Methyl Sulpho Oxide) extract showed intermediate activity against gram-positive bacteria. Crude seed extract and seed oil has shown some moderate activity whereas, no activity was observed in any leaf extracts. Higher resistance of gram-negative bacteria to external agents is attributed to the presence of lipopolysaccharides in their outer membrane, which make them inherently resistance to antibiotic, detergent and hydrophilic dyes. The reason for higher sensitivity of the gram-positive bacteria than negative bacteria could be ascribed to the differences between their cell wall compositions (*Sanjay et al.*, 2011).

The *Jatropha* leaf extract was effective in controlling the fungal pathogens *Sclerotium* sp. and *Colletotrichum musae*. Furthermore, the latex of *Jatropha* contains an alkaloid (Jatrophine) which is used as a hemostatic and wound dressing and is said to be efficacious in treating scabies, wasp and bee stings, treat bleeding gums, toothache, anti-inflammation, eczema and ringworm. In addition, antibacterial activity of extracts of *Jatropha* seed oil, root and stem bark was also reported by many workers (*Aiyelaagbe et al.*, 2007; *Jinda*, 2009 and *Tarun et al* 2012).
Table (4.3): The mean values inhibition zones (cm) of antimicrobial activities of the varies concentrations of *Jatropha* oil

<table>
<thead>
<tr>
<th>Conc. (hexane)</th>
<th>0%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>100%</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Bacillus})</td>
<td>0.673</td>
<td>0</td>
<td>1.83</td>
<td>0</td>
<td>0</td>
<td>3.25</td>
</tr>
<tr>
<td>(\text{Staph.})</td>
<td>0.648</td>
<td>1.55</td>
<td>0.94</td>
<td>0</td>
<td>0</td>
<td>4.05</td>
</tr>
<tr>
<td>(\text{E. coli})</td>
<td>0.651</td>
<td>1.94</td>
<td>1.40</td>
<td>0</td>
<td>0</td>
<td>2.83</td>
</tr>
<tr>
<td>(\text{Salmonella})</td>
<td>0.676</td>
<td>0</td>
<td>1.61</td>
<td>0</td>
<td>0</td>
<td>4.51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variance</th>
<th>Average</th>
<th>Sum</th>
<th>Count</th>
<th>SUMMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.65</td>
<td>1.69</td>
<td>5.08</td>
<td>3</td>
<td>Bacillus</td>
</tr>
<tr>
<td>2.72</td>
<td>2.18</td>
<td>6.54</td>
<td>3</td>
<td>Staph.</td>
</tr>
<tr>
<td>0.52</td>
<td>2.06</td>
<td>6.17</td>
<td>3</td>
<td>E. coli</td>
</tr>
<tr>
<td>5.22</td>
<td>2.04</td>
<td>6.12</td>
<td>3</td>
<td>Salmonella</td>
</tr>
<tr>
<td>1.04</td>
<td>0.87</td>
<td>3.49</td>
<td>4</td>
<td>(25%)</td>
</tr>
<tr>
<td>0.14</td>
<td>1.45</td>
<td>5.78</td>
<td>4</td>
<td>(50%)</td>
</tr>
<tr>
<td>0.58</td>
<td>3.66</td>
<td>14.64</td>
<td>4</td>
<td>Antibiotic</td>
</tr>
</tbody>
</table>

### ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>(P)-value</th>
<th>(F) crit</th>
<th>(F) crit</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Rows})</td>
<td>0.393</td>
<td>3</td>
<td>0.131</td>
<td>0.161</td>
<td>0.919</td>
<td>4.757</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Columns})</td>
<td>17.339</td>
<td>2</td>
<td>8.669</td>
<td>10.633</td>
<td>0.011</td>
<td>5.143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Error})</td>
<td>4.892</td>
<td>6</td>
<td>0.815</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22.624</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Plate (4.1) Effect of oil conc. (25%) on *Staphylococcus*.

Plate (4.2) Effect of oil conc. (25%) on *E.coli*.
Plate (4.3) Effect of oil conc. (50%) on *Bacillus*.

Plate (4.4): Effect of oil conc. (50%) on *Staphylococcus*. 
Plate (4.5) Effect of oil conc. (50%) on *E.coli*

Plate (4.6): Effect of oil conc. (50%) on *Salmonella*. 
CHAPTER FIVE
CONCLUSION AND RECOMMENDATION

5.1 Conclusions
The present study was to carry out preliminary investigation on the physicochemical characteristics and the antibacterial activity of *Jatropha* oil. Most of the physicochemical characteristics were out of the standards to be suitable source of biodiesel. All strains of bacteria were affected by the concentration of 50%, however just *Staphylococcus* and *E. coli* were affected by concentration of 25%, but the activity was less than antibiotic,. The two concentrations that did not affect bacteria were 75% and 100%, and.

The use of oil as an antibiotic explained that it is a broad spectrum in the elimination of bacteria gram positive and negative.

5.2 Recommendations
- Although the nature and number of active components are not clear and the extract method of the *Jatropha* oil may not be perfect, but the findings put the basis for further studies to work to prepare an optimize methods for studying of the physicochemical composition of the oil compare to the biodiesel standards.
- Detect the active ingredients suitable as an antibacterial for wider range of bacterial strains.
- Used the oil in manufacture of antibiotics by pharmacists.
- Used the oil as biodiesel for engines.
REFRENCES


EFSA (2012). Multi-country outbreak of *Salmonella* Stanley infections Update. EFSA Journal, 10(9):2893 [16 pp.].

Harry, M. (2006). Chair of the department of microbiology and immunology at the University of Michigan Medical School.


administration of probiotic *Escherichia coli* after birth reduces frequency of allergies and repeated infections later in life (after 10 and 20 years). Int. Arch. Allergy Immunol., 131(3):209-211.


**Peter, J.; Wan, P. J. and Wakelyn, P.** (1997). Technology and solvents for extracting


Xu, D. and Cote, J. C. (2003). Phylogenetic relationships between Bacillus species and related genera inferred from comparison of 3' end 16SrDNA and 5' end 16S-23S