ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF PHYSIC NUT (*Jatropha Curcus*) ON *E. coli, S. aureus and Candida albicans*

BY

EMOEKPERE MARY OMOZE	**AST/2382070744**
OKE JOY OLUWATOYIN	**AST/2382050931**
UGHE JUDITH EMIENHER	**AST/2382060022**

BEING A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE LABORATORY TECHNOLOGY, SCHOOL OF APPLIED SCIENCE TECHNOLOGY, AUCHI POLYTECHNIC, AUCHI

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DECEMBER, 2022

CERTIFICATION

This is to certify that this project titled "ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF PHYSIC NUT (*Jatropha Curcus*) ON *E. coli, S. aureus and Candida albicans* " is a work carried out by;

EMOEKPERE MARY OMOZE	**AST/2382070744**
OKE JOY OLUWATOYIN	**AST/2382050931**
UGHE JUDITH EMIENHER	**AST/2382060022**

in the department of Biological Science Laboratory Technology (Microbiology Option), Auchi Polytechnic, Auchi. Having completed my course work, it is accepted in partial fulfillment for the Higher National Diploma (HND) in Biology/Microbiology.

_____ _____

MR. ENUMA C. H. **DATE**
PROJECT SUPERVISOR

_____ _____

MR. OHIMAI A. **DATE**
(HEAD OF DEPARTMENT)

DEDICATION

With all sincerity of heart, we dedicate this project work to God Almighty, the giver of life, strength, knowledge, wisdom, understanding and ever loving kindness throughout our stay in school.

ACKNOWLEDGEMENT

All thanks to God Almighty for giving us the grace, wisdom and understanding to complete this project work.

Our special thanks go to our project supervisor Mr. Enuma C. H. for making this project work a successful one, may God bless you Sir.

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Our special appreciation goes to our parents for their prayer and financial support throughout our academic pursuit, may God in His infinite Mercy bless you and keep you save (Amen).

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ABSTRACT

The use of medicinal plants in solving health related issues particularly of microbial origin has remain an effective tools in addressing the problem of bacterial drug resistance as it

provides broad spectrum of activity. This study investigated the antimicrobial activity of aqueous and ethanolic extract of physic nut (*Jatropha curcas*) on *E coli. S. aureus* and *C. albicans.* The plant sample (*Jatropha curcas leaf*) was gotten from Auchi, washed and dried at room temperature (80^{o}C). the sample was brought to fine particles size using electric blender. Extraction was carried out using aqueous and ethanol. The antimicrobial activities were determined by Agar well and Disc diffusion methods. The result showed that *E. coli* on ethanolic extract has the highest zone of inhibition of 7.0mm followed by *S. aureus* which had 6.5mm, *C. albicans* had the least zone of inhibition of 3.0mm as against Gentamicin with 10.5mm which served as the control. It was concluded that *Jatropha curcas pose a broad spectrum of antimicrobial activities and thus, a potential candidate for a prospective antimicrobial treatment.*

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CHAPTER ONE

1.0 INTRODUCTION

Jatropha curcas Linn is commonly known as ‘physic nut’ is a non-food bioenergy plant and c*urrently* considered as alternative substitute to fossil. It is perennial shrub belonging to *Euphorbiaceae* family same as rubber and cassava trees (Misra and Misra, 2018). Originally, *Jatropha curcas* was native tree in South America and was induced to Thailand about 200 years ago by Portuguese who produced soap from Jatropha oil. Generally, Jatropha tree is 3-6 meter tall, smooth grey bark, having latex and heart green leaf. *Jatropha curcas L.* or physic nut is a drought resistant large shrub or small tree, producing inedible oil containing seeds. It is the commonest species found in Nigeria, but many species exist in different parts of the world. It is a multipurpose, drought resistant tree and can be cultivated in areas of low rainfall (Brittaine, 2018).

Jatropha curcas L. is a suitable plant for quick and efficient domestication compared with other woody species. Names used to describe the plant vary per region or country. It is most commonly known as "Physic nut". In Zimbabwe it is known as "Mufeta/ mujirimono" to mean it ‘oil tree’. In Nigeria it is known as "binidazugu/cinidazugu" and "lapa lapa" in Hausa and Yoruba languages respectively (DEG Jatropha Support Programme, 2019).

At present, the varieties being used to established plantations in Africa and Asia are inedible. Due to its toxicity, *J. curcas* oil is not edible and is traditionally used for manufacturing soap and tropical medicine. There have been increasing research investigations in the previous works about *J. curcas L.* to bridge the gaps concerning the chemical composition, antimicrobial activity and the biodiesel potential of this plant (Prakash, 2016), hence purpose of this research.

2.2 Statement of the problem

In recent years, human pathogens have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. The undesirable side effect posed by certain antibiotics and emergence of previously uncommon infections has led scientist in looking for new antimicrobial substance from various sources especially from medicinal plant.

1.3 Aim and Objectives of the Study

1.3.1 Aim

This study was aimed to ascertain the antimicrobial activity of Aqueous and Ethanolic extract of physic nut on *E. coli, S. aureus* and *Candida albicans.*

1.3.2 Objectives

The specific objectives of the study were to:

- Carryout phytochemical extraction on *Jatropha curcas* leaves using ethanol and aqueous solution.
- Demonstrates the inhibitory activity of the extract on *E. coli, S. aureus* and *Candida albicans.*

1.4 Significance of the Study

This project work will provides an up-to-date potential uses of different parts form *J. curcas L.,* which could be significant in providing insights for present and future research.

1.5 Justification of the Study

The demand for plant based therapeutics is increasing in both developing and developed countries because of growing recognition that they are natural products, non narcotic and easily

biodegradable, producing minimum environments hazards, having no adverse side effect and being easily affordable.

Jatropha curcas L. is an important herb used traditionally by the Chinese, and native American to treat many disease. It is also used as traditional medicine in many parts of the world such as Turkey, United States and France. Its potential antioxidant and healthcare applications as diuretic agent, in hyperglycemia reductions, as anti-depressant and anti-fatigue use have been claimed in several reports. Others use of *Jatropha curcas L*. Include teas and supplements to treat urinary related problems. The potentials use is very much related to its properties and mechanism of action of its plant's bioactive constituents such as flavonoids and terpenoids.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin of Physic Nut

A number of scientists have attempted to define the origin of physic nut, but the Source remains controversial. Martin and Mayeux (2017) identified the Ceara State in Brazil as a centre of origin but without giving any arguments. Dehgan and Webster (2011) cited Wilbur (2015) as follows; "It was without doubt that part of the flora of Mexico and probably of Northern Central America before the arrival of Cortez, and it most likely originated there the subsection, hence, appears to be one which originally was nearly or completely restricted to Mexico". According to other sources, the physic nut seems to be native to Central America as well as Mexico where it occurs naturally in the forest of coastal regions. However, Dehgan (2016) did not find true wild physic nut plants when collecting Jatrophas in Mexico. Those he found had always "escaped" from cultivated hedges. During a visit to Dehgan's Horticultural Systematics Laboratory, hundreds of herbarium specimens were checked for the distribution of physic nut in Mexico, Central America and the Caribbean. The materials collected were mostly from Mexico and all Central American countries. In the South American countries, physic nut occurs to a lesser extent. It is highly probable that the centre of origin of the physic nut is in Mexico and Central America since it is not found in these forms of vegetation in Africa and Asia but only in cultivated form.

2.2 Taxonomic and Botanical Description

The *Euphorbiaceae* family, which is considered one of the largest families of Angiosperms, covers about 7,800 species distributed in approximately 300 genera and 5 subfamilies worldwide. These species occur preferentially in tropical and subtropical

environments. Among the main genera belonging to this family, there is *Jatropha L.*, which belongs to the subfamily Crotonoideae, Jatropheae tribe and is represented by about 200 species. This genus is widely distributed in tropical and subtropical regions of Africa and the Americas. The name "Jatropha" is derived from the Greek words "jatros," which means "doctor" and "trophe," meaning "food," which is associated with its medicinal uses. The leaves have significant variability in their morphology from green to pale green, alternate to sub opposite, and three- to five-lobed with a spiral phyllotaxis. Flowers of *Jatropha curcas* produce nectar and are scented. The nectaries are hidden in the corolla and only accessible to insects with a long proboscis or tongue. The sweet, heavy perfume at night and greenish yellow colour of the flowers suggest that they are pollinated by moths (Nahar and Ozores-Hampton, 2018)

Table 1. Taxonomic Classification *J. curcas Linn.*

Kingdom:	**Plantae**
Subkingdom:	Angiosperms
Infrakingdom:	Streptophyta
Superdivision	Embryophyta
Division:	Tracheophyta
Subdivision:	Spermatophytina
Class:	Magnoliopsida
Superorder:	Rosanae
Order:	Malpighiales
Family:	Euphorbiaceae
Subfamily:	Crotonoideae
Tribe:	Jatropheae
Genus:	Jatropha
Species:	*Jatropha curcas Linn*

Source: (Nahar and Ozores-Hampton, 2018)

In inflorescences, the female flowers open one or two days before the male ones or at the same time as the earliest male flowers. Male flowers last only one day. Seed never sets in indoor cultivation unless the flowers are pollinated by hand. Plants raised from seed are more resistant to drought than those raised from cuttings, because they develop a taproot. Fruit development

from flowering to seed maturity takes 80–100 days. Plants from cuttings produce seeds earlier than plants grown from seed. Full production is achieved in the 4th or 5th year (Mubonderi, 2019)

Figure 1. Flower of *J. curcas L.;*
Sources: Blench, (2017)

Male and female flowers are produced on the same inflorescence, averaging 20 male flowers to each female. The petiole length ranges from 6.1–23.1 mm. The inflorescence can be formed in the leaf axil. Plants are monoecious and also possess hermaphroditic flowers occasionally. Flowers are formed terminally, individually, with female flowers usually slightly larger occurring in hot seasons. Where continuous growth, an unbalance of pistillate or staminate flower production results in a higher number of female flowers. More female flowers mean more fruits. Fruits are produced in winter when the shrub is leafless, or it may produce several crops during the year if soil moisture is good and temperatures are sufficiently high. Each inflorescence yields a bunch of approximately 10 or more ovoid fruits. A three *bi-valved cocci* is

formed after the seeds mature and the fleshy exocarp dries. The seeds are mature when the capsule changes from green to yellow. The whole genome of *J. curcas L* was sequenced by Kazusa DNA Research Institute, Chiba Japan in October 2018. It was reported, somatic chromosome numbers were counted from root-tip cells of four individuals per population and all had 2n=22 chromosomes, corresponding to the diploid level (x=11) in which all the plant populations were found diploid. This lack of variation in chromosome numbers contrasts with the high variability in other characteristics such as seed size, weight, and oil contents due to environment and genetic interaction (Lozano, 2017).

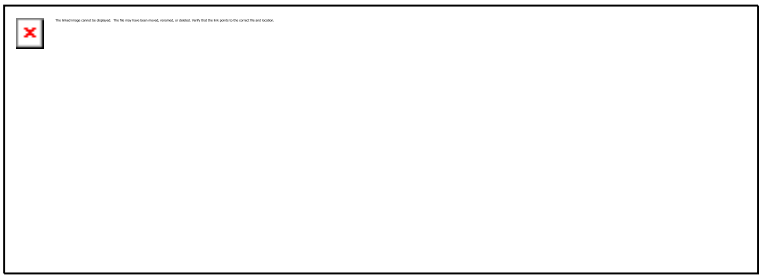


Figure 2. J. curcas L with ripen fruits;
Source: (Lozano, 2017).

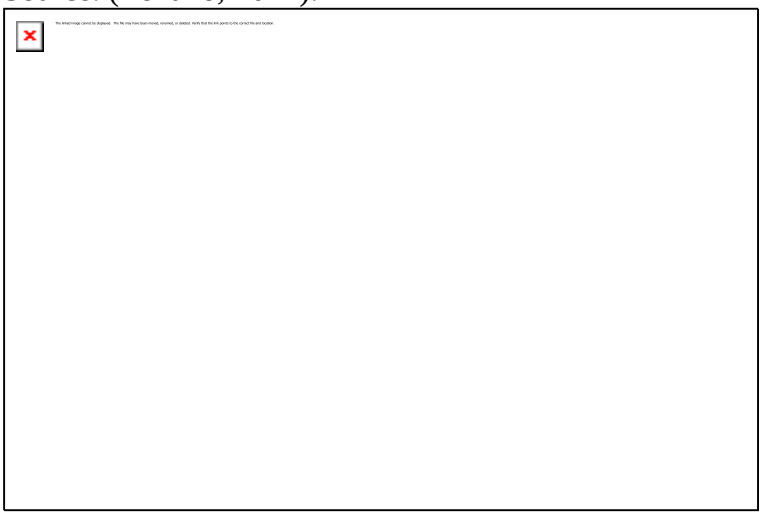
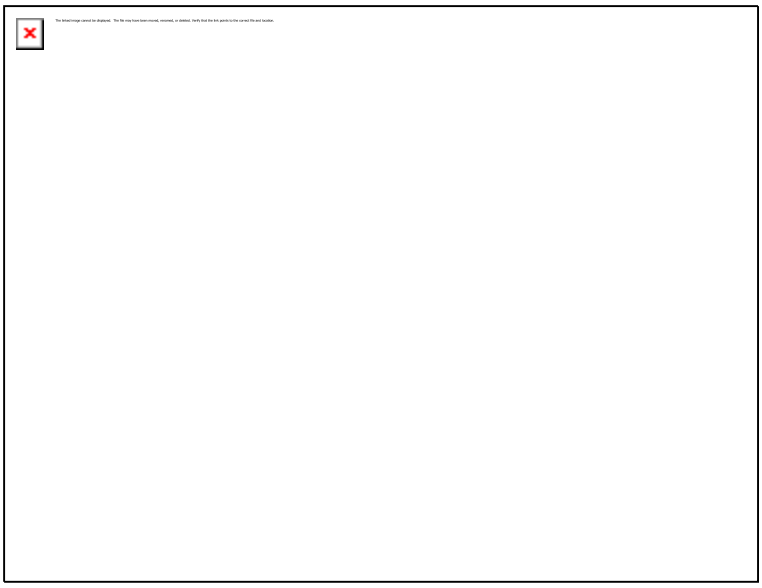


Figure 3. *J. curcas* L with ripen fruits;
Source: (Lozano, 2017).



Dried fruits of *J. Curcas L.*
Source: (Lozano, 2017).

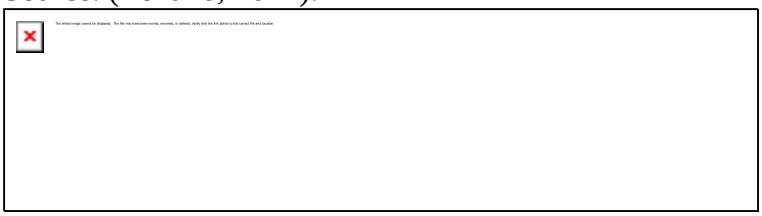


Figure 4. (a) Fresh and (b) dried seeds of *J. Curcas L.*
Source: (Lozano, 2017).

2.3 Ecological Requirements

Jatropha curcas grows almost anywhere, even on gravelly, sandy and saline soils. It can thrive on the poorest stony soil. It can grow even in the crevices of rocks. The leaves shed during the winter months (dry season) form mulch around the base of the plant. The organic matter from shed leaves enhance earth-worm activity in the soil around the root-zone of the plants, which improves the fertility of the soil. Regarding climate, *Jatropha curcas* is found in the tropics and subtropics and likes heat, although it does well even in lower temperatures and can withstand a light frost. Its water requirement is extremely low and it can stand long periods of drought by shedding most of its leaves to reduce transpiration loss. Jatropha is also suitable for preventing soil erosion and shifting of sand dunes (Arif and Ahimed, 2020)

J. curcas L. can be planted by two common methods; seed or seedling propagation and the cutting method. It was reported that vegetative propagation can be achieved by stem cuttings, grafting, budding and by air layering techniques. The investigation leads to the recommendation that cuttings should be taken preferably from juvenile plants and treated with 200 microgram per liter of indol butric acid IBA (rooting hormone) to ensure the highest level of rooting in stem cuttings. These vegetative methods have potential for commercial propagation of these plants and yields faster results than multiplication by seeds. The plant can grow in wastelands and grows on almost any terrain, even on gravelly, sandy and saline soils. Mycorrhizae have been observed on the roots; they promote growth, especiallly where phosphate is limiting. Complete seed germination is achieved within nine days. Adding manure during the germination has negative effects during that phase, but is favorable if applied after germination is achieved (Arif and Ahimed, 2020)

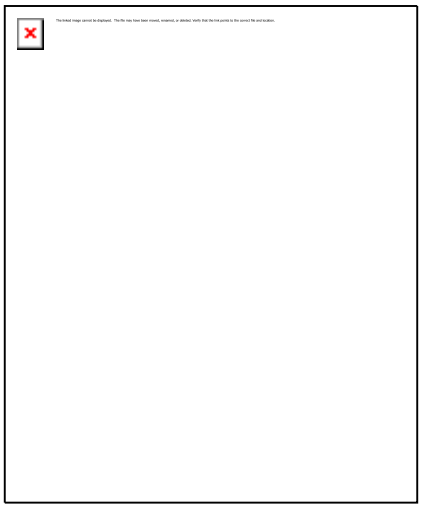


Figure 5a. Propagation of *J. curcas* by stem cutting.

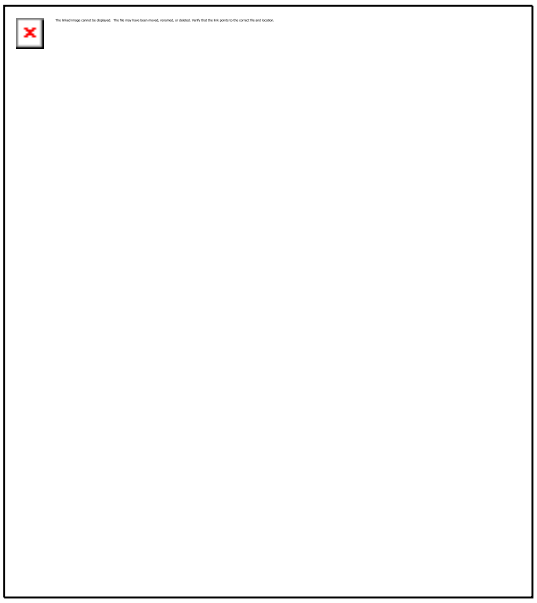


Figure 5b. Propagation of *J. curcas* by grafting.

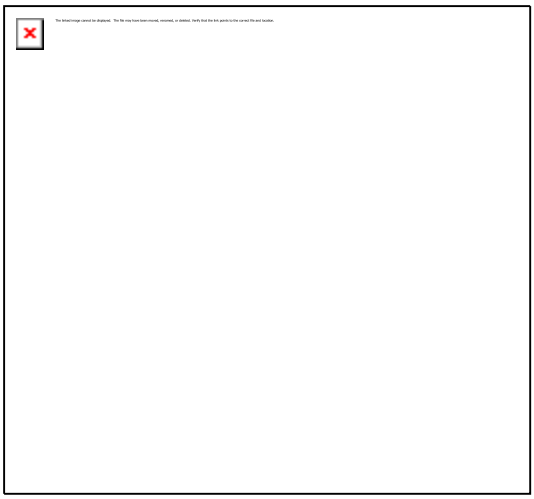


Figure 5c. Propagation of *J. curcas* by air layering techniques;
Source: (Arif and Ahimed, 2020)

Jatropha curcas thrives on a mere 250 mm of rain a year, and only during its first two years does it need to be watered in the closing days of the dry season. Ploughing and planting are not needed regularly, as this shrub has a life expectancy of approximately forty years. Jatropha is planted at densities ranging from 1 1010 to 2500 plants per hectare. Yield per tree is likely to increase with wider spacing but with a decline in yields per hectare. Spacing decisions should be

based on the environment, i.e. how it affects competition among trees for water, light and nutrients. Semi-arid, low-input systems should use wider spacing such as 3.0 x 2.0, 3.0 x 2.5 or 3.0 x 3.0 metres. Alternate planting in succeeding rows will minimize mutual shading. In addition, consideration should be given to access. At least 2.5 m between trees allows easier passage for fruit pickers, while a 5-metre alley at every fourth row facilitates access by carts. Planting holes of 30–45 cm wide and deep should be prepared and organic matter incorporated before planting. In the case of vegetative propagation of *J. curcas*, the method uses 40-50 cm long cuttings. Unlike seedlings, cuttings are planted during the dry season mostly two to three months prior to the commencement of rainy season. This is mainly because the plant has so much water that it can decompose if planted during the rainy season. This contains resonance with thin which the jatropha cuttings have a thin layer of wax that prevents the easy evaporation of water hence they have to be planted early to lose some water (Jingura *et al.*, 2011).

2.4 Chemical Composition of *Jatropha curacas L.* Components

Fruit Shell

The fruit shell describes the fruit pericarp, while the seed consists of the inner kernel and the outer husk or seed coat. As stated in Farmers Handbook prepared in Kenya when the fruit is crushed by a simple hand tool, the shells and seeds are separated. The shell is mechanically removed from the fruit in the first step during oil extraction. The chemical analysis of *Jatropha curacas L*. shell has shown that it is made up of 34%, 10% and 12% cellulose, hemicellulose and lignin, respectively. Volatile matter, ash and fixed carbon content of the shell have been shown to be 69%, 15% and 16%, respectively. These results show that *J. curcas L*. shells have very high ash content. This has an influence on the type of conversion technology that can be used to obtain energy from the shells. Jatropha shell ash fuses at temperatures above 750oc. Depending

on the magnitude of the ash content, the available energy of the fuel is reduced proportionately. At these high temperatures the ash reacts to form a slag, which can reduce plant throughput in combustion equipment. It was found that the caloric value of *J. curcas L.* shells is 11.1MJ kg-1. It was also found that the chemical composition of this plant shells seems to suggest that it is a good feedstock for biological conversion and for briquetting. Several conversion technologies have been studied using *J. curcas L*. shells as an energy feedstock. These include briquetting and combustion, pyrolysis and bio-methanation (Prakash, 2016).

Seeds

The matured seeds contain 21% saturated fatty acids and 79% unsaturated fatty acids, and they yield 25%–40% oil by weight. Additionally, the seeds contain other chemical compounds, such as saccharose, raffinose, stachyose, glucose, fructose, galactose, and protein. On average the seeds contain about 30-45% viscous oil which varies depending on where the jatropha is planted and the care it receives, water and nutrients (Prakash, 2016).

2.5 Uses of *Jatropha curcas L*

As Substitute of Fossil Diesel

It was extensively studied that seed oil from the plant is used as an alternative stationary engine or transportation fuel. This is due to its potential to substitute fossil diesel. The calorific value of seed oil is 39MJ kg-1 which is higher than anthracite coal and comparable to crude oil. Raw oil has been used as a substitute for petro-diesel both in modified and unmodified diesel engines. The use of raw oil in diesel engines was also studies in detail which has not shown satisfactory results due to its high viscosity. High viscosity of raw oil causes problems in its use in diesel engines. These include reducing the fuel atomization and increasing fuel spray, which would be responsible for engine deposits, injector coking, piston ring sticking and thickening of

lubricating oil. Despite the problems caused by its high viscosity for use in diesel engines, raw oil can have some other energy uses. It has been used in slow-speed stationary diesel engines such as pumps and generators with success. Tests with low heat rejection diesel engine showed that use of JCL oil results in higher brake specific energy consumption lower brake thermal efficiency (BTE), higher exhaust gas temperature and lower Nitrogen Oxides emissions than fossil diesel. The reduction in Nitrogen Oxides emission has environmental benefits. Pre-heating and blending raw oil with fossil diesel are techniques that have also been used to improve the use of raw *J. curcas L* as a fuel. Both techniques have the effect of reducing the viscosity of the seed oil (Prakash, 2016).

As Source of Biogas

Biogas has been produced from fruit shells. In addition, trials showed that seed husks can be used as a feedstock for a gasification plant. *J. curcas L* fruit shells and seed husks can be used for direct combustion. Since the shells make up around 35–40 percent of the whole fruit by weight and have a calorific value approaching that of fuelwood, they could be a useful by-product of jatropha oil production. As shown in Table 4 the calorific values *of Prosopis juliflora* (a fuelwood species of semi-arid areas) and jatropha fruit shells are similar. However, four times the volume of fruit shells is required to equal the heating value of fuel wood, due to their lower bulk density. The seed cake has a high energy content of 25MJ kg-1. Experiments have shown that some 60 percent more biogas was produced from jatropha seed cake in anaerobic digesters than from cattle dung, and that it had a higher calorific value (Brittaine, 2018)

Table 4. The calorific values of Prosopis juliflora (a fuelwood species of semi-arid areas) and jatropha fruit shells.

	Wood (Prosopis juliflora)	Biomass Briquettes	Jatropha fruit shell	Jatropha seed husk
Bulk density Kg/m3	407	545	106.18	223.09
Ash content% dm	1.07	8.7 7	14.88	3.97
Caloric value Kcal/kg	4018	4130	3762	4044

Adopted from: (Brittaine, 2018)

Seed husks have a higher heating value and greater bulk density which makes them more valuable than the fruit shells as a combustible fuel. However, the technology required to separate the seed husk from the kernel is more suited to large processing plants than small rural industry. The fruit shells can be dried and ground to a powder and formed into fuel briquettes. A trial found that 1 kg of briquettes took around 35 minutes for complete combustion, giving temperatures in the range of 525ºC–780ºC. The ash left after combustion of jatropha shell briquettes is high in potassium, which may be applied to crops or kitchen gardens. The fruit shells and seed husks also can be left around jatropha trees as mulch and for crop nutrition. For jatropha grown on degraded land, this has clear advantages because nutrient re-cycling – through returning the seed cake to the plantation – is unlikely to happen, due to the effort required and the higher utility to be gained from applying the seed cake to high-value crops (Brittaine, 2018)

Human Consumption

Jatropha can be toxic when consumed due to the seeds contain toxic Phorbol esters. However, a non-toxic variety of Jatropha is reported to exist in Mexico and Central America. This variety is used for human consumption after roasting the seeds/nuts, and "the young leaves may be safely eaten, steamed or stewed." They are favored for cooking with goat meat, said to

counteract the peculiar smell. This non-toxic variety of Jatropha could be a potential source of oil for human consumption, and the seed cake can be a good protein source for humans as well as for livestock (Adebowale and Adedire, 2016).

***Jatropha curcas L* Oil in Household Activities**

Traditionally, it is used for the manufacture of candles and soap, as lamp oil and as fuel for cooking. It is a poor lubricant as it dries quickly. Throughout the tropics and warm subtropics J. curcas is increasingly planted for bio-fuel purposes. The oil is either used directly in adapted engines powering local grain mills, oil presses, water pumps and small generators, or first refined by trans-esterification with methanol or ethanol to produce regular fuel suitable for high-performance diesel engines (Adebowale and Adedire, 2016).

2.6 Medicinal Properties of Jatropha Plant

Jatropha species are used in traditional medicine to cure various ailments in Africa, Asia, and Latin America or as ornamental plants and energy crops. Several known species from genus Jatropha have been reported for their medicinal uses, chemical constituents, and biological activities such as *Jatropha curcas, Jatropha elliptica, Jatropha gossypiifolia*, and *Jatropha mollissima*, among others. Although the leaves are toxic when consumed, the green pigment that comes out of the leaves and the latex that comes from the stem can be used to stop bleeding wounds on both humans and livestock. Apart from being used as a live fence, the plant is used as a repellant agent. Some people believe that jatropha protects the home from evil spirits and snakes. In Mutoko, Zimbabwe, witchcraft is a common social phenomenon hence jatropha is believed to have the power to repel witches and bad omens (Adebowale and Adedire, 2016).

2.7 Pharmacological Activities

Anti-bacterial activity: Acetone, chloroform, ethanol and methanol extracts of *J. curcas* root bark has been reported to inhibit the growth of both gram-positive (*Staphylococcus aureus*) and gram negative bacteria like *Klebsiella pneumonia, Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Escherichia coli* (Sundari *et al*., 2011).

Anti-fungal activity: Various plant parts of *J. curcas* have antifungal activity against *Aspergillus fumigatus, A. flavus, A. niger, Bacillus subtillis, Phymatotrichopsis omnivora, Candida albicans* etc. which are responsible for many diseases in human being and plants (Kubmarawa *et al*., 2017).

Antiviral activity: The water extract of the branches of *J. curcas* strongly inhibit the HIV-induced cytopathic effects with low cytotoxicity (Matsuse *et al*., 1999). Latex of *J. curcas* possesses inhibitory property against *Water melon mosaic virus* (Tewari and Shukla, 1982).

Anti-inflammatory activity: Topical application of *J. curcas* root powder in paste form in mice and rats has been reported to possess anti-inflammatory activity by Mujumdar and Misar (2004).

Anti-oxidant activity: Root bark extract of *J. curcas* were capable of scavenging hydroxyl in a concentration dependent manner and have a stronger hydroxyl scavenging activity of compared with ascorbic acid (Sundari *et al*., 2011).

Coagulant and anticoagulant activities: Latex is a blood coagulant whereas dilute latex is anticoagulant. Curcin from seed produces deleterious effects to the blood (Osoniyi and Onajobi, 2003).

Anti-diarrhoeal activity: The petroleum ether and methanol extract of *J. curcas* roots shows anti-diarrhoeal activity in various species of albino mice (Mujumdar *et al*., 2000).

Pregnancy terminating effect: Pregnancy terminating effect of methanol, petroleum ether and dichloromethane extracts extract of *J. curcas* fruits in rats have been scientific reported by Goonasekera *et al*. (1995).

Wound healing activity: Wound healing activities of stem bark of *J. curcas* has been reported in literature, Sachdeva *et al*. (2011) had scientifically evaluated wound healing potential of white soft paraffin base ointment containing 5 and 10% (w/w) extract of stem bark of *J. curcas* using incision and excision wound model in albino rats.

Insecticidal, larvicidal and anthelmintic activity: Various plant parts of *J. curcas* have been reported to possess insecticidal and larvicidal and anthelmintic activity. Insect pests of stored grains (*Sitophilus zeamais* and *Rhyzorpertha dominica*) are susceptible to seeds and pericarps of *J. curcas* (Silva *et al*., 2019). Ethanol extract of leaves of *J. curcas* may be as useful for developing a safe and ecofriendly therapeutic agent to combat the problems of tick *Rhipicephalus* (*Boophilus*) *annulatus* and tick-borne diseases (Juliet *et al*., 2019). *Jatropha curcas* is a potential source of herbal mosquito control agent. Larvicidal activities methanol extract of leaves, crude protein extract and purified toxin, Jc-SCRIP, from the seed coat of *J. curcas* has the larvicidal potential against *Aedes aegypti*, *Anopheles arabiensis, Aedes aegypti* and *Culex quinquefasciatus* (Karmegam *et al*., 1997) Aqueous extract of leaves of *J. curcas* possesses anthelmintic activity against *Pheritima poshtuma* (Ahirrao *et al*., 2008).

Phytochemicals: The leaf, bark and latex of *Jatropha* contains alkaloids such as jatrophine, jatropham, curcacycline A, curcain, tannins, glycosides, flavonoids and sapogenins with anti-cancerous properties (Thomas *et al*., 2008). The seeds of *J. curcas* contains some toxic compounds such a protein (curcin) and phorbol-esters diterpenoids (King *et al*., 2020). The diterpenes isolated from *Jatropha* species belongs to rhamnofolane, daphnane, lathyrane,

tigliane, dinorditerpene, deoxy preussomerin and pimarane skeletal structures and the majority of the diterpenes exhibited cytotoxic, antitumor and antimicrobial activities *in vitro***.** Jatrophone, spruceanol and jatrophatrione exhibited antitumor properties against P338 lymphocytic leukaemia and japodagrol against KB carcinoma cells. Curcusone exhibited anti-invasive effects against cholangiocarcinoma cells. The phorbol esters (Jatropha factor C1-C6) and jatropherol exhibited insect deterrent/cytotoxic properties. Jatrophalactam, faveline derivatives, multifolone, curcusone, jatrophone derivatives etc. have shown *in vitro* cytotoxic activity. Japodagrin, jatrogrossidione derivatives and jatropholone derivatives exhibited antimicrobial activities. Jatropha diterpenoids having a wide spectrum of bioactivity could form lead compounds or could be used as templates for the synthesis of new compounds with better biological activity for utilization in the pharmaceutical industries (Devappa *et al*., 2011). Three deoxypreussomerins, palmarumycins CP1, JC1 and JC2, have been isolated from the stems of *J. curcas* (Ravindranath *et al*., 2004) which possess a wide range of biological properties including antibacterial, antifungal, herbicidal, antibiotic and antitumor activities (Wipf *et al*., 2001). *Jatropha curcas* seed oil chemically consists of triacylglycerol with linear fatty acid chain. Palmitic acid, stearic acid, oleic acid and linoleic acid, lauric acid**,** meristic acid, arachidic acid, arachidonic acid and behenic acid are some important fatty acids present in *J. curcas* seed oil (Adebowale and Adedire, 2016) identified a new chemical compound jatrophasin A (3,4,4',5'-tetrahydroxyl-3'-methoxyl-bisepoxylignan) with strong anti-oxidative activity from the seeds of *J. curcas*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Area of study

The study was conducted in Auchi, Etsako West LGA, Edo State, Nigeria. Auchi is located in the Northern part of Edo State within the coordinates of latitude 07^0 04^1N and longitude 06^0 16^1E. it is situated in the South-South geographical zone of Nigeria with a population of over 500,000 people according to the 2015 population census. It is approximately one hundred and thirty kilometer (130km) away from Benin City, the capital of Edo State. Auchi is the headquarters of Etsako West Local Government and has witnessed territorial development owing to rural-urban migration. It is bounded to the north by Jattu, to the south by Aviele, to the east by Iyakpi and to the west by Owan Local Government Area. It is also the seat of the Federal Polytechnic, Auchi.

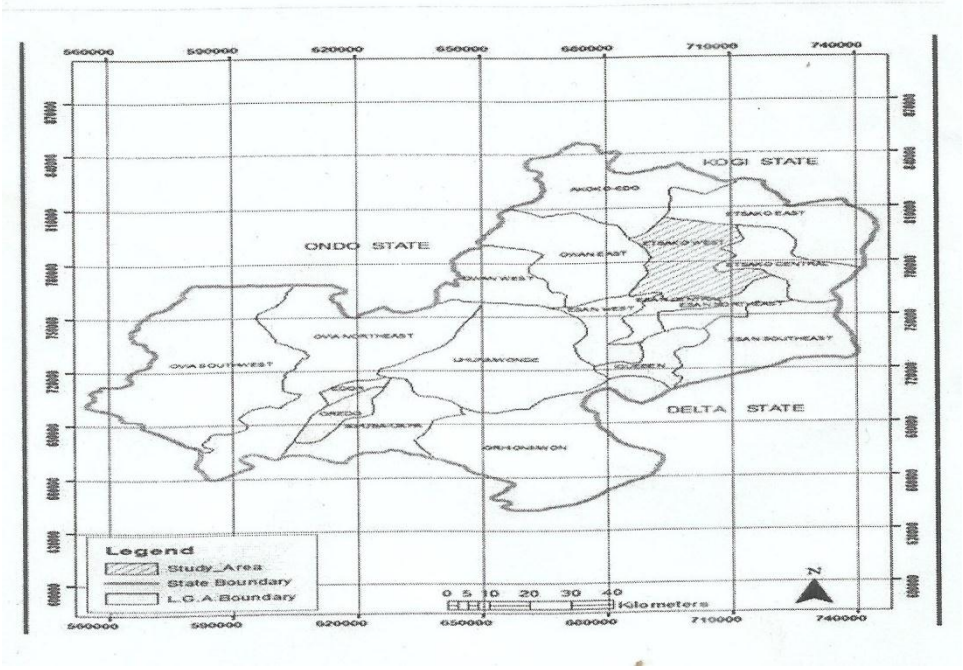


Figure 3.1 Map of the Edo State showing Etsako West L.G.A (source: produced from Arcmap10.1.20.2013.

3.2 Sample collection

Fresh leaves of *Jatropha Curcas* were collected from farm land in Aristotle, Auchi polytechnic, Auchi located at Etsako West Local Government area of Edo State. The sample were transported to the microbiology laboratory of Federal Polytechnic, Auchi, Edo State, where the plant was examined, identified and authenticated as *Jatropha Curcas* by a botanist in the Department of science Laboratory Technology.

3.3 List of Apparatus and Experimental Reagents

Sample *(Jatropha curcas)*, Distilled water, Ethanol, Gentamicin, isolates *(Escherichia coli*, *staphylococcus aureus*, *Candida albicans*), Muller hector agar, Aluminum Foil, cotton wool, Petri dish, What man NO.1 Filter paper, Cork borer, Gas cylinder, Ruler, Syringe Forceps, wire loop, masking tape, beaker, Autoclave, Rotary evaporator, Incubator, Hand gloves, mortar and pestle, Blender.

3.4 Sterilization of materials

All materials used in the course of this project such as glass waves were properly washed with detergent and water to remove dirt and contaminants and were properly dried. The washed glass ware were sterilized in a portable Laboratory autoclave temperature 121^{o} C for 15minutes. All media used were also sterilized at the same temperature.

3.5 Sample Extraction

Samples of *Jatropha curcas* was collected, examined and identified. The leaves of *Jatropha curcas* were allowed to cur-dry at room temperature for fourteen (14) days until the leaves became brittle, and ground into fine powder in a clean mortar using a clean pestle. Ten grams (10g)) of the leaves powder(sample) was weighed using a weighing balance and poured into two (2) bottles, labeled, soaked in 200ml of distilled water, 200ml of Ethanol respectively

the preparations were allowed to stand undisturbed, but with occasional agitation for 72 hours and filtered using What man NO.1 Filter paper. The residues were discarded and the filtrates (extracts) was purported to dryness in rotary evaporator;

- Beaker 1- *Jatropha curcas* + Distilled water at 100^{o} C
- Beaker2 – *Jatropha curcas* + Ethanol at 60^{o} C

3.6 Preparation of Agar media

3.6 1 Preparation of Muller Hilton Agar

Muller- Hilton Agar was prepared by dissolving 10g of Muller Hilton agar power in 200ml of distilled water in a clean conical flask. The mouth of the flask was plugged with non-absorbent cotton wool wrapped with aluminum Foil paper that was extended up to the neck of the flask and shaken very well to dissolve it was sterilized in an autoclave at 121^{o}C for 15minutes and allow to cool at 47^{o}C before pouring into plates especially in the required amount and allowed to solidify.

Muller-Hilton agar was used for susceptibility test for physic nut extract.

3.7 Antimicrobial Activities

Anti microbial activities were studied by disc diffusion method and the Agar well diffusion method, Each of the inocula *(E. coli, Staphylococcus aureus, Candida albicans)* was streaked on the solidified agar media. Holes were marked using the cork borers in 6 Petri dishes, then different concentrations of the holes using a sterile syringe. Gentamicin was used as control Plates were then kept to stand in the incubator at 37^{o}C for 24hours.

The combination of the samples *(Jatropha curcas)* was also tested to know their anti microbial effects on some clinical isolates (*Staphylococcus aureus* and *E. coli*) and incubated at 37^{o} C for 24hours Zones of inhibition were measured and recorded.

3.7 1 Minimum Inhibitory Concentration

The minimum Inhibitory concentration of the plant extracts *(Jatropha curcas)* against the sensitive organisms were determined using the agar disc method.

Serial dilution of plant extracts were prepared to obtain 200, 100, 50, 25, and 12.5mg/ml. Each of the inoculations was streaked using a wire loop into each Petri dish containing agar and allowed to set. A 3mm cork borer was used to bore wells. Serial dilutions were wacked into the marked cells. The plates were incubated at 37^{o}C for 24 hours. The growth were observe to determined the sensitivity of each organism using clear zones of no microbial growth. The combination of both plants was carried out using the same method.

The least concentration of the plant extracts with the lowest inhibitory effect was taken as the minimum inhibitory concentration (MIC) of that plant extract *(Jatropha curcas)* against *Escherichia coli, Staphylococcus aureus, Candida albicans.*

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

4.1 Result

The Antimicrobial activity of sample (*Jatropha curcas*) is tabulated below. Table 1 represents the zone of inhibition of the *Jatropha curcas* on isolates using Agar well method.

Table 2 represent the zone of inhibition of *Jatropha curcas* leave extract on isolates using the Agar Disc diffusion method

Table 3 represents the minimum inhibitory and bactericidal concentration of the plant extracts on isolates.

Table 4.1: Zone of the *Jatropha curcas* on isolates using Agar well method.

Extract	Isolates	Zone of inhibition (mm)
Ethanol	*Escherichia coli* *Staphylococcus aureus* *Candida albicans*	7.0 6.5 3.0
Water (Aqueous)	*Escherichia coli* *Staphylococcus aureus* *Candida albicans*	6.5 6.0 3.0
Control (Gentamyein)	*Escherichia coli* *Staphylococcus aureus*	10.0 10.5

Table 4.2: Zone of inhibition of *Jatropha curcas* leaves extract on isolates using the Agar Disc diffusion method.

Extract	Isolates	Zone of inhibition (mm)
Ethanol	*Escherichia coli* *Staphylococcus aureus* *Candida albicans*	7.0 6.0 2.5
Water (Aqueous)	*Escherichia coil* *Staphylococcus aureus* *Candida albicans*	5.0 5.4 2.5

Table 4.3: Minimum inhibitory and bactericidal concentration of the plant extract on bacterial isolates

Concentration of Extract (mg/ml)

Isolates	Minimum laboratory concentration (mg/ml)	Minimum Bactericidal concentration (mg/ml)	extract
Staphylococcus aureus	25	25	AE
Escherichia coli	12.5	6.25	EE
Candida albicans	12.5	7.25	EE

AE = Aqueous Extract (Distilled water)

EE= Ethanol Extract

4.2 Discussion

The use of plant extract with known antimicrobial properties can be of huge benefits in pharmaceutical industries (Devappa *et al.*, 2011). The results of the present study support the use of *Jatropha curcas* in the treatment of human related diseases. This might be due to the presence of essential chemicals such as alkaloids, flavnoids, glycoside, amongst others (King *et al.,* 2009).

The antimicrobial activities of decoted leaves extract of *Jatropha Curcas* were determined by Agar well and Disc diffusion methods on *S. aureus, E. coil* and *C. albicans* following the protocol demonstrated by Oyagade *et al.,* (1999), using aqueous solution and ethanol as solvent and the result were presented based on the zone of inhibition.

The result showed that *E. coil* on ethanolic extract of *Jatropha Curcas* has the highest zone of inhibition of 7.0mm followed by *S. aureus* which had 6.5mm, *C. albicans* had the least zone inhibition of 3.0mm as against gentamicin with 10.5mm which served as the control.

This study is in consonant with the research carried out by Ekundayo *et al.,* (2011) on *S. aureus* and *Klebsiella pneumoniae* using ethanol and aqueous extracts of *Jatropha Curcas*.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Jatropha curcas pose a broad spectrum of antibacterial activities and thus, a potential candidate for a prospective antimicrobial treatment of human related diseases. Furthermore, it possesses stronger antibacterial properties against *Escherichia coli, Staphylococcus aureus and Candida albicans*. Due to their broad spectrum of activity, the local use of *Jatropha curcas* for various medicinal purpose is therefore encouraged.

5.2 Recommendations

- It is recommended that the use of *Jatropha curcas* should be massively encouraged in drugs just as antibiotics. Also, the leaf should be combined to further strengthen their antibacterial properties.
- Furthermore, further studies should be conducted on *Jatropha curcas* against broader microbial isolates

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