PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON THE SEEDS OF *JATROPHA CURCAS* LINN. (EUPHORBIACEAE)

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DECLARATION

I declare that the work in this Dissertation entitled 'Phytochemical and Biological Studies on the Seeds of *Jatropha curcas* Linn. (Euphorbiaceae)' were carried out by me in the Department of Pharmacognosy and Drug Development. The information derived from the literature were duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

_____ _____ _____

Name of Student Signature Date

CERTIFICATION

This dissertation entitled 'Phytochemical and Biological Studies on the Seeds of *Jatropha curcas* Linn. (Euphorbiaceae)' by Amos YUSUF meets the regulations governing the award of degree of Master of Science in Pharmacognosy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dr. U. A. Katsayal (Signature) _____

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Head, Department of Date _____

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DEDICATION

This work is dedicated to God Almighty and the loving memories of my father, Yusuf Madaki (1945-2015), and my sisters, Nana Yusuf Madaki (1972–1998) and Nyakachat Yusuf Madaki (1985–2006). May God bless their souls.

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ABSTRACT

Jatropha curcas Linn. (Euphorbiaceae) is a shrub that grows up to 5m in height. It thrives well in tropical countries. It grows in the wild as an uncultivated, non-food plant. It is traditionally used as a living fence to protect gardens from animals.

An ethnobotanical survey of the seed of *J. curcas* was carried out in parts of Zaria and Zonkwa in Kaduna State of Nigeria. The seeds are used in traditional medicine for analgesic, antimicrobial, purgative, laxative and aphrodisiac purposes. Sometimes, the seeds serve as food when cooked. Some of the common toxic effects of direct consumption of the seeds include purging, body weakness, convulsion and abdominal pains.

Pharmacognostic study of the seed was carried out including macroscopy, microscopy and the determination of some physical standards. A study on the phytochemical constituents and biological activities of *J. curcas* seeds was carried out. The thin layer chromatographic analysis using random solvent systems showed rich composition of chemical constituents like flavanoids, steroids and triterpenes using various solvent systems.

The seed extract was tested for analgesic activity using the acetic acid induced writhing response in mice. Chemomicroscopic and fluorescence analyses of the seed were carried out.

Using agar well diffusion method, the methanolic extract of *J. curcas* seeds and four fractions of the extract; petroleum ether fraction, ethyl acetate fraction, n-butanol fraction and aqueous fraction, were used for antimicrobial test. Two fold serial dilution of the extract and fractions were made to obtain 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml,

12.5mg/ml, 6.25mg/ml, 3.13mg/ml and 1.57mg/ml. The test organisms were; *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes* and *Candida albicans*.

The seed oil was found to have an acid value of 5.61 and a saponification value of 297.33. Preliminary phytochemical studies revealed the presence of flavonoids, carbohydrates, saponins, tannins and steroids. Acute toxicity test revealed that the methanolic extract has an LD_{50} of 471.17 mg/kg in albino mice, making it very toxic.

The seed methanolic extract significantly ($P<0.05$) reduced writhes induced by acetic acid. The results of the analgesic test were comparable to the standard drug (paracetamol 100mg/kg). Although not all organisms were susceptible throughout, it was discovered that the methanolic extract, aqueous fraction, ethyl acetate fraction, n-butanol fraction and petroleum ether fraction generally exhibited activity against the test organisms at 50-200µg/ml concentrations.

Thus, the results obtained justified the use of *J. curcas* seeds in traditional medicine for analgesic and antimicrobial purposes.

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ABBREVIATIONS

APTT	-	Activated partial prothrombine time
ANOVA	-	Analysis of variance
cm	-	Centimetre
g	-	Grammes
ha	-	Hactre
HCN	-	Hydrogen cyanide
IBA	-	Indole Butyric Acid
kwh	-	Kilowatt hour
mt/ha	-	Metric tonnes/hactre
µg	-	Micro grams
ml	-	Millilitres
mm	-	Millimetres
KOH	-	Potassium hydroxide
PT	-	Prothrombine time
NaOH	-	Sodium hydroxide
TLC	-	Thin Layer Chromatography
vol	-	Volume
v/v	-	Volume by volume
wt	-	Weight
w/w	-	Weight by weight

CHAPTER ONE

1.0 INTRODUCTION

1.1 Medicinal Potentials of *Jatropha curcas* Seeds

The trend of developing drugs from natural sources scientifically is not new. Most drugs are either herbal based or were developed as synthetic products from the natural sources. The use of natural/crude herbs or drugs to cure ailments is the oldest medicinal practice the world over. However, some natural plant products that were discovered to be poisonous were found useful as arrow poison or fishing poison (Taylor, 2000).

Critics of herbal medicine are quick to point out that many plants are toxic. Plants such as oleander and foxglove fall in this category. The use of oleander branches at barbeques is toxic to people. The plant foxglove, which is used to make cardiac glycoside medicines, is also toxic. Therefore, recommending these plants for medicinal purpose is quite unpopular. However, the use of traditional medicines in dosage ranges that have been determined by centuries of trial and error could likely be beneficial without side effects (Acharya and Shrivastava 2008).

The yew tree, *Taxus*, has a legendary connection to death. Its seeds, leaves, and bark are highly poisonous to humans. However, it has in recent times earned a different reputation as a potential preserver of life. In the 1960s, researchers working for the U.S. National Cancer Institute discovered that the bark of *Taxus brevifolia*, the Pacific yew, contained a toxic ingredient that could be harnessed on a cellular level to inhibit the progress of some cancers (Chiej, 1984).

It is baffling how traditional healers, without the use of elaborate equipment or any formal schooling can be so adept at identifying plants, discern their uses and find

effective remedies among the thousands of plants that surround them. They also manage to combine several species that may work together synergistically such that remedies could be administered at doses that cure, but kill when administered in other doses (Acharya and Shrivastava 2008).

Today there are at least 20 distinct chemical substances derived from plants that are considered as important drugs currently in use in one or more countries in the world. Many years ago a plant chemical was discovered in a tropical plant, *Cephaelis ipecacuanha*, and the chemical was named emetine. A drug was developed from this plant chemical called Ipecac which was used to induce vomiting if someone accidentally swallowed a poisonous or harmful substance. In the United States of America it has been replaced by other drugs but Ipecac can still be found in Pharmacies in many third world countries (Taylor, 2000).

In recent times researches are aimed mostly at isolating specific constituents or chemicals in plant drugs responsible for the desired pharmacological activity. Several drugs on sale today are simple synthetic modifications or copies of the naturally obtained substance. A good example of this is the plant chemical quinine, which was discovered in a rain forest tree, *Cinchona ledgeriana*, over 100 years ago (Taylor, 2000).

Man has been inter-twined with medicinal plants since ancient times. In fact, the use of medicinal plants by native cultures has shaped traditional medicine. In most cases, traditional medicine is passed on orally, generation after generation (Dias *et al.,* 2012).

1.2 **Statement of Research Problem**

The use of the seeds of *Jatropha curcas* in traditional medicine is not as common locally as other parts of the plant, although it thrives well even as a non-cultivated species of

plant. Due to the scientific and general knowledge about the toxicity of the seeds, research on some of the reported potential uses of the seeds as herbal remedy is limited.

1.3 **Justification of the Study**

It is very important to carry out research on the seeds of *J. curcas* in order to have scientific basis of lending more credence to its reported potential uses in traditional medicine.

1.4 **Research Hypothesis**

The seed of *J. curcas* has significant analgesic and antimicrobial properties.

1.5 **Aim and Objectives**

To establish the phytochemical properties of the seeds of *Jatropha curcas* and provide some rationale for the analgesic and antimicrobial use of the plant by some traditional medicine practitioners. The specific objectives are:

i. To conduct an ethnobotanical survey on the seed of *J. curcas*.
ii. To evaluate some pharmacognostic parameters of the seeds of the plant.
iii. To carryout preliminary phytochemical analysis including thin layer chromatographic study of the seed extract of *J. curcas*.
iv. To evaluate analgesic and antimicrobial activities of the seed extracts of the plant.

CHAPTER TWO

2.0. LITERATURE REVIEW

Jatropha curcas Linn. (Euphorbiaceae) is a perennial poisonous shrub (up to 5m in height). It is a native of Central America, but has over the years spread to other tropical and subtropical countries. It is an uncultivated, non-food wild specie traditionally used as a living fence to protect gardens from animals (Gadekar, 2006).

The botanist, Carl Von Linne, first classified the plant in 1753 and gave it the botanical name "*Jatropha curcas"* derived from two Greek words "jatros" meaning "doctor" and "trophe" meaning "nutrition". This is because Linne realized the potential of the plant for a wide range of medicinal purposes (Isawumi, 1978).

Scientific Classification

KINGDOM: Plantae

DIVISION: Embryophyta

CLASS: Spermatopsida

ORDER: Malpighiales

FAMILY: Euphorbiaceae

GENUS: *Jatropha*

SPECIES: *Jatropha curcas* Linn.

Plate I: *Jatropha curcas* with fruits on a local farm in Dakace area of Zaria in the month of May, 2014.

As a plant known for its versatility in traditional medicine, *Jatropha curcas* has different vernacular and common names;

English: Barbados nut, physic nut, purging nut tree, black vomit nut.

Hausa: Mamulu, Bi ni da zugu, Ci ni da zugu.

Igbo: Olulu idu, Owulu idu.

Yoruba: Botiye, Lapalapa, Funfun (Isawumi, 1978).

2.1. **Botanical Features of *Jatropha curcas***

It has a glabrous, erect, branched shrub about 2-5m high. It has the following features:

Branches: stout, cylindrical and green.

Leaves: entire, orbicular ovate, angular or somewhat 3-5 lobed, 10-18cm long, 8-16 cm wide, acuminate, base is cordate, the petiole is long (8-12 cm).

Flowers: greenish or greenish white, unisexual, 7 to 8mm in diameter.

Fruits: capsules, at first fleshy, becoming dry, black, rounded with 2-3 one-seeded divisions, 3-4cm long.

Seeds: mature seed is oblong and covered within black seed coat. It appears milky in colour when seed coat is removed.

Plate II: *Jatropha curcas* seeds

Plate III: *Jatropha curcas* seeds without seed coat

2.2. **Ethnobotanical Uses of *Jatropha curcas***

There have been a lot of reports on the folkloric use of *J. curcas* as a herbal remedy in Nigeria. It is a very well known plant as at least one or two parts of it is used in various ways medicinally in the localities it grows. All parts of *Jatropha* (seeds, leaves and bark) have been used in traditional medicine and for veterinary purposes for a long time (Duke and Ayensu 1985). The seeds, as well as the fruits, are used as contraceptives in south Sudan (List and Horhammer, 1979).

Extracts of *J. curcas* seeds are used in folk remedies for cancer. Homeopathically, it is used for the treatment of numerous illnesses including cold sweats, diarrhoea and leg cramps. It is reported to have the following properties: aborticificient, anodyne, antiseptic, diuretic, emetic, haemostatic, narcotic, purgative. The nut is used in folk remedy for burns, convulsions, cough, dermatitis, inflammation, pneumonia, rash, rheumatism, scabies, sores, stomach ache, syphilis, tetanus, ulcers, whitlows and yellow fever (Duke and Wain, 1981; List and Horhammer, 1979).

Furthermore, the seeds have been used as purgative, antihelminthic, abortificient as well as for treating ascites, gout, paralysis and skin diseases. The seed oil is also used as an ingredient in treating rheumatic conditions, itch, parasitic skin diseases, fever, jaundice and gonorrhoea. It is also used as a diuretic agent and a mouth–wash (Duke and Ayensu, 1985).

In some African countries, people are accustomed to chewing the seeds when in need of a laxative. The seeds are highly effective against *Strongyloides papillosus* infection in gouts (Adam and Magzoub, 1975).

The latex can be applied topically to bee and wasp stings. The Mauritanians massage ascetic limbs with the seed oil. Cameroon natives apply the leaf decoction as remedy for arthritis (Watt and Breyer–Brandwijk, 1962). In Columbia the leaf decoction is used orally for veneral diseases (Morton, 1981). Costa Ricans apply the latex to burns, hemorrhoids, ringworm and ulcers. The seeds are used for dropsy, gout, paralysis and skin ailments (Watt and Breyer-Brandwijk, 1962). The seed is viewed as aperient; the seed oil is emetic, laxative, purgative and used for skin ailments, while the root is used as decoction for mouthwash to alleviate bleeding gums and toothache. Other uses are for eczema, ringworm and scabies treatment (Perry, 1980; Duke and Ayensu, 1985).

Almost all parts of the plant contain hydrogen cyanide (HCN). Except in extreme cases, animals don't feed on it. All parts of the plant are considered toxic, particularly the seed. It contains a poisonous constituent, curcin, considered similar to ricin found in *Ricinus communis*, containing castor oil. Curcin is found mainly in the seeds but some little quantity is also present in the fruit and sap (Adam and Magzoub, 1975). But the seed is heat liable, it is safe for consumption once boiled (Birgit, 2006).

2.3. **Reported Economic Uses of *Jatropha curcas***

The seeds in the Misantla area of Veracruz in Mexico are well appreciated by the population once boiled and roasted. This could either be because there is a non-toxic variety of *Jatropha curcas* in Mexico and central America or the seeds become edible once processed by cooking (Birgit, 2006). It has been reported that the seeds are edible once the embryo has been removed (Levingston, 2006).

The young leaves may be eaten safely, steamed or stewed. They are flavours for cooking with goat meat as it is said to counteract its peculiar smell (Ochse, 1931). Though

purgative, the nuts are sometimes roasted and eaten. Leaves are pounded and applied to repel flies around horse's eyes in India. The oil has been used for illumination, soap, candles, adulteration of olive oil and making Turkey red oil. The nuts can be strung on grass and burnt like candle nuts (Watt and Breyer-Brandwijk, 1962). The shrub is used as a host for Lac insect in Mexico. When roots are burnt, the ashes are used as salt substitute (Morton, 1981). The seeds of *J. curcas* have received great attention mainly due to the fact that its oil is considered a rich source of biofuel.

In addition, Turkey red oil (otherwise known as sulphonated castor oil) is made by adding sulphuric acid to pure *J. curcas* oil. Turkey red oil is the only oil that completely disperses in water. Its complete dispersal in water makes it useful for making bath oil products. It was the first synthetic detergent after ordinary soap (Gadekar, 2006). A colourant can also be derived from the seed. The sap stains linen and can be used as ink for markers (Michelle and Rook, 1979).

When oily seeds are processed into oil, it can be directly used as fuel for combustion engine or may be subjected to trans-esterification to produce high quality biodiesel usable in standard diesel engines. *Jatropha* seeds contain 27-40% oil (Achten, *et al*., 2007). Biofuel is an alternative fuel produced from renewable vegetable oils, animal fats or recycled cooking oils by transesterification reaction. Due to increase in environmental concern and diminishing petroleum reserves, biodiesel has drawn significant attention (Ma and Hanna, 1999). Non-edible oils like *J. curcas* oil are attractive due to high seed yield and high oil content (Wood, 2005).

2.4. **Reported Biological Profile of *Jatropha curcas***

Some compounds with antitumor activities were reportedly found in this plant (Vanden-Berg *et al*., 1995). The oil and aqueous extract from oil have potential as insecticides. For instance it has been used in the control of insect pests of cotton including cotton bollworm and on pests of pulses, potato and corn. It has also been reported that the methanol extracts of *Jatropha* seed (which contains biodegradable toxins) could be used in the control of bilharzia-carrying water snails (Kaushik and Kumar, 2004).

The roots are used as mouth wash for bleeding gums, toothache and skin disease like ringworm, scabies and eczema (Oliver-Bever. 1986; Mujumdar *et al*., 2001). It contains anti-tumor compounds like jatropham and jatrophone (Duke and Ayensu, 1984).

The roots are utilized extensively in West African ethnomedicinal practice in different forms to cure; fever, mouth infections, jaundice, guinea worm, sores and joint rheumatism (Irvine, 1961; Oliver-Bever 1986). The latex has also shown antibacterial activity against *Staphylococcus aureus* (Thomas, 1989).

Aiyelaegbe *et al*., (2007) lends credence to the folkloric use of *J. curcas* in treating microbial infections as the methanolic extract of the root bark showed potent broad-spectrum activity against *Gardnerella viginalis, Neisseria gonorrhoea, Staphyllococcus aureus* and found to be more active than gentamycin with respect to *Escherichia coli, Klebsiella aerogenes* and *Pseudomonas aeruginosa*. The stem bark also inhibited the growth of bacteria and fungi which is an indication of its broad-spectrum antimicrobial potential as reported in previous work (Igbinosa *et al*., 2009). Uche and Aprioku (2008) reported the methanolic extract of *J. curcas* leaves to possess significant anti-inflammatory activity on egg albumin-induced oedema of the paw of albino rats and

analgesic activity on acetic acid-induced writhing in mice, the results of which are comparable to piroxicam and parecetamol. Prothrombine time (PT) and activated partial thrombine time (APTT) tests on plasma confirmed the latex of *J. curcas* has both procoagulant and anticoagulant activities as was experimented for its reported folkloric use as a haemostatic or styptic by Osoniyi *et al*., (2003).

Curcin, a poisonous protein found in the seed of *J. curcas,* has been reported to have antitumor effect with related antitumor mechanisms to N-glycosidase activity. It is a novel cysteine containing Ribonucleic Inhibiting Protein (RIP), which might be ideal for the preparation of immunoconjugates with promising use as a chemotherapeutic agent for the treatment of various cancers (Lin *et al*., 2003). Although curcin and phorbol esters have been reported as the toxic agents in the seeds of *J. curcas* (Adam and Magzoub; 1974), Gübitz *et al.,* (1999), Goel *et al.,* (2007) later found that some derivatives of phorbol esters have antimicrobial and antitumor properties, as well as molluscicidal and insecticidal effects.

The direct consumption of the seeds is rare because of its reported toxic effect such as excessive stooling and vomiting (Becker and Makkar, 1998). Thus, it is mostly used topically for skin diseases such as eczema and scabies. Sundari *et al.* (2011) found that the extract of *J. curcas* seed have natural phytochemicals that could serve as agents against bacterial and fungal phytopathogens which could be used for agricultural applications at a low cost and safe practice.

It has also been found that *J. curcas* seed extracts could inhibit the mycelial growth of *Colletotrichum musae*, the causative agent of anthracnose disease in bananas (Thangavelu *et al.* 2004).

2.5. **Reported Chemical Proflile of *Jatropha curcas***

The chemical compounds isolated from *J. curcas* can be used in various industrial applications. Depending on the variety, the decorticated seeds contain 40–60% of oil (Liberalino *et al*., 1988; Gandhi *et al*., 1995; Sharma *et al*., 1997; Wink *et al*., 1997; Makkar *et al*., 1997; Openshaw, 2000), which is used for many purposes such as lighting, as a lubricant, for making soap (Rivera-Lorca and Ku-Vera, 1997).

Jatropha oil contains approximately 24.60% of crude protein, 47.25% of crude fat, and 5.54% of moisture contents (Akintayo, 2004). The oil fraction of *Jatropha* contains saturated fatty acids, mainly palmitic acid (16:0) with 14.1% and stearic acid (18:0) with 6.7%. Unsaturated fatty acids consisted of oleic acid (18:1) with 47.0%, and linoleic acid (18:2) with 31.6%. The oil with high percentage of monounsaturated oleic and polyunsaturated linoleic acid has a semi-drying property (partially hardens when the oil is exposed to air). This semi-drying oil could be an efficient substitute for diesel fuel (Augustus *et al*., 2002).

Table 2.2 gives a summary of other compounds reported from different parts of *J. curcas.*

Table 2.1: Some chemicals substances isolated from different parts of *J. curcas*

Plant part	Chemical Composition	References
Aerial parts	Organic acids (*o* and *p*-coumaric acid, *p*-OH-benzoic acid, protocatechuic acid, resorsilic acid, saponins and tannins	Hemalatha and Radhakrishnaiah (1993)
Stembark	β-Amyrin, β -sitosterol and taraxerol	Mitra *et al*. (1970)
Leaves	Cyclic triterpenes, stigmasterol, stigmast-5-en-3β, 7 β -diol, stigmast-5-en-3 β,7α-diol, cholest-5-en-3 β, 7 β -diol, cholest-5-en-3fl,7α-diol, campesterol, β -sitosterol, 7-keto- β -sitosterol as well as the β -d-glucoside of β -sitosterol. Flavonoids apigenin, vitexin, isovitexin. Leaves also contain the dimer of a triterpene alcohol ($C_{63}H_{117}O_9$) and two flavonoidal glycosides	Mitra *et al*. (1970), Khafagy *et al*. (1977), Hufford and Oguntimein (1987)
Latex	Curcacycline A, a cyclic octapeptide Curcain (a protease)	Van den Berg *et al*. (1995) Nath and Dutta (1991)
Seeds	Curcin, a lectin, Phorbolesters, Esterases and Lipase	Stirpe *et al*. (1976) Adolf *et al*. (1984), Makkar *et al*. (1997) Staubmann *et al*. (1999)
Kernel and press cake	Phytates, saponins and a trypsine inhibitor	Aregheore *et al*. (1998), Makkar and Becker (1997), Wink *et al*. (1997)
Roots	β-Sitosterol and its β -d-glucoside, marmesin, propacin, the curculathyranes A and B and the curcusones A–D, diterpenoids jatrophol and jatropholone A and B, the coumarin tomentin, the coumarino-lignan jatrophin as well as taraxerol	Naengchomnong *et al*. (1986, 1994)

2.6. **Reported Toxicity of *Jatropha curcas* Seeds**

The toxicity of the whole seed from *J. curcas* has been known for a long time, and has been attributed to a protein component which was isolated from the seed and designated as curcin by Felke (1914). He proposed that curcin was a type of toxalbumin which has also been found to inhibit protein synthesis of intact cells. Curcin is toxic to animals and inhibit protein synthesis in a cell-free system (an in-vitro biosystem that can be used to study reactions that happen within the cell under reduced complex interactions found within the whole cell), but not in whole cell where all cellular components are present. It is unable to penetrate the cell wall and this is thought to be because it lacks a carrier moiety or the galactose-binding groups which bind to cell membranes as the case is in ricin (Stirpe *et al*., 1976).

All cases of systematic poisoning have resulted from ingestion of plant material (particularly seeds). Phytotoxins are well absorbed from the gastro intestinal tract. Curcin is partly metabolized in the digestive tract with onset of symptoms developing one or more hours later (Kingsbury, 1964).

In severe intoxications, dehydration and haemorrhagic gastroenteritis can occur with CNS and cardiovascular depression and collapse. Curcin is said to be highly irritant and remains in the seed after the oil has been expressed. Several cases of *J. curcas* nut poisoning in humans after accidental consumption of the seeds have been reported with symptoms of giddiness, vomiting and diarrhoea and in the extreme condition even death has been recorded (Becker and Makkar, 1998).

No case of toxicity has been reported from topical application of the oil against skin diseases in folk medicine. The high concentration of phorbol esters present in *Jatropha*

seed has been identified as the main toxic agent responsible for *Jatropha* toxicity (Adolf *et al*., 1984; Makkar *et al*., 1997). Complete removal of the toxins is therefore necessary before *Jatropha* oil can be used in industrial applications or in human medicine, the oil must be shown to be completely innocuous before it is used commercially. Mart´ınez-Herrera *et al*. (2006) studied the nutritional quality and the effect of various treatments (hydrothermal processing techniques, solvent extraction, solvent extraction plus treatment with $NaHCO_3$ and ionizing radiation) to inactivate the antinutritional factors in defatted *Jatropha* kernel meal of both toxic and non-toxic varieties from different regions of Mexico. Ionizing radiation treatment could serve as a possible additional processing method for inactivation or removal of certain antinutritional factors such as phorbol esters, phytates, saponins and lectins (Siddhuraju *et al*., 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Ethnobotanical Survey of *Jatropha curcas* Seeds

An ethnobotanical survey was conducted on the traditional medicinal uses of the seeds of *Jatropha* among local folks using a structured questionnaire (appendix A). The survey covered farmers, herdsmen, traders and herbalists who have first hand information on the medicinal uses of the seeds. The questionnaire was designed to obtain basic information such as the known traditional medicinal uses of the seeds, mode of preparation, route of administration and toxicity.

The seeds were used so as to make identification of the plant easy for the local folks some of which preferred anonymity even though they cooperated while others cooperated fully. The respondents were mostly non-English as such Hausa being the common language was used for communication, after which all relevant information was translated to English and documented.

3.2. Collection and Preparation of the Plant Material

Mature seeds of *J. curcas* Linn. were collected from Dakace and Kufena villages of Zaria Local Government, Kaduna state, Nigeria.

The plant was authenticated in the Herbarium Unit of Biological Sciences Department, Ahmadu Bello University, Zaria, by the Herbarium keeper, Malam U.S. Gallah where a specimen with a voucher number 1911 has been deposited.

The seeds were dried under shade and powdered using pestle and mortar in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical

Sciences, Ahmadu Bello University, Zaria, Nigeria. The powdered sample was stored in an air tight container until needed.

3.3. **Chemicals**

Methanol, and petroleum-ether (60 - 80^{0}C) from Sigma Aldrich chemical company, U.S.A. were used for extraction. Reagents and chemicals such as Molisch's reagent, Fehlin's Solution A and B, ammonia, benzene, nitroprusside, glacial acetic acid, n-butanol and ethyl acetate from Loba Chemie Laboratory Reagents and Fine chemicals Mumbai, India, were used for experiments. Paracetamol from Pfizer Pharmaceutical company, U.S.A. was used for analgesic study.

3.4. **Test Animals**

All animals, comprising albino mice and rats of either sex weighing 20-25 g were obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University. They were kept in cages in the Animal House of the Department and acclimatized to the environment for 2 weeks before the commencement of experiments.

3.5. **Extraction of Plant Material**

The seed oil was extracted with petroleum ether (60 - 80^{0}C) in a Soxhlet extractor. The marc was dried and extracted with absolute methanol in a Soxhlet extractor. The methanol extract was evaporated to dryness in an evaporation dish on a water bath (60^{0}C) for one hour. It was dissolved in water and filtered. The filtrate was then shaken gently with 1L of petroleum ether and allowed to settle before the petroleum ether layer was separated. For the purpose of the antimicrobial test, it was further fractionated with ethyl acetate and n-butanol in addition to the petroleum ether and aqueous fractions.

3.6. **Pharmacognostic Evaluation of *J. curcas* Seed**

The seed was subjected to macroscopical and microscopical evaluation:

3.6.1. **Macroscopic evaluation**

The seed coats were removed by gentle pounding of the seeds using morter and pestle. The macroscopic evaluation of the seed samples included visual appearance of the seeds to naked eye, odour, size, shape, taste and texture according to standards described by WHO (1998).

Colour: The seeds were examined under sunlight.

Shape and size: The length of the seed was determined.

Odour: The odour and taste of a crude seed samples are extremely sensitive criteria based on individual's perception. The seeds were crushed by applying pressure and the odour was determined.

Taste: A small quantity of the seed was chewed to determine the taste.

Texture: The texture was examined by taking a small quantity of material and rubbing it between the thumb and forefingers to determine whether it is smooth, rough and gritty.

3.6.2. **Microscopical evaluation**

In order to obtain fine sections of the seeds with the hard seed coats, the seeds were fixed in formalin-acetone-50% ethanol (9:1) for 24 hours and it was ensured that the whole of the seeds was immersed in the fixative. The seeds were fixed, dehydrated in an ethanol series (50, 60, 70, 80, 90 and 95%). After fixation, samples of the seeds were softened in maceration solution prepared with concentrated glacial acetic acid and 30% hydrogen peroxide (1:1 v/v) and kept for 6 hours. Distilled water was then used to completely remove the solution. After this procedure, the seeds were again dehydrated in an ethanol

series (10, 20, 30, 40, 50, 60, 70 %). Fine sections of the seeds were cut with metronome and embedded after being softened in the maceration solution described by Franklin (1945) and washed with distilled water to completely remove the solution prior to fixation (Johansen 1940).

The seed powder used for powder microscopy was obtained by removing the seed coat and grinding the seed with mortar and pestle.

3.6.3. **Physico-chemical study**

Moisture content: Three grammes of the powdered seed sample was placed on an evaporating dish and dried at 105^0C for 6 h and weighed. The drying was continued until the difference between two successive weighing was not more than 0.25%. Constant weight was reached when two consecutive weighing after drying for 30 min. in a desiccator showed not more than 0.01 g difference (WHO, 1998).

Total ash: 4.0 g of the finely ground and dried material was accurately weighed and placed in a previously ignited and tarred crucible. The material was spread in an even layer and ignited by gradually increasing the heat until it is white, indicating absence of carbon. It was then cooled in a desicator and weighed. The residue was moistened with 2 ml of water, dried on a water bath, then on a hot plate and ignited to constant a weighed. The residue was allowed to cool in a suitable desicators for 30 min. and weighed immediately (WHO, 1998).

Water-soluble ash: It is the difference in weight between total ash and the residue left after treatment of total ash with water. 25 ml of water was added to the crucible containing the total ash and boiled for 5 min. The insoluble matter was collected on an ashless filter paper. Washed with hot water and ignited in a crucible for 15 min. The ash

obtained was weighed and subtracted from the weight of total ash. The percentage of ash was calculated (WHO, 1998).

Acid-insoluble ash: 25 ml of hydrochloric acid was added to the crucible containing the total ash. It was covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in vacuum desiccators for 30 min., and weighed without delay (WHO, 1998).

Alcohol soluble extractives: Alcohol extractive value was determined by weighing 5.0 gm of coarsely powdered seeds, which was macerated with 100 ml of the absolute methanol in a glass stopper closed flask for 24 h with frequent shaking during the first 6 h and allowed to stand for 18 h. It was filtered rapidly and 25 ml of the filtrate was transferred in to a tarred flat bottom dish and evaporated to dryness on a water bath. It was dried at 105^0C for 6 h and cooled in a vacuum dessicator for 30 min. and weighed without delay.

Water soluble extractives: The water extractive value was determined by weighing 5.0 gm of the sample and macerated with 100 ml of chloroform water (1:400) in a glass stopper closed flask for 24 h with frequent shaking during the first 6 h and allowed to stand for 18 hrs. It was filtered rapidly taking care not to lose any solvent and then 25 ml of the filtrate was transferred in to a tarred flat bottom dish and evaporated to dryness on

a water bath. It was dried at 105^0C for 6 h and cooled in a vaccum dessicator for 30 min (Indian pharmacopeia, 1996).

3.6.4. **Chemomicroscopic and florescence analysis**

Chemomicroscopic analysis: The seed powder was tested with various reagents such as phloroglucinol, hydrochloric acid, Million's reagent and Sudan IV. The sample was viewed under microscope for colour inferences indicating the presence or absence of proteins, lipids tannins etc.

Florescence analysis: The florescence character of the seed powder was studied both in daylight and UV light (254 and 366 nm) after treatment with different reagents like glacial acetic acid, hydrochloric acid, sodium hydroxide and sulphuric acid (Kokoski *et al.*, 1958).

3.7. **Phytochemical Analysis of *J. curcas* Seed Extract**

The extract was subjected to various tests for phytochemical evaluations by using methods of Evans (2002) and Sofowora (1993).

3.7.1. Test for carbohydrates

(a) Molisch's test

Three drops of Molisch's reagent were added to the methanol extract dissolved in distilled water. This was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test for carbohydrates.

(b) Fehling test

Of the methanol extract 0.5 g was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

3.7.2. Test for anthraquinone derivatives

(a) Borntrager's test

Of the methanol extract 0.2 g was shaken with 10 ml of benzene and then filtered. 5 ml of the 10% ammonia solution was then added to the filtrate and thereafter shaken. Appearance of a pink, red or violet colour in the ammoniacal (lower) phase was taken as the presence of free anthraquinones

(b) Modified Borntrager's test

To 0.5g of the methanol extract 5 ml of 10% hydrochloric acid was added and boiled for 3 minutes to hydrolyze the glycosides to yield aglycones which are soluble in hot water only. The solution was filtered and cooled. The filtrate was shaken with 5ml of benzene. The benzene layer was filtrated off and shaken gently with 5ml 10% ammonia solution. A rose-pink or cherry-red colour in the lower ammonical layer indicates combined anthracene.

3.7.3. Test for cardiac glycosides

(a) Kella-Killiani test

Of the methanol extract 0.5g was dissolved in 2ml glacial acetic acid containing traces of ferric chloride and filtered. The filtrate was transferred to a test tube. The test tube was held at an angle of 45^0 and 1ml of sulphuric acid was run down the side. Formation of a purple ring interface indicates cardiac glycosides

(b) Legal test

Of the methanol extract 0.5g was dissolved in a 2ml of pyridine in the fumed cupboard. 0.5ml of 2% nitroprusside and 0.5ml of sodium hydroxide were added. The production of a deep-red colour indicates the presence of cardenolides.

(c) Kedde test

To 0.5g of the methanol extract was added 1ml of 2% 3,5-dinitrobenzoic acid in 95% alcohol. 0.5ml of 5% sodium hydroxide was added. The appearance of a purple-blue colour indicates the presence of cardenolides in the ring.

(d) Salkowski test

Of the methanolic extract 0.5g was dissolved in chloroform and 0.5ml of concentrated sulfuric acid were added. The formation of a reddish-brown colour at interface indicates the presence a steroidal ring.

3.7.4. Test for saponins

(a) Frothing test

Of the extract 1g was boiled with 5 ml of distilled water and filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins.

(b) Haemolysis test

In test tubes 2ml of 1.8% aqueous sodium chloride were taken. 2ml of methanol extract was added to one portion and same volume of distilled water was added to the other portion. 0.5ml of blood was added to each portion and observed for 30 minutes. Haemolysis in the tube containing the sample indicates the presence of saponins.

3.7.5. Test for steroids and terpenoids

Lieberman-Burchard's test

(a) Steroids: 2 ml of acetic acid was added to 0.2 g of the methanol extract. The solution was cooled well and followed by the addition of 1 ml conc. H_2SO_4 carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e. aglycone portion of cardiac glycoside.

(b) Terpenoids: To 0.2 g of the sample 1 ml of acetic anhydride was added, followed by the addition of conc. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids.

3.7.6. Test for flavonoids

(a) Shinoda test

About 0.5g each of three portions of methanolic extract was dissolved in 2ml of 50% methanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by few drops of concentrated HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.

(b) Sodium hydroxide test

About 5ml of the dissolved methanol extract was filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

3.7.7. Test for tannins

(a) Lead sub-acetate test

Three drops of lead sub-acetate was added to a solution of the methanol extract dissolved in water. A coloured precipitate indicates the presence of tannins.

(b) Ferric chloride test

About 0.5 g of the methanol extract was dissolved in 10 ml of distilled water and then filtered. 3 drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

(c) Bromine water test

Three drops of bromine water were added to a solution of the methanol extract dissolved in water in a fumed cupboard. A buff coloured precipitate indicates the presence of condensed tannins only.

3.7.8. Test for alkaloids

About 1.0 g of the extract was dissolved in 10ml of 1% aqueous HCl on water bath and then filtered.

(a) Meyer's test

Three drops of Mayer's reagent was added 2ml of the extract in a test tube. Appearance of cream-coloured precipitate will be an indication for the presence of alkaloids.

(b) Dragendorff's test

Three drops of Dragendoff's reagent were added to 2ml of the extract. An orange-red precipitate was taken as presence of alkaloids.

(c) Wagner's test

To 2ml of the methanol extract was added three drops of Wagner's reagent. A brown precipitate indicates the presence of alkaloids.

(d) Picric acid test

To 2ml of the methanol extract was added a three drops of 1% picric acid solution. Yellow coloured precipitate indicates the presence of alkaloids.

(e) Tannic acid test

Three drops of tannic acid were added to 2ml of the methanol extract. A brown precipitate indicates the presence of alkaloids.

3.8. **Evaluation of *J. curcas* Seed Oil**

The seed oil of *J. curcas* was tested according to standards described in WHO pharmacopoeia.

3.8.1. **Determination of acid value**

Of *Jatropha curcas* oil 10g of was weighed in a 250ml conical flask. 50ml of a mixture of equal volumes of ethanol (95%) and ether was added. After the addition of 1ml of phenolphthalein solution the mixture was titrated with N/10-potasium hydroxide solution with constant shaking until a pink colour persisting for some seconds was obtained. The volume of potassium hydroxide required for the titration was recorded as v and the acid value was found using the expression according to WHO (1998):

$$\frac{v \times 0 \cdot 0561 \times 1000}{weight\ of\ oil}$$

3.8.2. **Determination of saponification value**

Jatropha curcas oil (2g) was weighed in a 250ml flask and 25ml of alcoholic KOH solution was added and refluxed for 1hr. 1ml of phenolphthalein was added and the

mixture was titrated with N/2-HCl. The required volume of the acid was taken as x. The procedure was repeated without the oil and the volume of the acid required for the titration was recorded as y. Saponification value was calculated using the mathematical expression according to WHO (1998):

$$\frac{(y-x) \times 0 \cdot 2805 \times 100}{weight\ of\ oil}$$

3.8.3. **Solubility test**

Jatropha curcas oil (0.2ml) was shaken in 5ml of various solvent to observe its solubility. The solvents used include water, methanol, chloroform and petroleum ether.

3.8.4. **Sodium hydroxide test**

About 1.0 ml of 1% copper sulphate solution was mixed with 5 drops of the oil. 5 drops of 10% sodium hydroxide solution was then added. A clear blue solution indicates the presence of glycerin (WHO, 1998).

3.8.5. **Test for cholesterol**

The seed oil (0.5ml) of *J. curcas* was treated with 0.2ml of acetic anhydride and a drop concentrated sulphuric acid was added. A deep green colour indicates the presence of cholesterol (WHO, 1998).

3.9. **Thin Layer Chromatography of *Jatropha curcas* Seed Extract**

Various solvent systems were developed in order to obtain the best separation of compounds. The methanol extract and aqueous fraction were dissolved in methanol while the petroleum-ether fraction was dissolved in petroleum-ether. Precoated plates were activated in the oven at about 100^0C for 30mins and then cooled at room temperature.

The extracts and fractions were spotted on the plates at different points on the same origin.

Solvent systems used for the methanol extracts and fractions were Chloroform : Ethyl acetate (1:1), Chlroroform : Acetone (4:1), Acetic acid : Methanol (9:1) and Acetic acid : Acetone (4:1). Chloroform (100) was used as the solvent system for developing the oil.

The spotted plates were developed in chromatographic tanks with various solvents. The plates were removed, dried and the spots located with the general spray reagent, 20% H_2SO_4 in methanol, and their R_f values calculated (Touchstone, 1992) as described by Osadebe and Uzochukwu (2006).

3.10. **Acute Toxicity Test for *Jatropha curcas* Seed Extract**

Acute toxicity test was carried out using Lorke (1983) method. Briefly, after five days of aclimatization of animals, they were administered three different doses of the methanol extract of *J. curcas* seed (dissolved in tween 80) intraperitoneally; 10mg/kg (group 1), 100mg/kg (Group II) and 1000mg/kg (group III). They were put under observation for signs and symptoms of toxicity, including death, for 24hrs.

The experiment was carried out in two phases:

Phase I - An initial investigation was made to establish the range of doses producing the toxic effect.

Phase II - Based on the result in phase I, four specific doses were administered in order to determine LD_{50}. The doses given are: 140, 225, 370 and 600mg/kg. The LD_{50} was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

Mathematically, it can be expressed as

$$a = \sqrt{cd}$$

where a = acute toxicity (LD_{50})

c = highest non-lethal dose

d = lowest lethal dose

3.11. **Pharmacological and Antimicrobial Evaluation of *Jatropha curcas* Methanolic Seed Extract and Fractions**

3.11.1. **Analgesic activity of *J. curcas* seeds**

Acetic acid-induced writhing response in mice was used to test for analgesic activity according to the method of Coster *et al*., 1959.

Five mice per group were used. Group I (control) received normal saline (0.5ml/kg *i.p.*); Group II (positive control) received paracetamol (100mg/kg *i.p.*); Group III, IV and V received doses of 10mg/kg, 40mg/kg and 80mg/kg of the methanolic extract respectively. Group VI, VII and VIII also received similar doses of the aqueous fraction respectively. The same procedure was followed for group IX, X, XI for the petroleum ether fraction. After 30 minutes, 0.6% acetic acid (10ml/kg) solution was administered intraperitioneally to all animals in different groups. The number of writhes occurring between 5 – 15mins after acetic acid injection were counted. The reduction in writhing in test animals compared to negative control group was taken as an antinociceptive response.

3.11.2. **Antimicrobial activity *J. curcas* seeds**

The methanolic extract of *J. curcas* seeds was fractionated into four fractions; petroleum ether, ethyl acetate, n-butanol and aqueous fractions. The methanol extract and all the fractions were used to test for antimicrobial activity.

(a) Test organisms: Standard cultures of *Escherichia coli*, *Salmonella typhi* (gram –ve bacteria), *Streptococcus pyrogenes*, *Staphylococcus aureus* (gram +ve bacteria) and the fungus *Candida albicans* were used for the antimicrobial activity test.

(b) Antimicrobial assay: Broth cultures of the test organisms were prepared by suspending a portion of the colonies of each organism in nutrient broth and incubated aerobically at 37^0C for 18 hours. The respective suspensions were adjusted to a turbidity of 10^8 colony forming units per ml (CFU/ml) using 0.5 McFarland standards for visual comparison.

(c) Antimicrobial screening: The antimicrobial screening of the extract was carried out using agar well diffusion method. Sterile nutrient agar plates were flooded with 0.1ml of the bacterial suspensions in duplicates. These were streaked uniformly on the surface of the culture media. Wells of 6mm diameter were punched on each plate with sterile cork borer. 0.1ml of the stock extract and fractions was added to each well and allowed to stay for 1 hour to enhance diffusion through the media. The plates were incubated (inverted) aerobically at 37^0C for 24 hours. At the end of the incubation period, the diameters of the zones of inhibition of growth were measured using a transparent ruler and recorded. The extracts were tested in duplicates and mean zones of inhibition were calculated (Akerele *et al*, 2011). 30µg/ml of Streptomycin was used as positive control for bacteria and 10µg/ml of Ketoconazole was used for the fungus.

(i) Minimum Inhibitory Concentration (MIC): The MIC was determined using broth dilution method as reported by Vellokobia *et al*, (2001). Two fold serial dilution of the extract and fractions were made to obtain 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.13mg/ml and 1.57mg/ml. 0.2ml suspension of standard

inoculum of each organism was inoculated to the different concentrations of the extracts. The extracts were then incubated at 35^0C for 24 hours for bacteria, and at 24^0C for 3 days for fungi (Sandle, 2014), after which they were observed for inhibition of growth. Inhibition of growth was indicated by a clear solution. The MIC was defined as the least concentration of the extract inhibiting the visible growth of each organism.

(ii) Minimum Bactericidal/Fungicidal Concentration (MBC/MFC): The contents of the MIC tubes and the preceding tubes in the serial dilution were subcultured into appropriately labelled nutrient agar plates by dipping a sterile wire loop into each tube and streaking on the surface of each agar plate respectively. The plates were then incubated at 35^oC for 24 hours for bacteria (Sandle, 2014), and at 24^0C for 3 days for fungi, after which they were observed for colony growth. The lowest concentration of the subcultures with no growth was considered to be the minimum bactericidal/fungicidal concentration of the extracts (Abdullahi *et al.* 2011).

CHAPTER FOUR

4.0 RESULTS

4.1. Ethnobotanical Survey of *J. curcas* Seeds

A total of 38 respondents within the age range of 29-70 years were covered by the survey, all of whom were males. About 18 of them were in Dakace and 6 in Kufena areas of Zaria Local Government; 6 in Samaru area of Sabon Gari Local Government and 8 in Zonkwa, Zangon Kataf Local Government of Kaduna state. From the survey, the common diseases treated traditionally with *Jatropha curcas* seeds are as follows: 25.64% of respondents said they use it as an antimicrobial agent for skin infections such as eczema, ringworm, skin rashes; 23% reported to use it as analgesic against headache, scorpion sting; 5.12% use it for remedy against sexually transmitted infections. Other used it traditionally for constipation, stomachache, and weak erection (see Fig. 4.1).

The seeds of *J. cucas* are used in wound treatment and are also used as laxative and purgative. It was also found that the seed oil serves as an ingredient in local pomade while seeds are eaten as food after boiling. The ethnobotanical uses of the seeds of *J. curcas* in folk medicine could be summarised as follows: analgesic, purgative, laxative, antimicrobial, antibiotic, anodyne and aphrodisiac. The common toxic effects of direct consumption of the seeds include excessive stooling and purging, abdominal pain, fever, general body weakness and sometimes convulsion in the case of children (Fig. 4.1).

In Dakace town of Zaria, Kaduna State, various parts of *J. curcas* have traditional medicinal uses. The leaf is boiled and drunk as a remedy for fever, squeezed and applied topically for skin diseases such as eczema and ringworm. Another remarkable use of the leaf, from preliminary discussions with some gardeners in Dakace is skin cancer

treatment, a disease popularly known in Hausa as “ciwon daji”. The leaf is sqeezed and rubbed on the affected part. The latex is used as a mouthwash and also applied with cotton wool on the tongue as remedy against rashes. The stem is used as a chewing stick. It must however be noted that because animals do not feed on the plant (except in extreme hunger), some people don’t readily take the leaf decoction or use the stem as a chewing stick.

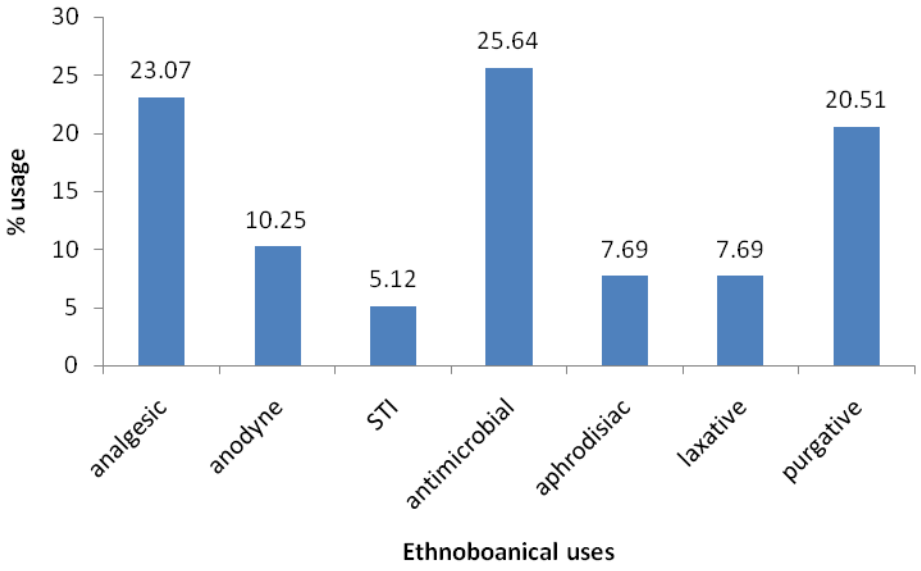


Fig. 4.1: Percentage use of *Jatropha curcas* seeds in traditional medicine

The respondents reported using various forms of administration of *J. curcas* seed in traditional medicine; 30% administer it as powder, 30% use it as whole seed, mostly chewing it to serve as laxative, and 8% use the seed oil topically (Fig. 4.2).

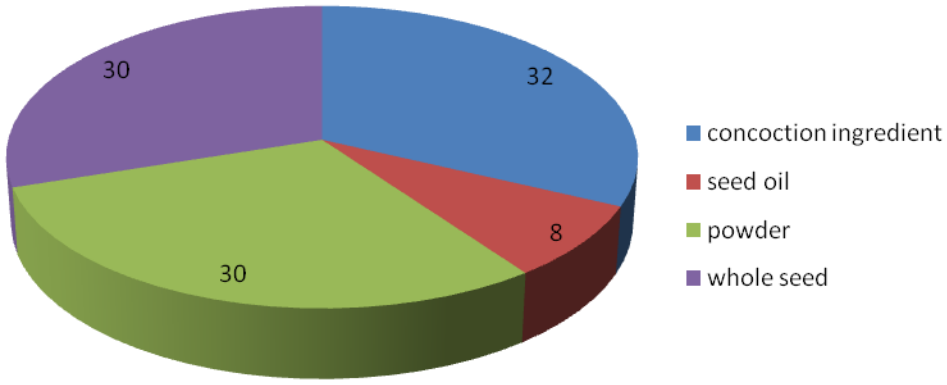


Fig. 4.2: Forms of administration of *Jatropha curcas* seeds in traditional medicine

4.2. **Pharmacognostic Evaluation of *J. curcas* Seeds**

4.2.1 **Macroscopic study**

Colour: dark brown when fresh and black when dry. The seeds appear milky white when the seed coat is removed.

Shape: oval or oblong.

Size: the seed ranges between 3 – 4 cm with an average length of 3.5cm.

Taste: it has a sweet taste.

Odour: weakly aromatic

Texture: the seed coat smooth but a bit rough within. It cracks like a shell when dry.

Number of seeds per fruit: 3 – 4

4.2.2. **Microscopic study**

After mounting the finely cut slides and powder of the seed on the micrioscope, the slides were observed and pictures of the observed features were taken (plates IV, V). The testa of the seed contains oval cells which form schizogenous cavities at adjacent positions. The schizogenous cavities are dense from the outer part of the seed and less towards the centre of the seed where the cells are more compacted. The seed powder shows numerous oval cells with oil droplets.

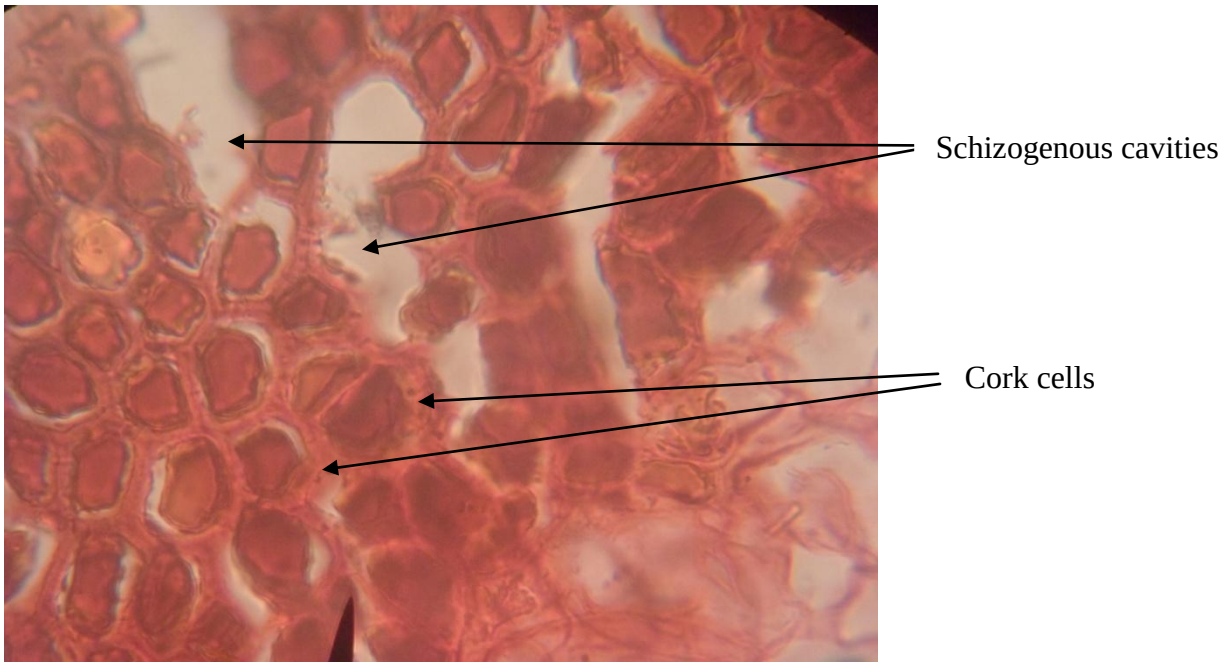


Plate IV: Transverse Section *Jatropha curcas* seed showing schizogenous cavities and cork cells (x400**)**

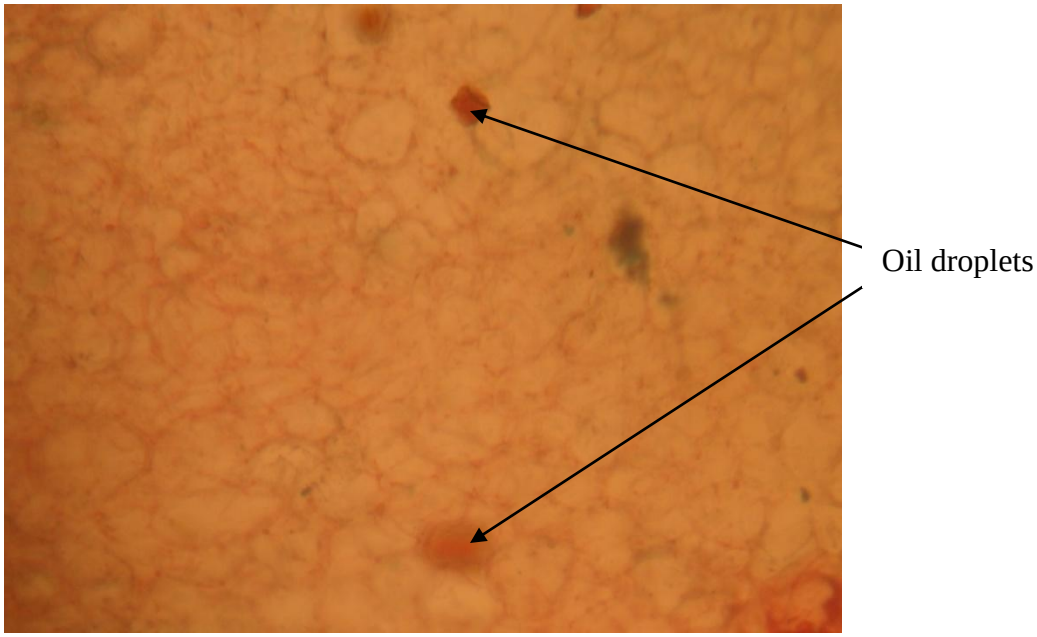


Plate V: *Jatropha curcas* seed powder showing oil droplets (x100)

4.2.3. **Physico-chemical parameters of *J. curcas* seed**

The seeds were observed to have alcohol extractive value of 18.00 ± 0.57 %, while the water extractive value was 11.00 ± 2.10 %. Table 4.1 shows the summary of all the physic-chemical parameters evaluated.

Table 4.1. Physico-chemical parameters of *J. curcas* seed

Parameter	**Value (% w/w)***
Moisture content	4.67 ± 0.32
Total ash value	7.50 ± 0.50
Water soluble ash	5.00 ± 1.00
Acid insoluble ash	3.00 ± 1.00
Alcohol soluble extractives	18.00 ± 0.57
Water soluble extractives	11.00 ± 2.10

***Mean value of three determinations.

4.2.4. **Chemomicroscopic and fluorescence analysis of *J. curcas* seeds**

Bright dissolving crystals were observed when HCL was added to the seed powder which comfirmed the presence of calcium oxalate. A pink colour showed the presence of lipids when Sudan IV was added. Table 4.2 shows a summary of test results.

Table 4.2: Chemomicroscopic study of *J. curcas* seed powder

Reagent	Observation	Inference
Phloroglucinol + conc. HCl + seed powder	No red colour	Lignin absent
HCl + seed powder	Bright dissolving crystals	Calcium oxalate present
Million's reagent + seed powder	Yellow colour	Protein present
Sudan IV + seed powder	Pink colour	Lipid present
Ferric chloride + seed powder	Greenish-black colour	Tannin present
66% H_2 SO_4 + iodine + seed powder	No blue colour	Cellulose absent
Iodine + seed powder	No blue-black colour	Starch absent

When treated with concentrated HCl, both powdered and oil samples appeared yellow in daylight. However, there was no fluorescence in either the powder or oil under UV light. Under UV light, only the powder samples treated with concentrated H_2SO_4 and concentrated NHO_3 had reddish brown and milky white fluorescence respectively. Table 4.3 has the results of the fluorescence analysis observed under daylight and UV light.

Table 4.3: Flourescence analysis of *J. Curcas* seed powder and oil

Treatment	Daylight		UV light	
	Powder	**Oil**	**Powder**	**Oil**
Conc. HCl	Faint yellow	Faint yellow	No fluorescence	No fluorescence
Conc. H_2SO_4	Reddish brown	Deep Red	Reddish brown	Yellow
Conc. HNO_3	Yellow	Reddish brown	No florescence	No florescence
10% NaOH	Brownish	Milky	Milky-white	No florescence
Glacial acetic acid	No change of colour	Faint Yellow	No florescence	No florescence
Distilled water	Whitish	-	Milky-white	-

4.3. **Phytochemical Analysis of *Jatropha Curcas* Seed Extract**

The phytochemical analysis of the methanol extract of *J. curcas* seeds showed it to contain carbohydrates, flavonoids, steroids, tannins, and cardiac glycosides as summeriaed on Table 4.4.

Table 4.4: Phytochemical constituents of methanol extract of *J. curcas* seed

Constituent	Test	Result
Carbohydrate	Molisch's	Present
	Fehling's	Present
	Borntrager's	Absent
	Modified Borntrager's	Absent
Cardiac Glycosides	Kella-Killiani	present
	Legal	Absent
	Kedde	Absent
	Salkowsky	Present
Saponins	Frothing	Present
	Haemolysis	Present
Steroids & Triterpenes	Lieberman-Burchard's	Present
Flavonoids	Shinoda	Present
	Sodium hydroxide	Present
Tannins	Lead sub-acetate	Present
	Ferric chloride	Present
	Bromine water	Present
Alkaloids	Meyer's	absent
	Dragendorf's	Absent
	Wagner's	absent
	Tannic acid	Absent

4.4. **Evaluation of *J. curcas* Seed Oil**

The oil of *Jatropha curcas* seed produced partial froth on shaking in water and settled into two immiscible liquids. However, the oil is soluble in chloroform as a clear miscible solution was formed on mixing. Acid value was found to be 5.61 mg/g. NaOH test revealed the presence of glycerin by the formation of a clear blue solution. Table 4.5 and appendix B contain summary of other parameters evaluated for the oil.

Table 4.5: Evaluation of *J. curcas* oil.

Parameter	Result/Observation	Inference
Solubility		
Water	Partial froth was formed on shaking which settled to form two immiscible layers with the oily layer at the top.	Not soluble in water.
95% ethanol	A whitish/misty solution was formed on shaking which settled to form two distinct layers, with the oily layer at the bottom.	Not soluble in ethanol.
Chloroform	A clear miscible solution was formed.	Soluble in chloroform.
Petroleum ether	Complete miscibility was observed.	Soluble in pet-ether
Chemical test		
NaOH test	A clear blue solution was formed.	Glycerin presence.
Cholesterol	No formation of a deep green colour.	Cholesterol absent.
Filter paper test	The oil spread a few cm from the drop point leaving the covered area translucent.	Sample is oil
Acid value	5.61mg/g	
Saponification value	297.33mg/g	
Colour	Yellow	
Taste	Tasteless	
Smell	Characteristic of vegetable oils	

4.5. **Thin Layer Chromatography of *J. curcas* Seeds**

The TLC plates were sprayed with 20% H_2SO_4 in methanol. The methanolic extract and petroleum-ether fraction had the highest number of spots with chloroform-acetone (4:1) and acetone-acetic acid (1:4) solvent systems. The of the spots were noted and their R_f calculated (Tables 4.6-4.9). The plates were also recorded as plates VII-XI.

Table 4.6: Thin Layer Chromatographic profile of methanolic extract of *Jatropha curcas* seed.

Solvent system	No. of spots	Colour of spots		R_f value
		Day light	After spray*	
chloroform:ethyl acetate (1:1)	3	-	Light green	0.01
		-	Blue green	0.51
		-	Green	0.97
chloroform:acetone (4:1)	5	-	Purple	0.07
		Brown	Reddish brown	0.21
		-	Purple	0.78
		Brown	Brown	0.90
		Brown	Brown	0.97
acetone:acetic acid (1:4)	5	Brown	Reddish brown	0.28
		Green	Green	0.74
		-	Brown	0.80
		-	Light green	0.90
		Brown	Dark brown	0.94
methanol:acetic acid (1:9)	5	-	Purple	0.14
		Brown	Reddish brown	0.28
		Purple	Purple	0.48
		Pink	Pink	0.77
		Brown	Brown	0.98

*sprayed with 20% H_2SO_4 in methanol and heated at 110^oC for 5 minutes.

Table 4.7: Thin Layer Chromatographic profile of aqueous fraction of *Jatropha curcas* seed.

Solvent system	No. of spots	Colour of spots		R_f value
		Day light	After spray*	
acetone:acetic acid (1:4)	4	Green	Green	0.18
		Green	Green	0.34
		-	Light purple	0.70
		Brown	Brown	0.97
methanol:acetic acid (1:9)	3	-	Green	0.25
		Green	Green	0.51
		-	Brown	0.71

*sprayed with 20% H_2SO_4 in methanol and heated at 110^oC for 5 minutes.

Table 4.8: TLC profile of petroleum ether fraction of *Jatropha curcas* seed.

Solvent system	No. of spots	Colour of spots		R_f value
		Day light	After spray*	
chloroform:ethyl acetate (1:1)	4	Pink	Pink	0.10
		-	Pink	0.51
		-	Purple	0.73
		Light green	Green	0.97
chloroform:acetone (4:1)	7	-	Purple	0.10
		-	Purple	0.28
		Brown	Reddish brown	0.74
		Purple	Purple	0.80
		Brown	Brown	0.90
		Brown	Dark brown	0.94
		Brown	Dark brown	0.98
acetone:acetic acid (1:4)	4	Brown	Brown	0.07
		-	Light brown	0.19
		-	Light brown	0.89
		Brown	Dark brown	0.97
methanol:acetic acid (1:9)	4	Brown	Reddish brown	0.25
		-	Purple	0.48
		Pink	Pink	0.79
		Brown	Reddish brown	0.99

*sprayed with 20% H_2SO_4 in methanol and heated at 110^oC for 5 minutes.

Table 4.9: Thin Layer Chromatographic profile of *J. curcas* seed oil.

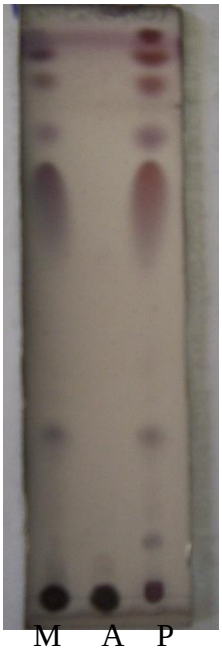
Solvent system	No. of spots	Colour of spots		R_f value
		Day light	After spray*	
Chloroform (100)	5	Brown	Brown	0.16
		Brown	Brown	0.24
		Brown	Brown	0.35
		Brown	Brown	0.48
		Brown	Brown	0.93

*sprayed with 20% H_2SO_4 in methanol and heated at 110^oC for 5 minutes.

M A P

Plate VI: TLC plate developed in Chloroform : Ethyl acetate (1:1)

M – methanolic extract, A – aqueous fraction, P – petroleum ether fraction



M A P

Plate VII: TLC plate developed in Chloroform : Acetone (4:1)

M – methanolic extract, A – aqueous fraction, P – petroleum ether fraction

M A P

Plate VIII: TLC plate developed in Acetic acid : Methanol

M – methanolic extract, A – aqueous fraction, P – petroleum ether fraction

Plate IX: TLC plate developed in Acetic acid : Acetone (4:1)

M – methanolic extract, A – aqueous fraction, P – petroleum ether fraction

Plate X: TLC plate of *J. curcas* seed oil developed in Chloroform (100)

4.6. **Acute Toxicity Test of *J.curcas* Seed Extract**

The LD_{50} of the methnolic extract of *J. curcas* was found to be 471.17mg/kg.

4.7. **Biological Activity Test for *J. curcas* Seed Extract and Fractions**

4.7.1. **Analgesic test**

The number of writhes reduced from 19.0 ± 2.8 in the group administered normal saline to 8.0 ± 0.8 in the group administered the standard drug, paracetemol (100 mg/kg) giving 57.90 % inhibition of writhes. At the highest dose level of 80 mg/kg, the methanolic extract gave 78.95 % reduction in number of writhes, while the aqueous and petroleum ether fraction had 68.4 % and 84.21 % inhibition levels respectively (see Table 4:10 and Fig. 4.3 to 4.5).

Table 4.10: Analgesic activity of *J. curcas* seed extract and fractions

Drug/sample	**mean number of writhes (% inhibition)**		
	10 mg/kg	40 mg/kg	80 mg/kg
Normal saline	19.0 ±2.80		
Paracetamol	8.0 ±0.80 (100 mg/kg)		
Methanol extract	12.0 ±2.20 (57.90)	6.0 ±1.60 (68.43)	4.0 ±1.70 (78.95)
Aqueous fraction	14.0 ±3.10 (26.32)	12.0 ±1.90 (36.85)	6.0 ±0.70 (68.40)
Petroleum ether fraction	12.0 ±2.70 (36.84)	8.0 ±1.00 (57.89)	3.0 ±0.90 (84.21)

(Values are expressed as mean ± standard error of mean. Values in parenthesis are percentage inhibition of acetic acid induced writhing)

4.7.2. **Antimicrobial Test**

At 50-200 mg/ml, ethyl acetate fraction was consistent in inhibiting the growth of all gram +ve and gram –ve bacteria. These zones of inhibition are comparable to those exhibited on same organisms by streptomycin. At 200 mg/ml, the methanol extract showed zones of inhibition of 22mm, 20mm and 16mm on *E. coli*, *S. aureus* and *S. pyrogenes* respectively. However, the least concentration of 12.5 mg/ml did not inhibit microbial growth in the methanolic extract, as well as all other fractions. The n-butanol fraction at 25 mg/ml inhibited the growth (12mm) of *Candida albicans* at a close range to ketoconazole (15mm) which made it the only fraction that inhibited the growth of the fungus at that concentration. Table 4.11 represents the zones of inhibition of other fractions.

Table 4.11: Zones of inhibition of microbial growth of *J. curcas* seed extract and fractions

Extract Samples	Extract Concentration (mg/ml)	Zone of Inhibition (mm)				
		Candida albicans	*Escherichia coli*	*Salmonella typhi*	*Staphylococcus aureus*	*Streptococcus pyrogenes*
Methanolic extract	200	20	22	-	20	16
	100	18	18	-	15	13
	50	14	14	-	13	-
	25	-	12	-	-	-
	12.5	-	-	-	-	-
Petroleum ether fraction	200	20	-	-	-	15
	100	16	-	-	-	14
	50	13	-	-	-	12
	25	-	-	-	-	-
	12.5	-	-	-	-	-
Ethyl Acetate fraction	200	15	25	18	18	25
	100	13	20	16	15	21
	50	11	15	13	13	16
	25	-	-	-	-	14
	12.5	-	-	-	-	-
n-butanol fraction	200	20	18	14	16	14
	100	18	16	12	14	12
	50	14	18	-	12	-
	25	12	12	-	-	-
	12.5	-	-	-	-	-
Aqueous fraction	200	22	25	14	-	-
	100	20	18	12	-	-
	50	16	14	-	-	-
	25	14	-	-	-	-
	12.5	-	-	-	-	-
Ketoconazole	10µg	15	-		-	-
Streptomycin	30µg	-	24	18	14	16

Key: - = No activity

Following the determination of the zones of inhibition, the Minimum Inhibitory Concentrations (MIC) of *J. curcas* seeds methanol extract and the fractions were also determined. The methanol extract and ethyl acetate fraction both had the same MIC (50 mg/ml) on *E. coli* and *S. aureus*. In the case of the fungus, *Candida albicans*, the methanol extract, n-butanol and aqueous fractions all had an MIC of 50 mg/ml. the summary of the result is shown on table 4.12.

Table 4.12: Minimum Inhibitory Concentrations (MIC) of the *J. curcas* seed extract and fractions

Extract Samples	Extract Concentration (mg/ml)	*Candida albicans*	*Escherichia coli*	*Salmonella typhi*	*Staphylococcus aureus*	*Streptococcus pyrogenes*
Methanolic	200	-	-	+	-	-
extract	100	-	-	++	-	M
	50	M	M	+++	M	+
	25	-	+	++++	+	++
	12.5	+	++	++++	++	+++
Petroleum	200	-	+	+	+	-
ether fraction	100	-	++	++	++	-
	50	M	++	+++	+++	M
	25	+	+++	++++	+++	+
	12.5	++	+++	++++	+++	++
Ethyl Acetate	200	-	-	-	-	-
fraction	100	M	-	M	-	-
	50	+	M	+	M	-
	25	++	+	++	+	M
	12.5	+++	++	+++	++	+
n-butanol	200	-	-	M	M	M
fraction	100	-	M	+	+	+
	50	M	+	++	++	++
	25	+	++	++	+++	+++
	12.5	++	+++	+++	+++	+++
Aqueous	200	-	-	-	+	+
fraction	100	-	M	M	++	++
	50	M	+	+	++	+++
	25	+	++	++	+++	+++
	12.5	++	+++	+++	++++	++++

Key: M = MIC; - = no growth; + = scanty growth; ++ = moderate growth; +++ = dense growth

The MBC/MFC were determined and the bactericidal and fungicidal concentrations of the methanol extract and the fractions were found mostly at 100-200 mg/ml. However, only the ethyl acetate fraction had bactericidal effect on *Salmonella typhi* at 200 mg/ml. The methanol extract had an MFC at 100 mg/ml with no growth at all at 200 mg/ml. Table 4.13 has the summary of the results.

Table 4.13: Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) of *J. curcas* seed extract and fractions

Extract Samples	Extract Concentration (mg/ml)	*Candida albicans*	*Escherichia coli*	*Salmonella typhi*	*Staphylococcus aureus*	*Streptococcus pyrogenes*
Methanolic extract	200	-	-	+	-	Z
	100	Z	Z	++	Z	+
	50	+	+	+++	+	++
	25	++	++	++++	++	+++
	12.5	+++	+++	++++	+++	++++
Petroleum ether fraction	200	-	+	+	+	-
	100	Z	++	++	++	Z
	50	+	++	+++	+++	+
	25	++	+++	++++	+++	++
	12.5	+++	+++	++++	+++	+++
Ethyl acetate fraction	200	Z	-	Z	-	-
	100	+	Z	+	Z	Z
	50	++	+	++	+	+
	25	+++	++	+++	++	++
	12.5	++++	+++	++++	+++	+++
n-butanol fraction	200	-	Z	+	+	+
	100	Z	+	++	++	+
	50	+	++	+++	+++	++
	25	++	+++	+++	++++	+++
	12.5	+++	++++	+++	++++	+++
Aqueous fraction	200	Z	Z	+	+	+
	100	+	+	++	++	++
	50	++	++	++	++	+++
	25	+++	++	+++	+++	+++
	12.5	+++	+++	+++	++++	++++

Key: Z = MBC/MFC; - = no growth; + = scanty growth; ++ = moderate growth; +++ = dense growth

CHAPTER FIVE

5.0 DISCUSSION

Traditionally, from the ethnobotanical survey conducted, *Jatropha curcas* seeds are not so commonly used medicinally compared to other parts of the plant such as the leaves, stem bark and latex. But it is indeed used as a remedy for diseases such as headache, toothache, constipation and the treatment of wounds and skin ailments among some people in Dakace and Kufena areas of Zaria Local Government. However, not too many people directly use it for medicinal purpose mainly due to its non-edibility to animals. Its administration as a drug is more acceptable by herbalists or elderly persons believed to be more experienced. The most popular part of the plant used in Zonkwa is the latex which is applied topically against ringworm and other skin diseases such as eczema.

Nevertheless, according to a report by Offiah *et al.* (2011) seeds constitute about 48% of plant parts used as traditional medicine for diarrhoea. This underscores the general importance of the seeds in traditional medicine.

About 20 % of respondents use the seed as a purgative and another 7 % use it as laxative (fig. 4.1). About 1-2 seeds are usually chewed for this purpose. At convenient doses for some, it could be poisonous to others. It ranges from one to three seeds. As much as four seeds and above cause excessive stooling and body weakness.

Jatropha curcas is a well known plant in folk medicine in many parts of Africa. Most commonly used parts of the plant in traditional medicine include the root, stem bark and latex. The flowers are also used as remedy for animal diarrhoea (Offiah *et al.*, 2011).

The seed is known to be rich in oil and only second to *Elaeis guineensis* in oil content (Gaydou *et al.,* 1982).

Jatropha seeds contain phorbol esters, which are toxic to humans and many animals. A non-toxic genotype of *J. curcas* was reported from some rural communities in southern Mexico, suitable for human consumption (Heller, 1996).

Some of the respondents in Dakace village in Zaria and Zonkwa, Kaduna state reported that there could be toxic and non toxic varieties because some could chew more than three seeds just for its taste and would not experience any discomfort. It is not easy to distinguish the toxic variety from the non toxic variety (Valdes-Rodriguez *et al.,* 2013).

The toxic effects of direct consumption of the seeds are more severe in children as they suffer symptoms such as convulsion and even death in some cases. It was also discovered from the survey that the seeds are eaten as food when cooked. Phorbol esters, which are part of the toxic constituents of the seeds are heat liable (Adam and Magzoub, 1974).

The seeds of *Jatropha curcas* have not been given serious attention in terms of its phytochemical and pharmacologically active constituents. However, the phytochemical analysis showed presence of useful constituents such as flavonoids and steroids which may explain its analgesic property. Reports have shown that flavonoids, steroids and tannins have analgesic effects (Das *et al.,* 1989).

The seeds are rich in phytochemical constituents as other parts of the plant. A study by Aiyelaegbe *et al*. (2007) showed the methanolic extract of *J. curcas* roots to contain steroids, alkaloids, saponins, tannis and glycosides. This also is in agreement with previous report in literature (Rahila, *et al*., 1994). In another literature (Igbinosa *et al*., 2009) the phytochemical screening of *J. curcas* stem bark extract revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids. The leaf extract of *J. curcas* have also been reported to contain alkaloids, flavonoids, saponins, tannins and

phenolic compounds (Ahirrao *et al.*, 2011). Plants produce saponins to fight infections by parasites. When ingested by humans, saponins also seem to help the immune system and to protect against viruses and bacteria as reported by Roa and colleagues (1995).

The inferences drawn from the chemomicroscopic study of the seed powder revealed the presence of substances such as calcium oxalate, proteins, lipids and tannins (Table 4.2). These substances do not only serve as identification characters but lend further credence to the quality of the seed and the use of it as a source of protein when roasted and chewed.

Flourescence analysis can serve as an alternative criterion to TLC for identifying the authenticity of powdered drugs. Concentrated H_2SO_4 showed noticeable colours and fluorescence under day light and UV light respectively (Table 4.3).

The oil has been used in the control of insect pests of cotton including cotton bollworm and on pests of pulses, potato and corn (Kaushik and Kumar, 2004).

The oil of *J. curcas* has locally found use in treatment of eczema and other skin ailments by topical application. The potentiality of *J. curcas* oil in soap making is higher when the crude oil is primarily the raw material and less as a by-product of biodiesel production because the chemical processes involved in purifying the oil could be responsible for the reduction in saponification value. It is mostly treated chemically to remove or reduce the concentration of some constituents one of which is saponin (Achten *et al.*, 2007).

The best resolution produced seven spots for the TLC in chloroform-acetone (4:1). Beyond separation of compound using TLC, isolation and characterization of phytochemicals is also important. Through TLC technique a lot of compounds have been isolated in their purest forms. The solvent system that will yield the best resolution,

hence, is of paramount value. Chloroform-acetone (8:2) yielded the highest number of spots and a very good resolution. The chloroform-ethyl acetate (1:1) solvent system yielded good resolution but with fewer spots than the former. This is also a point to note that using a polar solvent such as methanol in extraction holds promise in improving the yield of phytochemical constituents which could be further isolated and characterized. With the highest number of spots in the petroleum ether fraction, it could be said that the extract is richer in non-polar constituents, which explains the absence of any spot in the aqueous fraction (plate VII).

The aqueous fraction though cannot be said to be void of chemicals as it has exhibited strong analgesic activity. It has given four and three spots in the acetone-acetic acid (1:4) and methanol-acetic acid (1:9) solvent systems respectively (Table 4.7). The fewer number of spots in the petroleum ether fraction of these solvent systems could be attributed to the polarity of the solvent systems where most constituents moved with the solvent with only few separating out (plate IX). The established profile can thus be used as a yardstick for identification, isolation and characterization of the chemical constituents of *J. curcas* seed extract.

Autopsy findings from ingestion of *J. curcas* seed are well known in veterinary practice and they include: severe gastro-enteritis, nephritis, myocardial degeneration, haemoglutination, sub-pericardial and sub-endocardial haemorrhages and sub-cortical sub-pleural bleeding. Some important noticeable symptoms include diarrhoea, inability to keep normal structure, nose bleeding, depression and lateral recumbence. The most marked pathological changes were catarrhal enteritis, erosions of the intestinal mucosa,

congestion and haemorrahages in small intestines, heart and lungs and fatty changes in the liver and kidneys (Adam, 1974).

In another experiment, post-morten examinations showed lesions in the liver, pancreas and spleen, hyperaemia of the intestine, sometimes ascites. The whole picture resembled that of rats poisoned with ricin (Stirpe, *et al*., 1976). The seed meal from Cape Verde and Nicaraguan varieties have been found to be toxic to fish, rats and chickens while the seed from a Mexican variety was not. However, the seed meal of *J. curcas* must be detoxified because both toxic and nontoxic varieties have been found to be high in protein and potential exists for incorporating *Jatropha* seed meal in the diet of monogastrics, fish and possibly humans (Makkar and Becker, 1999).

Detoxification of *J. curcas* seed has been a long practice in the parts of the world where the seeds are eaten. The practice of boiling or roasting has been traditionally employed by Mexicans to detoxify the seeds before consumption (Adam, 1974).

With an LD_{50} of 471.17mg/kg, the seed extract can be said to be very toxic. The bark, leaf, fruit and wood are reported to contain hydrogen cyanide (HCN). Curcin is a poisonous protein in the seed of *J. curcas.* According to Makkar and Becker (1999), extraction with 80 – 90% aqueous methanol or ethanol (for toxic varieties of *J. curcas*) holds promise for detoxification of *Jatropha* seed meal. The extract giving the LD_{50} 471.17mg/kg is methanolic which suggests the poisonous principle curcin could be responsible for the high toxicity. Though, hydrogen cyanide is not ruled out. However, the activity of the extract at 10, 40 and 80 mg/kg is impressive and far below the LD_{50}. These doses are not high enough to cause toxicity. *J. curcas* seed could be a useful

chemotherapeutic agent provided it is active at a non lethal dose (Adam, 1974). However, continuous use may be dangerous.

Scientifically, heat treatments have been used to detoxify the seeds where moist heat treatment was found to be more effective than dry heat treatment. Aregheore, *et al*., (1998) investigated lectin activity of *J. curcas* seed using latex agglutination method. It was found that at 121^0C of moist heat treatment for 10 and 20min, agglutination of the latex beads was observed but after 30min agglutination was not observed and conclusion was drawn that lectins can be completely inactivated using moist heat treatment at 121^0C for 30min. Makkar, *et al*. (1999) in a study on rats and fish fed diets containing *J. curcas* meal concluded that feed intake and protein utilization in animals fed heated *Jatropha* could be due to in activation of trypsin inhibitor and lectin by heat processing (121^0C for 30min and 66% moisture).

These results lend credence to the folkloric use of heat to detoxify *J. curcas* seeds. The poisonous principle, curcin, could be said to be heat liable. The seeds of the non-toxic varieties are eaten without processing. However, the risk of chewing the seed directly from the wild should not be taken because the potential of any variety being toxic or not could be due to geographical factors or some genetically determined biophysical processes (Makkar *et al.,* 1998).

J. curcas seed methanol extract (10-80mg/kg) showed statistically significant ($P<0.05$) reduction in mean number of writhes induced by acetic acid. Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognised as flower pigments in most angiosperm families (flowering plants). However, their occurrence is not restricted to flowers but include all parts of the plant. Flavonoids

are known to inhibit pain perception (Okwu and Josiah, 2006). Found to exhibit strong activity against pain (comparable to paracetamol), *J. curcas* seed extract can thus be said to posses analgesic properties probably due to its flavonoid content as Dias *et al.* reported (1989). The antinocipeptic activity can thus be said to be dose dependent and comparable to the standard drug, paracetamol (Table 4.10).

The presence of saponins in the seed extracts justifies its folkloric use in treating skin infections.

The ethnobotanical survey helped in guiding the research in establishing scientific basis for its use as a pain reliever and a remedy for skin diseases such as eczema and ringworm. The use of *Jatropha* generally for traditional medicinal purpose is not new. The plant parts are used in traditional folklore medicine in Africa, Asia and Latin America (Chopra *et al*., 1956; Martinez, 1959; Burkill, 1994). Saponnins are said to possess anti-microbial and anti-inflammatory activities and have effects on serum cholesterol (Oshowole *et al.*, 2008). The presence of these chemical constituents in the seeds supports its use in traditional medicine.

For the highest dose, 80mg/kg extract, the petroleum ether fraction of the extract gave the highest reduction in mean number of writhes, followed by the methanolic extract and the aqueous fraction (Table 4.10). But, averagely, the methanol extract had the highest and more consistent reduction in number of writhes in all the dose levels. This could be because certain phytochemical constituents are more active in the presence of others. It is worth noting that the methanolic extract of *J. curcas* is richer in secondary metabolites which may be due to the solvent's high polarity, hence, its ability to extract more components (Aiyelagbe *et al*., 2007).

The TLC of both fractions revealed the petroleum-ether fraction to have a richer profile than the aqueous fraction. This could explain the higher activity of the petroleum ether fraction in reduction of mean number of writhes compared to the aqueous fraction.

The antimicrobial test showed that the ethyl acetate fraction of the seed extract has a more general inhibitory property on the test organisms at 200, 100 and 50mg/ml concentrations, although the methanolic extract and the n-butanol fraction showed inhibitory action against *E. coli* at lower concentrations (25mg/ml) compared to the Ethyl Acetate fraction. *Salmonella typhi* was only susceptible to aqueous, ethyl acetate and n-butanol fractions but showed resistance to the methanolic extract and petroleum ether fraction.

All the test samples were active against the fungus *Candida albicans*. The aqueous fraction and n-butanol fraction were active against the fungus at 25-200mg/ml while the methanolic extract, Ethyl Acetate and petroleum ether fractions were active at 50-200mg/ml concentration.

Seeds containing bioactive agents such as saponnins, terpenoids, steroids, cardenolides, tannins and phenolics, thus, readily present themselves as a good source of raw material in modern and traditional medicine. Steroids are known for body building and performance-enhancing; cardenolides have anti-tumour effects on prostrate and breast cancers; saponnins are said to possess anti-microbial and anti-inflammatory activities and have effects on serum cholesterol and; alkaloids, terpenoids, tannins and phenolics are said to exhibit strong biological activities (Reinhold, 1987; Yeh, *et al.,* 2003; Stenkvist, 1999; Stenkvist, 2001; Oshowole *et al.,* 2008). The presence of these arrays of

phytochemicals in *J. curcas* seeds suggests that the seed phytochemicals will find relevance in the field of orthodox and traditional medicine (Ejelonu, *et al.*, 2000).

CHAPTER SIX

6.0. SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1. Summary

The ethnobotanical survey on the seeds of *Jatropha curcas* led to the discovery that it is a traditional remedy against skin diseases, stomach upsets, sexually transmitted infections. It also serves as analgesic, purgative and laxative.

Phytochemical analysis of the seeds showed important range of phytoconstituents which include flavonoids, saponins, steroids and terpenoids. Thin Layer Chromatographic analysis of the methanolic extract, petroleum ether and aqueous fractions showed a rich array of substances that could be further isolated and characterised.

The LD_{50} of the methanolic extract was determined and found to be 471.17mg/kg. Although very toxic, the methanolic extract and the aqueous and petroleum ether fractions of the seed exhibited analgesic activity at lower dose levels (10mg/kg, 40mg/kg and 80mg/kg) comparable to the standard drug – paracetamol.

Antimicrobial activity of the methanol extract of the seed and partitioned fractions (petroleum ether, ethyl acetate, n-butanol and aqueous) were tested and results showed that the methanol extract and all the fractions have antimicrobial properties against the fungus *Candida albicans* and the bacteria species; *Escherichia coli, Salmonella typhi, Staphylococcus aureus and Streptococcus pyogens*. The susceptibility of the organisms was dose dependent while some were resistant to the extract and fractions.

6.2. **Conclusion**

The present study has established:

i. The ethnobotanical survey of the seeds of *J. curcas* shows that the seeds are used locally for medicinal purposes such as purgative, antimicrobial and analgesic. The most common use of the seed in traditional medicine was found to be antimicrobial, then analgesic and purgative. The seeds are also used in traditional medicine as an ingredient in concoction with other parts of the plant or parts of other plants.

ii. The phytochemical analysis of *J. curcas* (Linn) seed extract has shown it to contain flavonoids, saponins, tannins and steroids.

iii. The seeds of *Jatropha curcas* exhibited antimicrobial properties against of *Escherichia coli, Salmonella typhi* (gram –ve bacteria), *Streptococcus pyrogenes, Staphylococcus aureus* (gram +ve bacteria) and the fungus *Candida albicans* thus providing a scientific support for its use in traditional medicine.

6.3. **Recommendations**

The seed of *J. curcas* is indeed a good source from which medicines could be developed traditionally and pure compound could be isolated for pharmacological purposes.

In view of the above mentioned, the following points are recommended:

i. attention must be focused on research for the isolation and characterization of biologically active constituents from *J. curcas* seed.

ii. further researches on other pharmacological activities of the constituents of *J. curcas* seed should be made.

iii. research should be geared towards developing easy ways of differentiating toxic varieties from non toxic ones.

iv. deliberate measures should be put in place to raise plantations of the non toxic varieties *J. curcas* with the view to boost more researches on its medicinal importance of the seed and its use as food and protein supplement for both humans and animals.

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APPENDICES

Appendix A. Sample of Ethnobotanical Survey Sheet For *Jatropha Curcas* (Linn.) Seeds

A. Respondent's Information:

i. Name ……
ii. Occupation ……
iii. Age ……
iv. Sex ……
v. Location/Address ……

B. Seed Information:

i. Vernacular name(s) of the plant from which the seed is obtained ……
……
……
ii. Medicinal uses of the seed ……
……
……
iii. Method of collection ……
iv. Method of preparation ……
……
v. Name of preparation ……
vi. Mode of administration ……
vii. Dosage ……
viii. Efficacy: Very effective/ Moderately effective/ Weakly effective
ix. Side effect(s) ……
x. Level of patronage of the drug: High/ Average/ Scarce

C. Toxic Information:

i. Effect(s) of direct consumption of the seed by humans or animals ……
……
ii. Antidote ……

D. Miscellaneous Information:

i. Non-medicinal use(s) of the seed ……
ii. Spiritual/ Occultic significance of the seed ……

Appendix B. Acid Value and Saponification Value of *Jatropha curcas* Seed Oil

Acid value:

weight of oil = 10g

average volume of KOH used, v = 10ml

$$\text{Acid value} = \frac{10 \times 0.00561 \times 1000}{10} = 5.936$$

Saponification value:

weight of oil = 2g

average volume of acid used with oil, x = 68.60ml

average volume of acid used without oil, y = 89.80ml

$$\text{Saponification value} = \frac{(89.80 - 68.60) \times 0.2805 \times 100}{2} = 297.33$$

Appendix C. Calculation of LD_{50}

Table for mortality rate of animals in acute toxicity test

PHASE I		PHASE II	
Doses (mg/kg)	**Mortality**	**Doses (mg/kg)**	**Mortality**
10	0/3	140	0/1
100	0/3	225	0/1
1,000	3/3	370	0/1
		600	1/1

Acute toxicity or LD_{50} was calculated using the relation

$$a = \sqrt{cd}$$

Where

$a = LD_{50}$

c = highest non-lethal dose = 370mg/kg

d = Lowest lethal dose = 600mg/kg

Therefore

$$a = \sqrt{370 \times 600 \text{ mg/kg}} = 471.17\text{mg/kg}$$

Appendix D. Analysis of Variance (ANOVA) for Analgesic Activity Test

Crude extract

Group	I	II	III	IV	V	TOTAL
	25	10	16	5	1	
	12	10	13	2	10	
	16	8	17	11	3	
	27	6	10	3	1	
	17	7	5	8	3	
$\sum X$	97	41	61	29	18	246
$\sum X^2$	2043	394	839	223	120	3619
$\overline{X}$	19.4	8.2	12.2	5.8	3.6	

$$C = \frac{(\sum x)^2}{rt}$$

C = correction factor

r = number of animals per group = 5

t = number of groups = 5

$$C = \frac{(246)^2}{(5)(5)}$$

$$= \frac{60516}{25}$$

$$= 2420.64$$

Total sum of squares, total SS = $\sum X^2 - C$

= 3619 – 2420.64

= 1198.36

Treatment SS = $\frac{97^2 + 41^2 + 61^2 + 29^2 + 18^2}{5} - 2420.64$

= 774.56

Error SS = total SS – Treatment SS

= 1198.36 – 774.56

= 423.80

ANOVA Table 1: Analgesic activity of crude extract

Source of variation	df	Definition (SS)	Mean (MS)	F
Treatment	5	774.56	154.91	6.94
Error	19	423.80	22.30	
Total	24			

F_{table} = 2.74 at $P<0.05$

Aqueous Fraction

Group	I	II	III	IV	V	TOTAL
	25	10	6	5	8	
	12	10	21	14	6	
	16	8	10	12	4	
	27	6	12	16	7	
	17	7	22	13	7	
$\sum X$	97	41	71	60	32	301
$\sum X^2$	2043	394	1205	790	214	4646
$\overline{X}$	19.4	8.2	14.2	12.0	6.4	

$$C = \frac{(301)^2}{(5)(5)} = \frac{90601}{25}$$

$$= 3624.04$$

Total SS = 4646 – 3624.04

= 1021.96

$$\text{Treatment SS} = \frac{97^2 + 41^2 + 71^2 + 60^2 + 32^2}{5} - 3624.04$$

Treatment SS = 526.96

Error SS = 1021.96 – 526.96

= 495

ANOVA Table 2: Analgesic activity of the aqueous fraction

Source of variation	df	Definition (SS)	Mean (MS)	F
Treatment	5	526.96	105.39	4.04
Error	19	495.00	26.05	
Total	24			

$F_{table} = 2.74$

Pet-Ether Fraction

Group	I	II	III	IV	V	TOTAL
	25	10	13	10	1	
	12	10	16	10	2	
	16	8	11	5	6	
	27	6	14	6	5	
	17	7	15	9	3	
$\sum X$	97	41	58	40	17	253
$\sum X^2$	2043	394	818	324	75	3672
$\overline{X}$	19.4	8.2	11.6	8.0	3.4	

$$C = \frac{(253)^2}{(5)(5)}$$

$$= \frac{64009}{25}$$

$$= 2560.36$$

Total SS = 3672 – 2560.36 = 1111.64

Treatment SS = $\frac{97^2 + 41^2 + 58^2 + 40^2 + 17^2}{5}$ – 2560.36

= 708.24

Error SS = 1111.64 – 708.24

= 403.4

ANOVA Table 3: Analgesic activity of the petroleum ether fraction

Source of Variation	df	Definition (SS)	Mean (MS)	F
Treatment	5	708.24	141.65	6.66
Error	19	403.40	21.23	
Total	24			

T_{table} = 2.74

Appendix E. Calculation of Physico-chemical Parameters of *Jatropha curcas* seed

Moisture content

Weight of plant material = 3.00g

Initial weight of dish + powdered plant sample = 44.76g

Final constant weight of dish + sample = 44.62g

Loss in weight = 44.76g – 44.62g = 0.14g

$$\% \text{ Loss on drying} = \frac{0.14}{3} \times 100$$

= 4.67%

Total ash value

Weight of plant material = 2.00g

Weight of empty crucible = 36.94g

Final constant weight of ash + crucible = 37.09g

Weight of ash = 37.09g – 36.94g = 0.15g

$$\% \text{ Total ash value} = \frac{0.15}{2} \times 100$$

= 7.5%

Water soluble ash

Weight of crucible = 36.94g

Weight of water insoluble ash + crucible = 37.04g

Weight of water insoluble ash = 37.04g – 36.94g = 0.10g

% Water insoluble ash = 0.10/2 x 100

= 5.0%

Acid insoluble ash

Weight of empty crucible = 36.94g

Weight of acid insoluble ash + crucible = 37.00g

Weight of acid insoluble ash = 37.00g – 36.94g = 0.06g

$$\% \text{ Acid insoluble ash} = \frac{0.06}{2} \times 100$$

= 3.0%

Alcohol soluble extractive

Weight of empty dish = 59.82g

Weight of dish + extract = 60.00g

Weight of extract = 60.00g – 59.82g = 0.18g

$$\% \text{ Yield} = \frac{0.18x5}{5} \times 100$$

= 18%

Water soluble extractive

Weight of empty dish = 59.82g

Weight of dish + extract = 59.93g

Weight of extract = 59.93g – 59.82g = 0.11g

$$\% \text{ Yield} = \frac{0.11x5}{5} \times 100$$

= 11%