PHYTOCHEMICALS AND ORGANIC CONSTITUENTS OF JATROPHA CURCAS

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A DISSERTATION SUBMITTED TO BIOCHEMISTRY UNIT OF BIOLOGICAL SCIENCES DEPARTMENT, MOUNTAIN TOP UNIVERSITY, IBAFO, OGUN STATE IN PARTIAL FULFILLMENT FOR THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE BSc (HONS) IN BIOCHEMISTRY.

NOVEMBER, 2020

CERTIFICATION

This is to certify that this project **PHYTOCHEMICALS AND ORGANIC CONSTITUENTS OF JATROPHA CURCAS** has been carefully supervised and approved as adequate for the partial fulfilment of the award of Bachelor of Science, B.Sc. (Hons) in Biochemistry of Mountain Top University.

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DEDICATION

I dedicate this work to God Almighty, for the gift of wisdom and understanding and also to my parents for their relentless support and motivation. I'm also grateful to my supervisor for the effort he put in, towards the successful completion of this work.

ACKNOWLEDGEMENT

My vehement gratitude goes to my Lord and God, who in his infinite mercies granted upon me the wisdom and understanding for the successful completion of this project.

I sincerely appreciate the efforts of my supervisor; PROF. A.I. AKINWANDE, for the time, energy and commitment he rendered towards this project. I especially want to appreciate him for his teachings and training and other unquantifiable inputs.

I also recognise the efforts of our Head of Department in biological sciences, for his tremendous support. I'm also grateful to the Technologist of chemistry laboratory in person of Mr Ojo for his support and assistance towards the course of this project.

My utmost appreciation goes to my mum, for all the financial, physical, spiritual and material support, without forgetting her advice and the seeing the need for me to be educated, may heaven continue to reward and protect you.

And to my colleagues; Keku Tosin, Essien Glory, Oluwanishola Anjola, Akunne Precious for their understanding and support during the course of this project.

Finally, I appreciate the teachings and advice of my uncle; Mr Akin Abioye for his support and advice, not forgetting the assistance of everyone that helped me towards the completion of this work, God bless you all.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Jatropha Curcas is a multipurpose drought resistance plant that belongs to the family Euphorbiaceae. It has been known that this plants possesses phytochemicals and other chemical constituents which are used in traditional practices to treat various illness and disease; most of which hasn't been yet identified; Before proper utilisation of the sap can be achieved, the components present must be known This study focused on analysing and evaluating the presence of phytochemicals and organic constituents in the sap of *J. curcas*. The sap was collected quickly from the plant and placed in an ice bath. The sample was subjected to phytochemical screening, which confirmed the presence of some phytochemicals which include: Tannin, steroid, flavonoids, saponin, alkaloids, cardiac glycosides etc. Gas Chromatography-Mass Spectroscopy (GC-MS), as well as Nuclear Magnetic Resonance (NMR) was also performed on the sap, which revealed different organic and chemical constituents present such as; 4-Ethylbenzamide, Tetradecanoic acid, 9-Octadecenamide (Z), 2-Furancarboxaldehyde 5-methyl.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUNG OF STUDY

Nature is always a golden sign to show the prominent phenomena of coexistence. Natural products from plants, animals, and minerals are the basis for treating human disease (Ramana *et al*, 2014). Medicinal plants demand are currently high and there is great progression in the rate of acceptance. Undeniably, plants has significant role in the provision of important services in ecosystems. Living organisms including human could not live a normal life without plants. Nevertheless, herbals particularly medicinal herbs continuously act as a general indicator of ecosystem health (Perinchery, 2020). Medicinal plants have undoubtedly been considered by human beings since ancient times. It has been noted in the past that humans recognised and exploited the plants around them and used them for food, clothing, shelter and fuel, in so doing they became more cognisant of the properties.

Jatropha curcas according to Prasad (2012) is a "multipurpose, drought resistant, perennial plant" that belongs to the family Euphorbiaceae. It has attained lots of economic importance due to its utilisation in industry and medicine. *J. curcas* has been applied in folklore medicine to treat different types of infections and diseases. Several active biological compounds have been isolated and categorised by different scientists. It is been utilised for different purposes, hence considered as a multipurpose plant, useful for both veterinary and traditional medicine practises (Abobatta, 2019). It is commonly grown for hedges and fences, and has been used by traditional doctors to cure series of illnesses and diseases, which include yellow fever, syphilis, rheumatic pains, incontinence, dysentery, gonorrhoea, spasms of infantile tetanus, carious teeth and to cuts and wounds as an antiseptic agent. The sap is produced abundantly, which is cloudy in appearance and soapy to touch. The chemical components present in *Jatropha curcas* seeds are pinhoen oil, resins,

toxalbumin, and curcin, and the leaves and latex contains sulphur oil and ricinoleic acid. However, the mechanism of action of the plant extracts is unknown (Akinwande *et al*, 1998).

"Jatropha curcas latex is stored in the laticifer tissue of approximately 10% of all angiosperms and is typically exuded as a white sticky sap upon physical tissue damage to the plants" (Konno, 2011). The latex contains numerous metabolites which are secondary like isoprenoids (terpenoids, rubber, cardenolides, etc.) (Heldt *et al*., 2011), phenolics alkaloids and proteins such as peptides, proteases, protease inhibitors, lectins, chitinases, and oxidases. Knowledge derived from studying these proteins, enzymes, peptides and hydrocarbons (products of enzyme) is the first step to pharmaceutical developments from the plant latex and a better understanding of the latex's biological function (Cho *et al*., 2009).

The latex-rich shrub *Jatropha curcas* is fast becoming a prevalent biodiesel crop because of its easy propagation, marginal land adaptation, rapid growth, short gestation period, drought resistance and endurance and avoidance by herbivores (Moniruzzaman *et al*., 2016). The two prevalent means of extracting broad and representative populations with fewer contaminants from plant samples are phenol extraction and TCA/acetone extraction; samples from plant have low proteins and high proteases, oxidative enzymes, saccharides, and commonly contaminants are the secondary metabolites (Isaacson *et al*., 2006),which may greatly hinder the isolation of high quality proteomic samples.

According to Kumar and Tewari (2015), the origin of 'Jatropha' is from Greek words 'jatros' and 'tropes' (nutrition) which implies medicinal uses. It is commonly known as physic nut, purging nut, Barbados nut and nutmeg plant in English. Other vernacular names of *J. curcas* are pourghere (French),

The parts of the medicinal plants that may be used are different types of seeds, root, leaf, fruit, skin, flowers or even the whole plant. The active compounds in most parts of medicinal plants like *Jatropha Curcas*, *Eugenia Caryophyllus* etc have direct or indirect therapeutic effects and are used as medicinal agents. In the body of these plants, certain materials are produced and stored that are referred to as active compounds (substances), which have physiological effects on the living organisms (Oskoueian, *et al*, 2011). The use of plant raw materials by humans is mainly utilised as medicinal herbs, needed to maintain health and cure diseases (Djordjevic, 2017). Plants that have medicinal benefits are used for treatment because they have certain properties, including

synergistic actions. There might be possible interaction between the components of the plant with potential beneficial or adverse effect to each or eradication of the unsafe effects to both. Compounds derived from plants may improve radically difficult illnesses such as cancer. Another characteristic of plant constitutes is that they have the capacity to avert progression of some illnesses. Orthodox and allophatic medicines toxicity and side effects plays a significant role in the speedy rise in the demands and number of manufactures of herbal drugs and also decrease in the use of chemical drugs (Rasool Hassan, 2012). As time goes on there was important discoveries regarding the use of some medicinal plants for treatment of some illnesses, therefore, facts become accepted and the empirical framework concerning usage of these plant was slowly withdraw. The first written evidence regarding usage of medicinal plants for drug preparation was discovered on a Sumerian clay slab from Nagpur dated over 5000 years ago (Qiu, 2007). Schippmann et al (2006) indicated that the first human to use plants as medicine before 27BC are the Egyptians and Chinese. Additionally the Greek are familiar with the medicinal properties of some plants hence, Hippocrates, founder of medicine and Aristotle used medicinal plants for treatment of illnesses.

The last couple of years have brought about numerous uses of *J. curcas* such as utilisation of the oil of the plant for biodiesel production rather than using edible crops (Henning, 2004). It is also relevant in reducing soil erosion in arid regions, and also thought to be drought resistant, possessing great yield potential, hence enabling it to thrive well under salinity and on marginal lands. Farmers has integrated *Jatropha* into unfertile areas as a favourable renewable energy crop.

1.2 STATEMENT OF THE PROBLEM

The objectives of the work is to explore the different phyto-chemical properties of the sap of *Jatropha curcas* at the site of collection. This can be divided into the following sub-problems:

1. Description of plant (*Jatropha curcas*) and its importance for economic development and its uses in medical practises.

2. To analyse the phytochemicals present in the sap of *Jatropha Curcas*.

3. Determination of the organic constituent present in the sap.

4. To understand the chemical structure of some compounds present in the sap.

1.3 AIM OF STUDY

To identify the phytochemicals and organic constituents present in the sap of *J.curcas*, using different methods.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OVERVIEW OF *Jatropha curcas*

Jatropha curcas according to Abdelgadir and Van Staden (2013) is a plant with multiple purpose that have the potential for production of biodiesel and medicines. Recently, a lot of interest has been shown on the seed oil for biodiesel production and this has encouraged cultivation of the plant on a large scale. It has been forecasted that millions of hectares will be used for *J. curcas* cultivation (Devappa *et al.,* 2010). Different studies has demonstrated the effectiveness of *Jatropha* covering various aspects for example, phytochemistry, medicinal properties, and pharmacology (Sharma and Singh, 2012). Taproot and four shallow lateral roots makes up the root system (Rodriguez *et al*., 2013). The flowers are in axillary clusters with a stalk of 3-5 cm long, thickly pubescent, yellowish-green, with prominent glandular discs. Male flowers have 5 ovate-elliptic sepals, less than 4 mm long and 5-oblong-obovate petals united in the lower half and hairy inside, 6-7 mm long, 8 stamens. Female flowers has oblong petals, large sepals and 4 mm long (Abdelgadir *et al., 2009*). Fruits are ovoid capsules 3-4 cm long, slightly trilobite, splitting into three cells. The seeds are three per fruit, large, oblong, 2 cm long and sweet tasting (Kochhar *et al.,* 2008).

2.1.1 TAXONOMY OF *Jatropha curcas*

According to ITIS standard report, the taxonomy of *Jatropha curcas* is shown in the Table 2.1 below:

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida

Order	Rosanae
Family	Euphorbiaceae
Genus	Jatropha
Species	Curcas

2.1.2 ECOLOGY, DISTRIBUTION AND HABITAT of *jatropha curcas*

The origin of *J. curcas* is centred around the North-eastern part of South America and desiccated regions of Mexico (Makkar *et al., 2009*). It is cultivated presently in abundance in many humid and sub-tropical Africa and Asia (Schmook *et al.*,1997). *J. curcas* grows under hash conditions where the temperature ranges between 15 and 40℃ (Kumar and Sharma. 2008).The plant grows well in well drained soils with good aeration and is well adapted to marginal soils with low nutrient content (Openshaw, 2000).

2.1.3DESCRIPTION

J. curcas is a dedicious oilseed shrub. Its genus is of the tribe *Joannesieae*, as a member of *Crotonoideae*, it belongs to the family Euphorbiaceae (Divakara *et al.,2010*). The leaves are green, thick, and it has a length of about 8.55cm, with a width of about 5cm, with heart shaped and long neck that reaches up to 11cm long. *J.curcas* possesses small greenish yellow flowers, and the fruits at ripening gives a yellow brown coloration, while at first stage the fruits are green; 2-3 oval black seeds are found in the fruits of *J.curcas*, that possesses a high oil content (about 32% - 40%) depending on the growing conditions and genotype (Kaushik, 2003).

2.1.4 FLOWERING AND POLLINATION

Jatropha curcas is a hermaphroditic plant that contains both the male and female flowers, in which the male flower surrounds the central female flower. It produces small greenish yellow flower usually bisexual, except a few male flowers. All the flowers can be opened at the same time, therefore cross-pollination can occur between flowers of the same plant or from other plants. Weather and temperature varies from country to country; In Egypt, *Jatropha curcas* flowering time is twice a year, firstly in April and secondly in December (Soliman *et al.*, 2015).

2.1.5 FRUITING AND SEED MATURITY

Jatropha curcas according to Abobatta (2019) produces fruit that are approximately 2.5cm long and contain three black seeds. It reaches full size after the completion of 90 days from pollination date approximately. After poliination the total periods can be divided into 30-45 days. Maturing of yellow fruits starts between 45-60 days however, ripening of the fruits begins after 60 days approximately.

2.2 *JATROPHA CURCAS* SEX TYPES

J. curcas possess three sex types of flowers which can be produced by similar or unlike plant, i.e., male, female and hermaphrodite. Hence, every *jatropha* plant probably possess a unique sex type which could be dioecious, hermaphrodite, monoecious, gynoecious, gynomonoecious, androecious, andromonoecious, or heteroicous. Plants that have both male and female sex organ produces alike plants with both male and female sex types in flower. Hermaphroditic plants produces male and female flowers, while dioecious plants produces male and female flowers on separate plants (Miller and Diggle, 2007). Sex-types discovered on *jatropha* are monoecious, heteroicous, and andromonoecious. Flowers containing each sex type affects pollination and also ramblingly upsets the construction of the seed of *jatropha*. The ones that possesses hermaphrodite flowers are favourable to yield, because they are capable of undergoing self-pollination (Hartati 2009).

2.3 CONSTITUENTS OF *JATROPHA CURCAS*

Jatropha species are rich source of phytochemicals such as lignans and cyclic peptides alkaloids. Alkaloids and proteins are present in appreciable amount in *J. curcas*; amino acids, carbohydrates, steroids, phenolics, flavonoids, tannins, diterpenes are also present in appreciable amount in diverse sections of the plant (Abdelgadir *et al.,* 2013). Abdelgadir *et al.,* 2013 stated the components present in different parts of *Jatropha curcas* plant in his study, it is shown below;

Table 2.2: List of constituents present in various parts of *J. curcas* (Abdelgadir *et al.,* 2013)

NO	Categories	Phytoconstituent	Plant part
1	Diterpene	Riolozatrione, acetoxyjatropholone.	Roots.
2	Phenolic	Caffeoylaldehyde, Syringaldehyde, 3-Hydroxy-4-methoxybenzaldehyde, 3-Methoxy-4-hydroxybenzoate.	Seed cake and root.
3	Proteins	Aquaporins, which is a functional protein.	Latex
4	Liganans, neoliganans, coumarins, coumarino-lignoids	Isoamericanin, isoprincepin, 6-Methoxy-7-hydroxycoumarin, marmesin, propacin, jatrophin,scopaletin, 5-Hydroxy-6,7-dimethyoxy coumarin.	Seed cake, root,aerial
5	Flavonoids	Nobiletin, Tomentin, flavonoid glycoside II, flavonoid glycoside I.	Root and aerial
6	Sequiterpenoids and triterpenes	Friedelin, taraxasterol, (z)-3-0-coumaroyloeanolic, β-amyrin, β-sitosterol, stigmasterol, daucasterol.	Latex, stem and root.
7	Alkaloids	Pyrimidine-2,4-dione, Diamide (curcamide), Imidazole (4-butyl-2-chloro-5-formyl-1H-imidazole),	Leaf, seed cake, root.

2.4 GENERAL USES OF *JATROPHA CURCAS*

Jatropha curcas is a plant with multiuse purposes which has shown so many importance in parched and humid regions as biofuel plants (Gour 2006). *Jatropha curcas* seeds can be made to form press cake which is considered as a biomass feedstock to produce energy or biogas. The press cake rich in protein is solely utilised as fertilizer. (Augustus *et al., 2002)*. Also powdered seed coat

derived from *J. curcas* is been utilised as an adsorbent for heavy metals like mercury exclusively from wastewater (Hsu *et al.*, 2014). Furthermore it is applied to stop soil erosion and as desertification plant. Hedges can be built from *J. curcas* because of its unpleasant taste to animals (Corte-Real *et al*., 2016).

It is also used to soften leather and lubricate machinery (e.g. chain saws). Availability of seed cake is utilised as a fuel for steam turbines to generate electricity. Apart from its relevance in bio-diesel application, the oil is utilised in cosmetic industries, for the manufacturing of soaps, detergent and candles. The extraction of the biodiesel after transesterification of the seed oil, leads to two main by-products that is glycerol and press or oil cake. Glycerol has numerous application in the industry, and functions as a substantial material in the synthesis of 1, 3 propane-diol and other polymeric materials (Sharma, 2008). The bark of *J. curcas* contains tannin. It also have the honey production potential as the flowers can attract bees

2.5 PHARMACOLOGICAL INFORMATION OF JATROPHA CURCAS

1. Anticancer activity

J. curcas is utilised in the managing and dealing with specific types of cancer in some part of Mexico (Alonso-Castro *et al*.,2011). Secondary metabolite such as Diterpenes can be synthesised by *J. curcas*, and these compounds has shown great potential in the inhibition of cytotoxin and tumor (Devappa *et al,* 2011). Protein with anticancer activities have been isolated, and subjected under study for many years. Ribosome-inactivating proteins (RIP) was isolated and identified by Stripe et al in 1976, and these proteins has proven to be a potential cell-killing agent that can inhibit cell-free protein synthesis.

2. Analgesic activity

Uche and Aprioku (2008) reported pain-relieving action of methanolic extract from J.curcas leaves in mice using the acetic acid-induced writhing test. The methanolic extract triggered momentous decline in the amount of acetic acid-induced writhing in mice compared to the pain-relieving outcome obtained from the reference drug paracetamol. Yusuf and Maxwell (2010), showed studies in the pain-relieving action of the methanolic leaf extract derived from J. curcas by means

of hot plate and acetic acid-induced wriggling impulse in mice as well as tail flick or dipping methods in rat. In hot plate and tail flick models, oral administration of the leaf extract at doses of 100, 200 and 400 mg kg−1 and the reference drug acetylsalicyclic 400 mg Kg−1 showed a potent analgesic effect by increasing the pain time dose dependent in mice and rats. In the acetic acid-induced wriggling response model, the extract decreased the number of abdominal contortions.

3. Wound healing activity

Folklore practices makes use of the numerous portions of J. curcas for wound healing in many parts of the world (Balangcod and Balangcod, 2011). Shetty *et al,* (2006) assessed the activity of crude bark extract of J. curcas in wounding healing of wistar albino rats. The extract precipitated the therapeutic progression by speeding up the wound shrinkage, granulation tissue breaking strength, skin breaking strength and parched granulation tissue mass and hydroxyproline levels.

4. Antidiabetic activity

Traditional preparation of boiled leaf decoctions (Jaiswal, 2010) or fruit burnt into ashes (Gbolade, 2009), water extract derived from the bark of *J. curcas* (Jayakumar *et al.*, 2010) are used for regulating blood glucose elevation in the body.

5. Hepatoprotective activity

(Balaji *et al,* 2009b) assessed the methanolic fractions from *J. curcas* leaves against hepatocellular carcinoma activated by aflatoxin B by oral administration in rats at prescribed amount of 100 and 200 mg/kg. The methanolic fractions reduced the levels of increased serum enzymes, lipid levels and bilirubin and elevated the levels of protein and uric acid. Methanolic fractions decreased the incidence of liver lesions, lymphocytic infiltrations and hepatic necrosis induced by aflatoxins.

6. Anti-inflammatory effects

Methanolic extract derived from the bark of *J. curcas* leaves exhibited anti-inflammatory effects on wister albino rats (Uche and Aprioku, 2008). Alcoholic extract derived from the leaves, stems, branches and leaves of *J. curcas* showed substantial anti-inflammatory action in severe carrageenan-induced rat paw edema (Nayak and Patel, 2010a). The extract from the leaf, stem bark, latex and root of *J. curcas* showed anti-inflammatory effect that is ascribed to their resilient iNOS inhibition (Oskoueian et al, 2011b).

7. Antimicrobial activity

Extracts from the diverse portions of *J. curcas* display antimicrobial activity. Namuli *et al*, (2011) reported microbial growth inhibition action of extracts derived from *J. curcas* such as methanolic and hexane extracts. According to Obasi *et al*, (2011), extract derived with methanol in adding to extract from the stem bark (chloroform, ethyl acetate and methanol) as well as extracts from the roots of *J. curcas* revealed a varied array of antimicrobial activity against specific microorganism responsible for sexual transmitted diseases. The seeds of the plant also showed antimicrobial activities.

8. Toxicity

Toxic and antinutritional components:

Toxic compounds are found in *J. curcas* as well as antinutritional compounds. The greatest lethal phytochemical that is contained in *J. curcas* are curcin and phorbol esters (Devappa *et al*, 2010a). Toxic components that can be found in the seeds include: phorbol esters, antinutritional phytate and the trypsin inhibition factor.

2.6 MEDICINAL IMPORTANCE OF JATROPHA

According to Bekalu (2020), different biologically active compounds have been identified, isolated and characterised from different sections of the plant. The mechanism of the plant action is considered in relationship to the numerous applications of *Jatropha curcas* in folklore practices. Chemical constituents such as phorbol esters, are accountable for the noxiousness of *J. curcas* to humans and animals respectively. It is useful for medicinal purposes in ancient times. The plant exhibit anti-inflammatory, antidiarrhoeal effect, antimetastatic, wound healing , insecticidal, coagulant, antitumor and anti-coagulant (dose dependent), disinfectant, antiparasitic, wound healing, pregnancy terminating activity. The foremost component of the plant are curcusone-B, curcain, curcin etc. Apart from these constituents, the plant also contains a toxic substance such as tetramethylpyrazine (TMPZ).

2.6.1 Roots

The roots of *J. curcas* are used to produce antidote for snake bites. The roots are used in decoction as mouth wash for bleeding gum and tooth ache as well as for eczema, scabies and ringworm. *J. curcas* roots is also utilised in curing diarrhoea, which is a common ethnobotanical practice in Konkan, a part of the Western coastal area of India. The roots are been used for pharmacognostic studies and estimation of antidiarrhoeal action in albino mice. Successive extraction of methanol fraction exhibited some activity counter to castor oil induced diarrhoea and intraluminal build-up of liquid. There is reduction in gastrointestinal motility after charcoal prepared meal administration in mice. The results indicated that the action of the extract containing methanol could be done by an arrangement of inhibition of elevated prostaglandin biosynthesis and abridged propulsive mobility of the small intestine (Bekalu 2020).

2.6.2 Latex

The sap of *J. curcas* exhibit anti-inflammatory activity in the action of wound healing process in the skin of mice (Muhammad Nur Salim *et al.*, 2018). The latex can also be used to cure alopecia, anasora, burns, dropsy, eczema, paralysis, yellow fever and inflammation (Heller, 1996). Its latex also contains alkaloids (jatrophine, jatrophen, jatrophore and curcain) which are anti-carcinogenic.

2.6.3 Stem

Urinary infections is cured using a young plant stem; also the tender twig can be used as a tooth brush to clean the teeth or used as toothpick. It is also used in treating gum problem (Bekalu *et al*., 2020).

2.6.4 Seed

The seed as well as the fruit are used as contraceptive in South Sudan. *J. curcas* is abortifaceous, anodyne, antiseptic, cicatrizant, diuretic, hemostat, depurative, lactagogue, purgative, narcotic and rubefacient. Folklore practises makes use of the seed to treat convulsion, dysentery, eczema,

cough, dermatitis, diarrhoea, dropsy, fever, paralysis, rashes, stomach aches, sores, yellow fever, uterosis, ulcer, tetanus, whitlows and erysipelas. The plant is rich in numerous phytochemicals such as vitex (bark), xylose (seeds), curcusones (white plant), sovitexin (leaf) (Bekalu *et al*., 2020).

2.6.5 Plant bark

The extracts from *J. curcas* activated the therapeutic progression by enhancing the skin breach strong point, granulation tissue weight and hydroxyproline levels. An important decrease in epithelisation period was also observed. The histopathological examination of granulation tissue expressed more advance phase of healing, with more collagen, which has organised to form bundles (Shetty S. *et al*., 2006).

2.7 Industrial application of *J. curcas*

J. curcas is a promising plant because of its usefulness and profitable by-products. The oil derived from the plant is used for different purposes and apart from its importance in biofuel production, the oil is utilised in the manufacturing of medicine, fertilizer, pesticides and soap (Benge 2006). It also serves or being implemented in the removal of adequate chemical and catalysts.

2.8 Social and environmental importance of *J. curcas*

Jatropha is implemented into rural development approach. By planting *jatropha*, hedges formed helps in securing parks and farming areas against roaming animals. The oils derived from the seeds are utilised in soap making, for igniting and food preparation purposes, as well as its uses as a source of fuel in specialised biodiesel engines. Even though the ideal environmental circumstances for *jatropha* manufacturing is found in warm sub-humid tropics and subtropics, *J. curcas* still has the capability to thrive in arid regions on degraded soils that have little or no potential availability of nutrients for agricultural purposes makes it especially attractive. It serves as a means to control soil erosion and to improve water infiltration as well as for livestock fees. Hence the *jatropha* scheme covers four main broad features of rural improvement which includes:

- Upgrade of women (indigenous or native soap making);
- Scarcity and decrease of products (preserving crops and other plant produce).
- Prevention of erosion (embedding hedges);

- Establishment of immobile engines and generation of energy for household and rural communities.

J. curcas is certainly a highly interesting plant, with significant uses, particularly as biofuel in helping to combat the energy crisis throughout the world and produce revenue in rural regions of unindustrialized countries. It has become globally competitive due to the fact that it belongs to a non-edible category and does not compete with food.

2.9 Latex sample collection

The plant sample in form of sap was provided by my supervisor Prof. A.I. Akinwande, which was placed in an eppendorf tube.

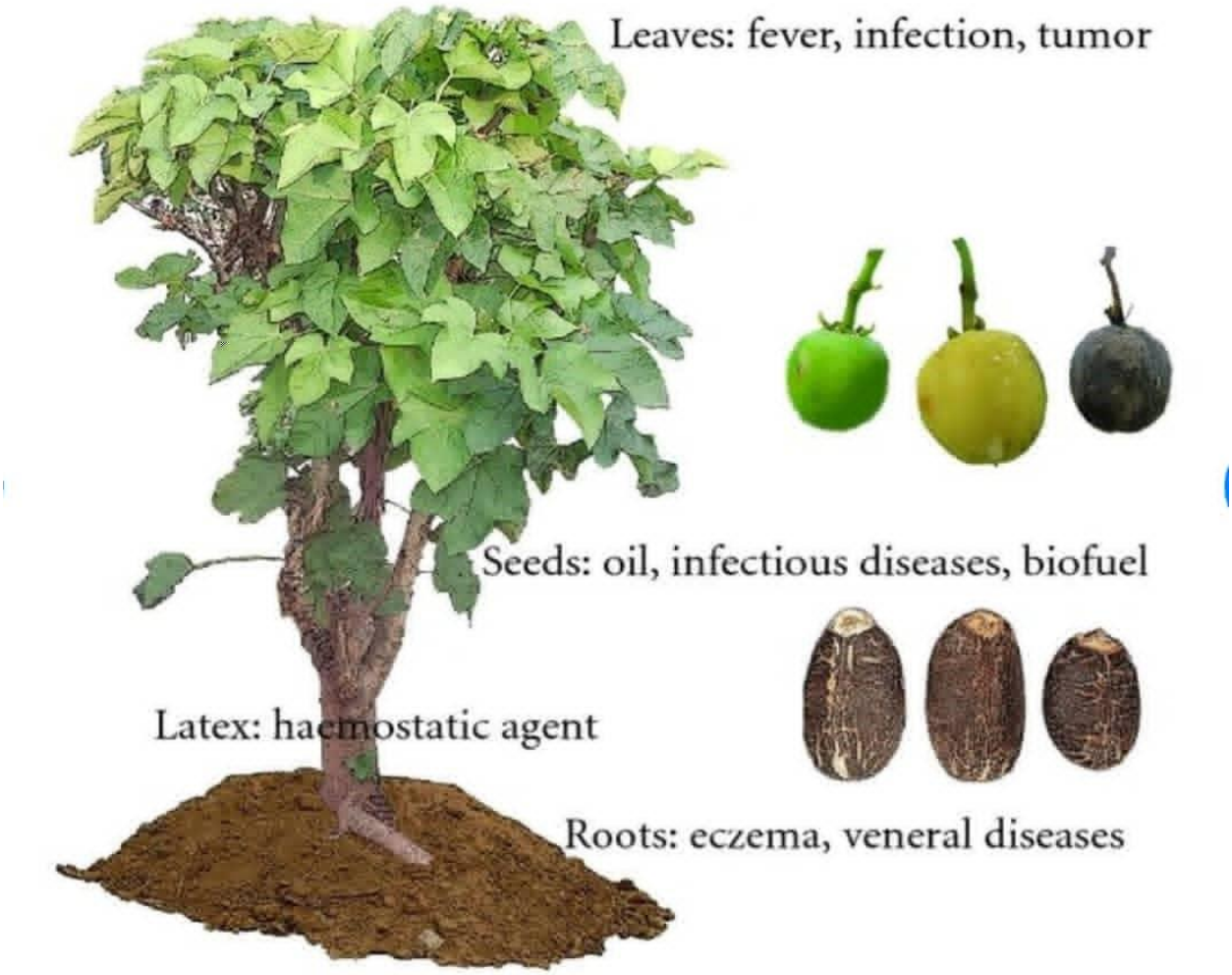


Fig 2.0: Diagram of *J. curcas* and uses

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3.1 MATERIALS

The instruments, scientific equipments used in the course of this study are as follows:

Refrigerator; Centrifuge; Filter paper; Analysis bottle; Beakers, Volumetric flask; Measuring cylinders; Funnels, Stirring rod; Spatula; Test tubes; Test tube racks; Pipette; Wash bottle.

The following are some chemicals used:

Chloroform; Sulphuric acid, Wagner's reagent (iodine in potassium iodide); Sodium Hydroxide; Hydrochloric acid; Absolute alcohol; Ferric chloride; Potassium Hydroxide; Acetic acid; Acetic Anhydride; Ninhydrin reagent; α-naphthol; Ether; Ammonia.

3.2 EXPERIMENTAL PLANT

The sap of *Jatropha Curcas* which is the experimental portion of the plant was used in the study and it was obtained taken just outside Mountain Top University, Ogun State premises. The sample was provided by my supervisor, which was placed in an eppendorf tube and placed in a refrigerator.

3.3 METHODS

3.3.1 PHYTOCHEMICAL ANALYSIS

PRINCIPLE

In quantitative analysis, the phytochemicals present in the sap can be tested to indicate their presence using different methods. The findings from quantitative phytochemical screening is a good way to identify the existence of different phytochemicals present in the sample.

PROCEDURE

3.31.1 Test for Flavonoids:

Alkaline reagent test: 2mL of the sample (sap of *Jatropha Curcas*) were treated with few drops of NaOH solution and observed for strong yellow pigmentation which vanished on the accumulation of hydrochloric acid that were been added (Sharma *et al.,* 2013).

3.31.2 Gum and Mucilage test:

The plant extract (sap) were dissolved in 20mL of distilled water and to this; 50ml of absolute alcohol were added with constant stirring (Sarma.and Babu, 2011).

3.31.3Test for Tannins:

The test sample was placed in a test tube and treated with 15% ferric chloride test solution. The subsequent colour was naked (Odebiyi and Sofowora, 1978).

3.31.4 Test for Alkaloids:

5mL of the test sample were dissolved in 5mL of chloroform and the solution were extracted with 2mL of diluted sulphuric acid and an acid layer was taken.

Wagner's test: To the 2mL of acid layer of test solution, 2mL of wagner's reagent were added (Gibbs, 1974).

3.31.5 Test for Cardiac glycosides:

Keller-killian's test: 0.5mL of glacial acetic acid were dissolved in 50mL of test solution containing one drop of ferric chloride solution. This was then under layer with 0.5mL of concentrated sulphuric acid (Aiyelaagbe and Osamudiamen, 2009).

3.31.6 Test for Terpenoids:

Salkowshi's test: The test sample was pipetted up to 1mL mark and poured into a test tube, after which 4mL of chloroform were added. 6mL of concentrated sulphuric acid were then carefully added to produce a layer (Sofowora, 1982).

3.31.7 Test for Phenols:

Ferric chloride test: 5mL of the test sample were added to an alcoholic solution, which was then added to 1mL of distilled water, then a small number of drops of 10% aqueous ferric chloride solution was added (Chandrashekar and Rao, 2013).

3.31.8 Test for Steroids:

Liebermann Burchard test: 2mL of glacial acetic acid, 2mL of acetic anhydride and three to four drops of concentrated sulphuric acid were added subsequently to 2mL of the test sample (Seema and Parwez, 2011).

3.31.9 Test for Protein and Amino acid:

Ninhydrin test: 2-3 drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) was added to a small quantity of the test sample and was heated (Mamta and Jyoti, 2012).

3.31.9.1 Test for Carbohydrates:

Molisch's test: 2mL of α-naphthol solution, concentrated sulphuric acid were poured through the sides, gradually into the solution in the test tubes and 4mL of the test sample were added.

3.31.9.2 Test for Fatty acid:

10mL of the test sample were mixed with 10mL of ether. The extract collected from the mixture was allowed to vaporise on filter paper and the filter paper was dried (Sarma and Babu, 2011).

3.3.2 GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS

PRINCIPLE

GC-MS is the coming together of two different analytical techniques, Gas Chromatography (GC) and Mass Spectroscopy (MS), is importance in the determination of complex organic and biochemical mixtures (Skoog *et al.*,2007). The GC-MS instrument is made up of two main components. The GC portion splits various compounds from a given sample based on their volatility; hence an inert gas which is the mobile phase carries the sample through a stationary phase. The MS portion determines and quantifies chemicals present according to their mass-to-charge ratio (m/z).

PROCEDURE

The analysis was performed using a 7820A gas chromatography coupled to 5975C inert mass spectrometer (with triple axis detector) and electron impact source (Agilent Technologies). The stationary phase of separation of the compounds was carried out on HP-5 capillary column coated with 5% of phenyl methyl siloxane.

The carrier gas was helium used at a constant flow rate of 1.573ml/min, an initial nominal pressure of 1.9514 psi and at an average velocity of 46cm/s. One microliter of the sample were injected in splitless mode at an injection temperature of 260 0C. Purge flow was 21.5ml/min at 0.50 min with a total gas flow rate of 23.355ml/min, gas saver mode was switched on.

The oven was initially programmed at 60 0c (1min), then ramped at 4 0c/min to 110 0C (3min), followed by temperature program rates of 8 0C/min to 260 0C (5min) and 10 0C/min to 300 0C (12min). Run time was 56.25min with a 3min solvent delay.

The mass spectroscopy was operated in electron-impact ionisation mode at 70eV with ion source temperature of 230 0C, quandrupole temperature of 150 0C and transfer line temperature of 280 0C. Scanning of likely organic compounds was from m/z 30 to 550 amu at 2.62s/scan; scan rate was recognised by relating the measured mass spectral data analysis with in NIST 14 mass spectral library.

3.3.3 NUCLEAR MAGNETIC RESONANCE ANALYSIS

PRINCIPLE

The nuclei present in all elements carries either a positive charge or a negative charge. When the spins of the protons and neurons comprising these nuclei produces a magnetic dipole along the spin axis, and the intrinsic magnitude of this dipole is a essential nuclear property called the nuclear magnetic moment.

PROCEDURE

IH NMR spectra was performed with a thermoscientific FOURIER 300 spectrometer operating at 3000000 MHz. Solutions were prepared using deionised water.

CHAPTER FOUR

4.0 RESULTS

4.1 SUMMARY OF PHYTOCHEMICAL ANALYSIS

Table 4.1 below shows the summary of the phytochemical analysis performed on the sap of *J. Curcas*.

Table 4.1: Phytochemical analysis result of sap of *J. curcas*

NO	Phytochemical Test	Result
1	Test for Flavonoid	+
2	Gum and Mucilage Test	+
3	Test for Tannin	+
4	Test for Alkaloid	+
5	Cardiac Glycoside Test	+
6	Test for Terpenoid	+
7	Phenol Test	+
8	Protein and Amino Acid Test	–
9	Test for Carbohydrate	+

10	**Fatty Acid Test**	**+**
11	**Test for Steroid**	**+**

4.2 SUMMARY OF GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS

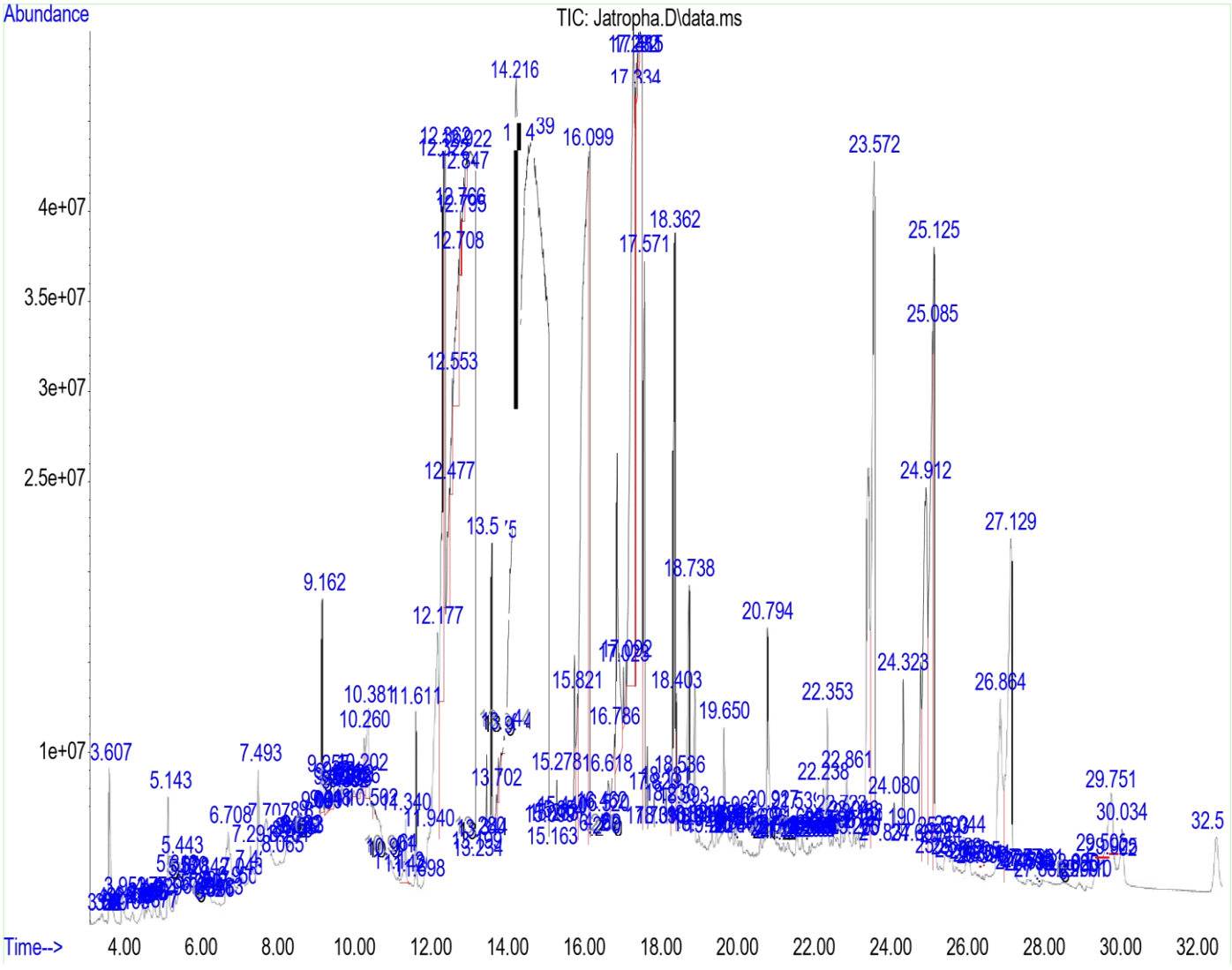
Table 4.2: Identification of compounds in the SaP of *J.curcas* by GC-MS

No	Retention time	Name of compound	Molecular formula	Peak Area (%)	Molecular weight
1	5.143	2-Furancarboxaldehyde, 5-methyl-	C_6H_6O2	0.20	110.1106
2	5.443	2-Hexanol, TMS derivative Silane, dimethyl (methylsilyl)	$C_9H_{22}Si$	0.60	174.3559
3	6.708	Pyrazine, 2-methoxy-6-methyl-	$C_6H_8N_2O$	0.46	124.1405
4	7.493	4-Ethylbenzamide, 4-Ethylbenzoic acid	$C_9H_{11}NO$ $C_9H_{10}O_2$	0.44	149.19 150.17
5	9.162	2-Undecanone	$C_{11}H_{22}O$	0.67	170.2918
6	10.381	Boronic acid, ethyl-, bis(2-mercaptoethyl ester)	$C_6H_{15}BO_2S_2$	1.66	194.1
7	13.55	Tridecanoic acid, 12-methyl-, methyl ester Methyl tetradecanoate	$C_{15}H_{30}O_2$	0.47	242.3975

8	14.216	Tetradecanoic acid n-Hexadecanoic acid	$C_{14}H_{28}O_2$ $C_{16}H_{32}O_2$	6.75	228.370 256.43
9	15.278	Pentadecanoic acid Tetradecanoic acid	$C_{15}H_{30}O_2$ $C_{14}H_{28}O_2$	0.05	242.4 228.370
10	18.362	Tetradecanoic acid, 2,3-dihydroxypropyl ester Tetradecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{17}H_{34}O_4$ $C_{17}H_{34}O_4$	1.57	302.449 302.45
11	18.738	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	0.62	281.476
12	19.650	Hexadecanoic acid, 2-hydroxy-1-(hy droxymethyl) ethyl ester Oxacyclododecan-2-one	$C_{19}H_{38}O_4$ $C_{11}H_{20}O_2$	0.27	330.503 184.278
13	20.794	2,3-Dihydroxypropyl elaidate 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	0.43	356.539
14	22.353	1,3-Dicaprin Carbamic acid, N-(3-chloro-4-methoxyphenyl)-,	$C_{23}H_{44}O_5$ $C_{11}H_{14}CLNO_3$	0.23	400.6 243.68
15	22.861	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	0.22	416.68
16	23.572	Dodecanoic acid, 1-(hydroxymethyl) -1,2-ethanediyl ester	$C_{27}H_{52}O_5$	2.01	456.699
17	24.328	Stigmasterol	$C_{29}H_{48}O$	0.33	412.69

18	25.125	2,4-Thiophenedicarboxylic acid, amino-3-methyl-, diethyl ester	$C_{11}H_{15}NO_4S$	2.78	257.31
19	26.864	Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{17}H_{34}O_5$	0.88	302.45
20	27.129	Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{17}H_{34}O_5$	1.64	302.45
21	29.751	9-Octadecenoic acid, (E)-Oleic Acid	$C_{18}H_{34}O_2$	0.71	282.46
22	30.034	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	0.29	625.032

Figure 4.2: GC-MS graphical representation analysis on the sap of *J curcas*.



4.3 NUCLEAR MAGNETICR ESONANCE ANALYSIS

Table 4.3 1H NMR chemical shifts of *Jatropha curcas*

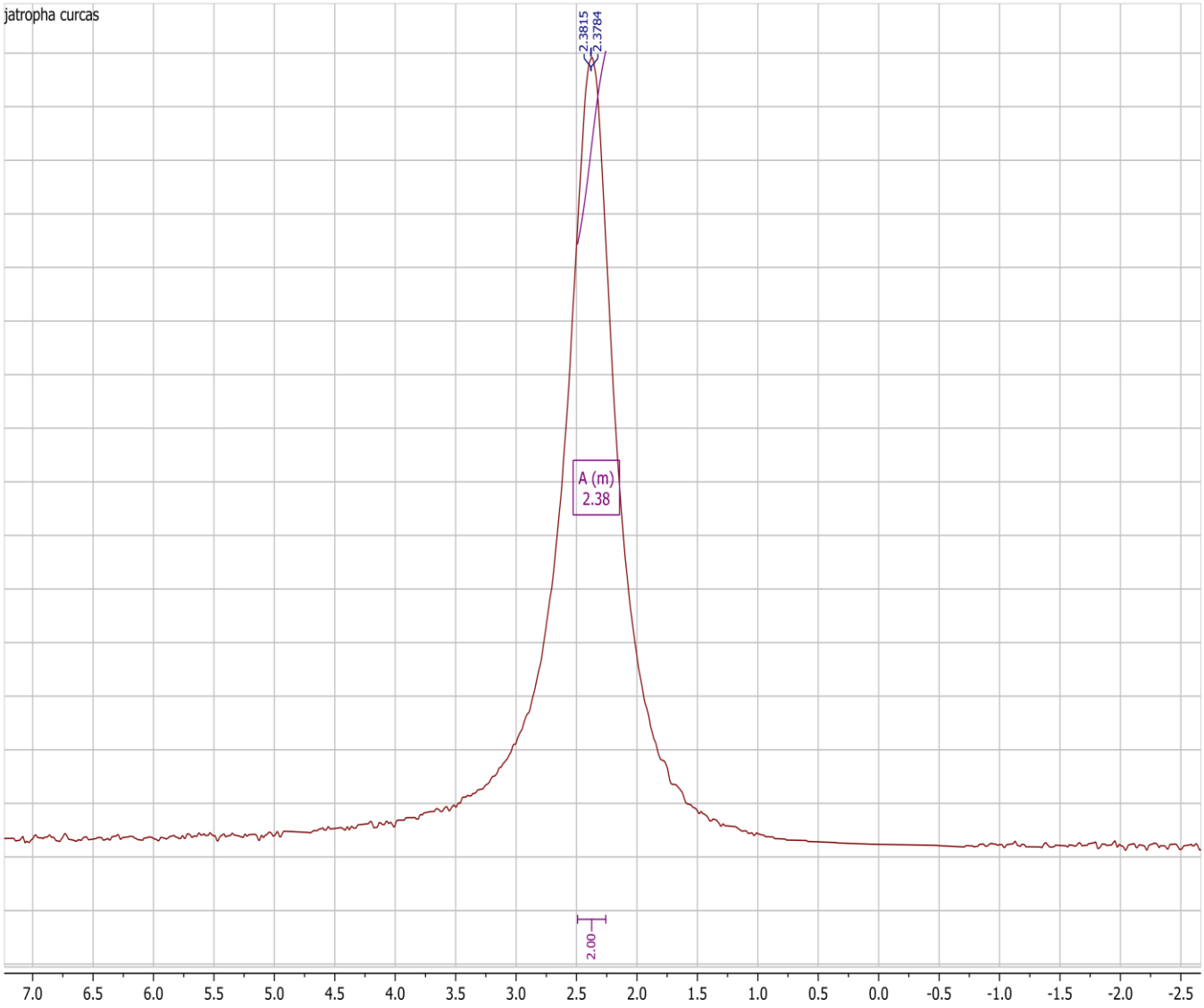
	Name	Shift	Range	H's	Integral	Class	J's	Method
1	A (m)	2.38	2.49...2.26	2	671513.70	m		Peaks

1H NMR (82 MHz,) δ 2.26 – 2.49 (m, 2H).

Table 4.4 1H NMR Concentration of *Jatropha curcas*

	ppm	Hz	pt
f1			
Now	3.1893	262.91	28449.87
A	2.5732	212.12	29281.95
B	2.1408	176.48	29865.94
|B-A|	0.4324	35.64	583.99
Intensity			
Now	334.955		
A	159.792		
B	72.6528		
|B-A|	87.1391		
B/A	0.454671		
A/B	2.19939		

Figure 4.3: NMR chemical shift analysis.



CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The GC-MS analysis shows the presence of twenty-two (22) prominent peaks with corresponding values from the Library which are chemically related compounds. Some of these compounds identified through the gas chromatography-mass spectrometry are known to have many important biological importance in addition to pharmaceutical importance. The compounds include: 2-Furancarboxaldehyde, 5-methyl-, 2-Hexanol, TMS derivative, Silane, dimethyl (methylsilyl),Pyrazine, 2-methoxy-6-methyl-, 4-Ethylbenzamide, 4-Ethylbenzoic acid, 2-Undecanone, Boronic acid, ethyl-, bis(2-mercaptoethyl ester), Tridecanoic acid, 12-methyl-, methyl ester, Methyl tetradecanoate, Tetradecanoic acid, n-Hexadecanoic acid, Pentadecanoic acid, Tetradecanoic acid, Tetradecanoic acid, 2,3-dihydroxypropyl ester, Tetradecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 9-Octadecenamide, (Z)-, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Oxacyclododecan-2-one, 2,3-Dihydroxypropyl elaidate, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, 1,3-Dicaprin, Carbamic acid, N-(3-chloro-4-methoxyphenyl)-,.gamma.-Tocopherol, Dodecanoic acid, 1-(hydroxymethyl),-1,2-ethanediyl ester, Stigmasterol.

The presence of 2-Furaldehyde is also used, in very small quantities, as a flavouring agent. 2-Furaldehyde is present in many food items as a natural product or as a contaminant. Pentadecanoic acid is among the rare fatty acid, even though it is not common in nature, it comprises of 1.2% of total milk fat from cow. Butterfat in cow's milk is its main dietary source and it is used as a marker for butterfat consumption.

Pentadecylic acid also occurs in hydrogenated mutton fat. Adewole et al., reported the GCMS analysis further revealed presence of many other important compounds; n-hexadecanoic acid, pentadecanoic acid, n-octadecanoic acid, eicosanoic acid, hexeicosanoic acid, docosanoic acid and 9, 15- octadecadienoate. The presence of this class of fatty acids further gives credence to the nutritional values of *Jatropha curcas* and possibly its potential ability to modulate the inflammatory pathways.

Tetradecanoic acid is a 14-C chain fatty acid that has a molecular formular of C14H28O2 which are lipid anchors in biomembranes (Wilfred et al,. 2019). Vanadium, (eta.7-cycloheptatrienylium)

(eta.5-2, 4-cyclopentadien-1-yl)-is a unstable organic compound with the molecular formula C12H12V-8 which boosts the uptake of nutrient in plants. cis-Vaccenic acid is a fatty acid with the molecular formula C18H34O2 that lowers total the level of cholesterol and triglycerides. Dodecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester is a fatty acid ester which is used as emulsLfiers for cream, milky lotion and hair conditioner is an important raw material in the cosmetic industry. Lauric anhydride is a fatty acid that increases the level of high density lipoprotein, study molar mass of unidentified constituent, treatment of wool in presence of cresol and in wafer form as a carrier in the study of drug release; is an important part of human diet and used in analytical chemistry. Tetrahydrofuran-2-carboxylic acid, dibenzofuran-3-ylamide is a volatile organic compound used in chemotherapy. In the food industry,

Heptadecanolide is an important component of food additive. Conclusion Plants stores up numerous phytochemical or phytonutrients that are very much useful to life processes and an important key to survival. From the phytochemical components of aqueous extract of *Jatropha curcas* it has been shown by Gas Chromatography-Mass Spectrometry investigation describes its relevance and usefulness in pharmaceuticals, and cosmetic and food industries.

NMR OF JATROPHA

Using NMR methods can be very useful for defining the molecular structure of a chemical as a whole. The result of merging data from infrared spectroscopy (to define the purpose of a compound) and NMR (gives an insight to the underlining information about the number of each type of hydrogen) is adequate to conclude more about an unknown structure (Pavia et al., 2001).

The resulting spectrum of ^{1}H NMR analysis that provides some important guidance in determining the structure with the value of Chemical shift and the TMS. The results of the analysis found that the existence of distinct peaks signals at 2.5732 ppm and 2.1404ppm with reference to the proton of acids H-C-OOH, esters H-C-OR and acetylene group of methylene protons bound to –O of the carboxylic acid ester group,-OOR that is the major methyl ester of TMP in the study. Signals at 4.016 ppm are for the methylene protons at the (methylene) carbon are formed in ester TMP. Based on information from the reference in Pavia et al. 2009 and Chemdraw software, the value is respectively 2.38 ppm.

Thus, the existence of the signal is then recognized that the ester product is ester TMP. Besides, proton signals at 2.26-2.49 ppm which gives the idea which denote the proton of C=C-H proton of

olefin that the values are also present in the analysis Chemdraw and the reference in Pavia et al. 2009.

5.2 CONCLUSION

The present study has established the distribution of the phytochemicals in the sap of *Jatropha curcas* and extract in terms of their polarities and thus provides a clue as to the exact constituents responsible for its numerous applications. The GC-MS analysis showed known constituents but were never reported previously as present in *Jatropha curcas*.

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