



Sudan University of Science & Technology



## College of Science

Department of Scientific Laboratories - Chemistry

Identification of an active component in *Guiera Senegalensis* plant

Used for healing diabetes wounds

التعرف علي المكون الفعالة في عشبة الغبيش المستخدمة في معالجة جروح السكري

A thesis Submitted in the partial fulfillment for the Requirements of B.sc (Honor) in  
scientific Laboratories –Chemistry

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## الإستهلال

قال تعالى :-

(وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُخْرَجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنَ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِّنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ<sup>ق</sup> انظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ<sup>ج</sup> إِنَّ فِي ذَلِكَ لَآيَاتٍ لِّقَوْمٍ يُؤْمِنُونَ).

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# Abstract

The African species Ghobash (*Guiera senegalensis* J. F. Gmel), is a small herb found mainly in West Africa. Its widespread use in traditional medicine. The aim of this study is to identify the bioactive ingredient responsible of healing diabetes wound. The aim were obtained using different process, First plant crude oil were extracted using the soxhlet apparatus and two organic solvent (Chloroform & Methanol). Then the natural product present in dry plant leave were investigate by phytochemical screening analysis tests which conform the main classes of secondary metabolites, namely terpenoids, alkaloids, flavonoids and stroides. The anti-bacterial activity test was carried out to show the bioactivities properties of the plant. The tests were positive in two types of bacteria (*staphylococcus aureus* & *pseudomonas aeruginosa*). The (GC/MS) analysis were carried out to separate and identify the components of the plant and their structures. The obtained results showed the presence of different active ingredients such as: flavonoids, alkaloids and steroids in high percentage. The expected component responsible of healing diabetes wound were suggested to be stigmasterol.

## الخلاصة

الغبيش نوع من أنواع النباتات التي تنمو في إفريقيا وهي عبارة عن عشبه صغيره توجد بصورة رئيسيه في غرب إفريقيا وهي واسعة الاستخدام في الطب البلدي .

الهدف من هذه الدراسة هو التعرف علي المكون الفعال المسؤل عن شفاء جروح السكري باستخدام طرق مختلفة.

في البدء تم استخلاص زيت النبات النقي من الأوراق الجافة للنبات باستخدام جهاز السكسوليت و مذيبين عضويين (الكلوروفورم و الميثانول) .

ثم تم التحقق من وجود عوائل المنتجات الطبيعية عن طريق المسح الفيزيوكيميائي الذي أكد وجود نواتج الأيض الثانوي وهي : التربينات و القلويدات و الفلافونويدات و الاسترويدات .

تم عمل كشف الفعالية ضد البكتريا الذي اظهر الخواص الحيوية للنبات باستخدام نوعين من البكتريا (الكروية العنقودية الذهبية و الزائفة الزنجارية ) حيث كانت النتائج ايجابية.

تم إجراء تحليل كروماتغرافيه الغاز و مطياف الكتلة لفصل مكونات النبات و التعرف عليها و علي صيغها الكيميائية. النتائج المتحصل عليها اظهرت وجود مكونات فعالة مختلفة مثل الفلافونويدات و القلويدات و الاسترويدات بنسب عالية . المركب المسؤل عن شفاء جروح السكري اقترح ان يكون ستجما استيرول .

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# Chapter One

Introduction and literature review

# 1.Introduction

## **1.1Introduction to natural product drug discovery Process:**

Natural products are products from various natural sources, plants microbes and animals.

Natural products can be an entire organism(e.g. a plant, an animal or a micro-organism), a part of an organism (e.g. leaves or flowers of a plant, an isolated animal organ), an extract of an organism or part of an organism and an exudates, or pure compound (e.g. alkaloids, coumarins, flavonoids, lignans, steroids and terpenoids) isolated from plants, animals or micro-organisms. However, in practice, the term natural product refers to secondary metabolites, small molecules (molecular weight < 1500 amu), produced by an organism, but not, strictly, necessary for the survival of the organism<sup>[1]</sup>.

### **1.1.1 Natural products in medicine**

The use of natural products, especially plants, for healing is as ancient and universal as medicine itself. The therapeutic use of plants, certainly, goes back to the Sumerian and the Akkadian civilizations in, about, the third millennium BC. Hippocrates (ca. 460–377 BC), one of the ancient authors who described medicinal natural products of plant and animal origins, listed approximately 400 different plant species for medicinal purposes. Natural products have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Ayurvedic and

Egyptian. Even now, continuous traditions of natural product therapy exist throughout the third world, especially in the orient, where numerous minerals, animal substances and plants are still in common use. According to the World Health Organization(WHO), some 3.4 billion people in the developing world depend on plant based traditional medicines. This represents about 88 per cent of the world's inhabitants, who rely mainly on traditional medicine for their primary healthcare. In China alone, 7295 plant species are utilized as medicinal agents. Nature has been a potential source of therapeutic agents for thousands of years. An impressive number of modern drugs have been derived from natural sources .Over the last century, a number of top selling drugs have been developed from natural products. Anticancer drug vincristine from *Vince rosea*, narcotic analgesic morphine from *Palaver somniferous*, ant malarial drug artemisin in from *Artemisia annua*, anticancer drug Taxol/ from *tax us brevifolia* and antibiotic penicillin from *penicillium ssp*. Apart from natural-product-derived modern medicine, natural products are also used directly in the 'natural' pharmaceutical industry that is growing rapidly in Europe and North America, as well as in the traditional medicine programme being incorporated into the primary health care systems of Mexico, The People's Republic of China, Nigeria and other developing countries<sup>[1]</sup>.

### **1.1.2 Drug discovery and natural products:**

Although drug discovery may be considered to be a recent concept that evolved from modern science during the 20th century, in reality the concept of drug discovery dates back many centuries, and has its origins in nature. Time and time again, humans have turned to Mother Nature for cures, and discovered unique drug molecules. Thus, the term natural product has become almost synonymous with the concept of drug discovery. In modern drug discovery and development processes, natural products play an important role at the early stage of ‘lead’ discovery, i.e. discovery of the active (determined by various bioassays) natural molecule, which itself or its structural analogues could be an ideal drug candidate. Natural products have been a wellspring of drugs and drug leads. It is estimated that 61 per cent of the 877 small molecule new chemical entities introduced as drugs worldwide during 1981–2002 can be traced back to or were developed from natural products. These include natural products (6 per cent), natural product derivatives (27 per cent), synthetic compounds with natural-product-derived pharmacophores (5 per cent) and synthetic compounds designed on the basis of knowledge gained from a natural product, i.e. a natural product mimic (23 per cent). In some therapeutic areas, the contribution of natural products is even greater, e.g. about 78 per cent of anti bacterial and 74 per cent of anticancer drug candidates are natural products or

structural analogues of natural products. In 2000, approximately 60 per cent of all drugs in clinical trials for the multiplicity of cancers were of natural origins. In 2001, eight (simvastatin, pravastatin, amoxicillin, clavulanic acid, clarithromycin, azithromycin, ceftriaxone, cyclosporin and paclitaxel) of 30 top selling medicines were natural products or derived from natural products, and these eight drugs together totalled US\$16 billion in sales. Despite the outstanding record and statistics regarding the success of natural products in drug discovery, ‘natural product drug discovery’ has been neglected by many big pharmaceutical companies in the recent past. The declining popularity of natural products as a source of new drugs began in the 1990s, because of some practical factors, e.g. the apparent lack of compatibility of natural products with the modern high throughput screening (HTS) programmes, where significant degrees of automation, robotics and computers are used, the complexity in the isolation and identification of natural products and the cost and time involved in the natural product ‘lead’ discovery process. Complexity in the chemistry of natural products, especially in the case of novel structural types, also became the rate-limiting step in drug discovery programmes. Despite being neglected by the pharmaceutical companies, attempts to discover new drug ‘leads’ from natural sources has never stopped, but continued in academia and some semi-academic research organizations, where more traditional approaches to natural product drug discovery have been

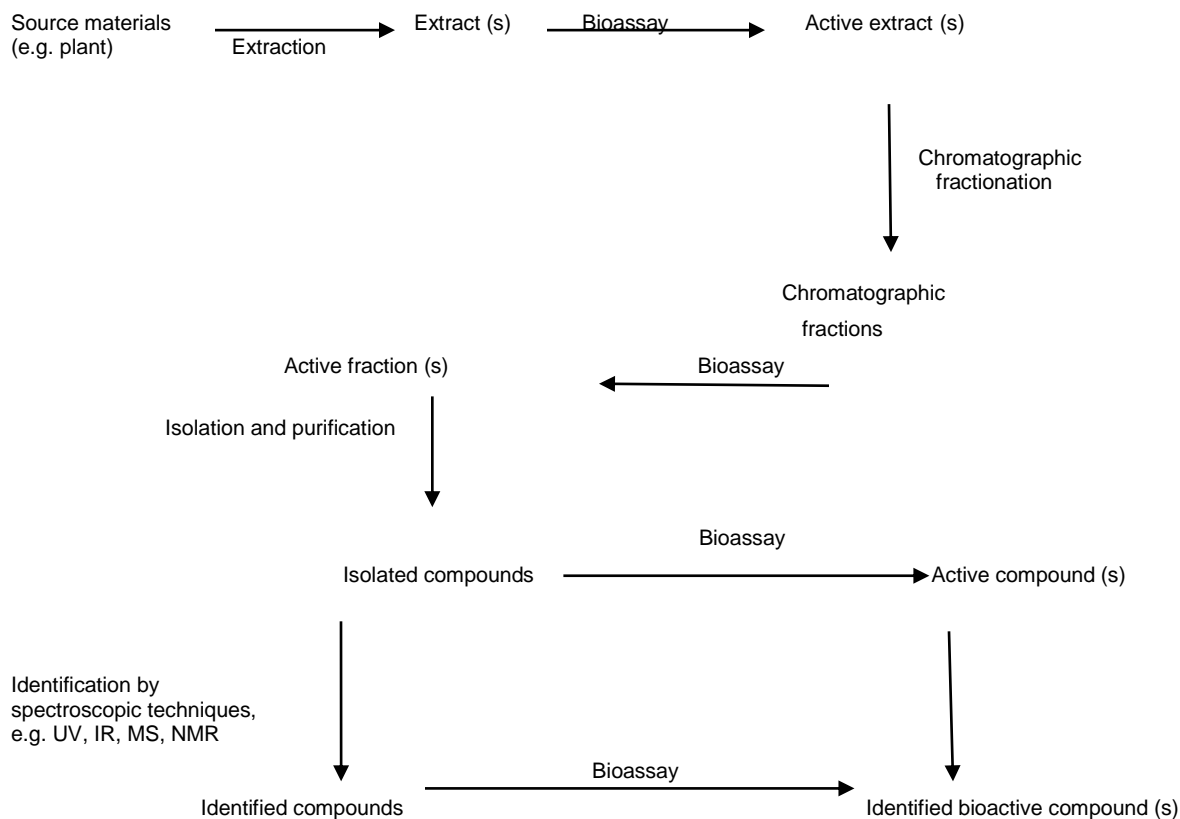
applied. Natural product drug discovery appears to be drawing attention and immense interest again, and is on the verge of a comeback in the mainstream of drug discovery ventures. In recent years, a significant revival of interests in natural products as a potential source for new medicines has been observed among academics as well as several pharmaceutical companies. This extraordinary comeback of natural products in drug discovery research is mainly due to the following factors. combinatorial chemistry's promise to fill drug development pipelines with de novo synthetic small molecule drug candidates is somewhat unsuccessful. The practical difficulties of natural product drug discovery are being overcome by advances in separation and identification technologies and in the speed and sensitivity of structure elucidation and, finally, the unique and incomparable chemical diversity that natural products have to offer. Moreover, only a small fraction of the world's biodiversity has ever been explored for bioactivity to date. For example, there are at least 250 000 species of higher plants that exist on this planet, but merely, five to 10 per cent of these terrestrial plants have been investigated so far. In addition, re-investigation of previously investigated plants has continued to produce new bioactive compounds that have the potential for being developed as drugs. While several biologically active compounds have been found in marine organisms, e.g. antimicrobial compound cephalosporin C from marine organisms (*Cephalosporium acremonium* and *Streptomyces* spp.) and antiviral

compounds such as avarolandavarone from marine sponges, e.g. Dysideaavara, research in this area is still in its infancy<sup>[1]</sup>.

### **1.1.3 The traditional way:**

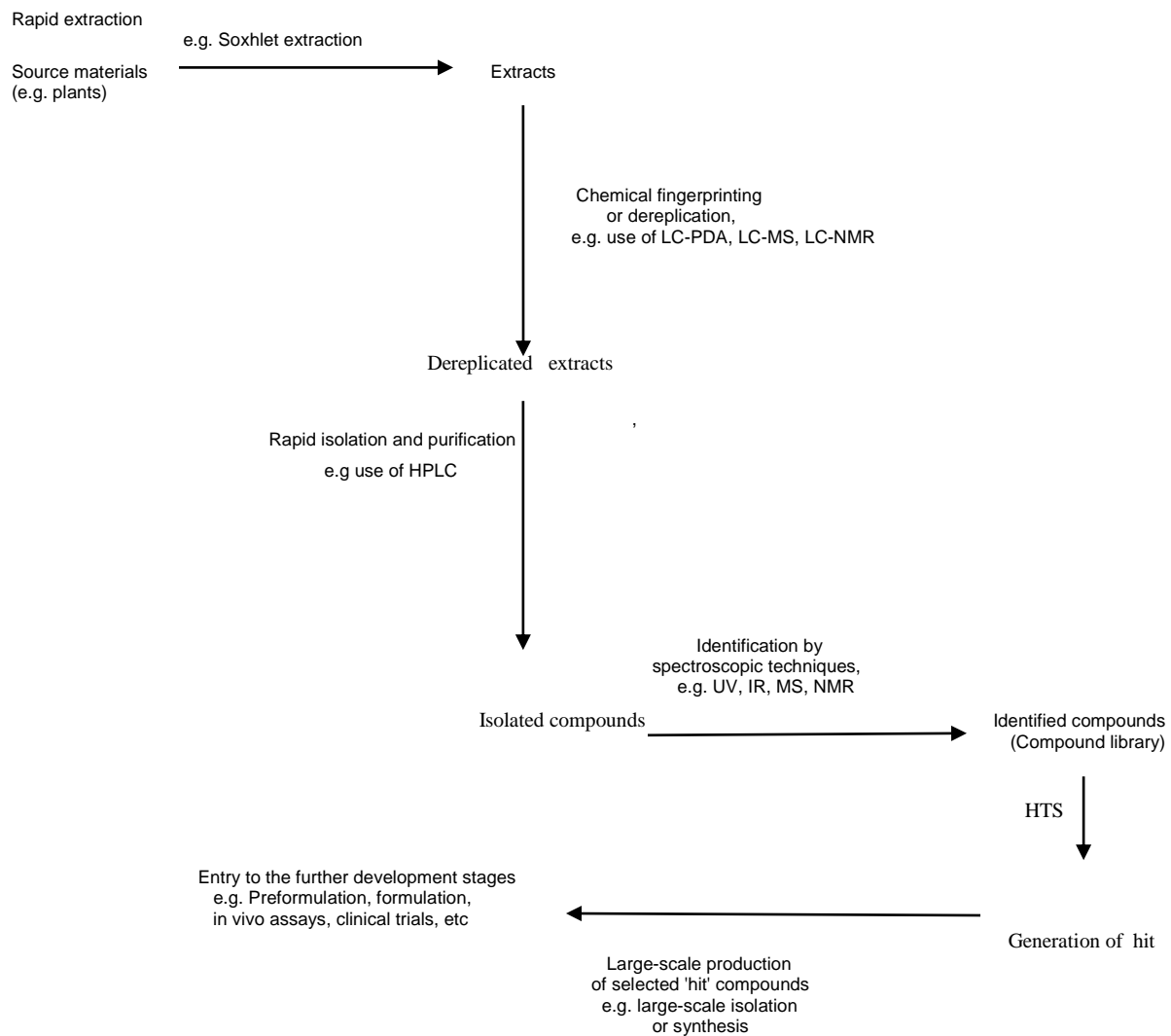
In the traditional, rather more academic, method of drug discovery from natural products, drug targets are exposed to crude extracts, and in the case of a hit, i.e. any evidence of activity, the extract is fractionated and the active compound is isolated and identified. Every step of fractionation and isolation is usually guided by bioassays, and the process is called bioassay-guided isolation. The following scheme presents an overview of a bioassay-guided traditional natural product drug discovery process. Sometimes, a straightforward natural product isolation route, irrespective of bioactivity, is also applied, which results in the isolation of a number of natural compounds (small compound library) suitable for undergoing any bioactivity screening. However, the process can be slow, inefficient and labour intensive, and it does not guarantee that a 'lead' from screening would be chemically workable or even patentable. <sup>[1]</sup>





Modern drug discovery approaches involve HTS, where, applying full automation and robotics, hundreds of molecules can be screened using several assays within a short time, and with very little amounts of compounds. In order to incorporate natural products in the modern HTS programmes, a natural product library (a collection of dereplicated natural products) needs to be built. Dereplication is the process by which one can eliminate recurrence or re-isolation of same or similar compounds from various extracts. A number of hyphenated techniques are used for dereplication, e.g. LC-PDA (liquid chromatography–photo-diode-array detector), LC-MS (liquid chromatography–mass detector)

and LC-NMR (liquid chromatography–nuclear magnetic resonance spectroscopy). While in the recent past it was extremely difficult, time consuming and labour intensive to build such a library from purified natural products, with the advent of newer and improved technologies related to separation, isolation and identification of natural products the situation has improved remarkably. Now, it is possible to build a ‘high quality’ and ‘chemically diverse’ natural product library that can be suitable for any modern HTS programmes. Natural product libraries can also be of crude extracts, chromatographic fractions or semi-purified compounds. However, the best result can be obtained from a fully identified pure natural product library as it provides scientists with the opportunity to handle the ‘lead’ rapidly for further developmental work, e.g. total or partial synthesis, dealing with formulation factors, in vivo assays and clinical trials.<sup>[1]</sup>



To continue to exploit natural sources for drug candidates, the focus must be on exploiting newer approaches for natural product drug discovery. These approaches include the application of genomic tools, seeking novel sources of organisms from the environment, new screening technologies and improved processes of sample preparation for screening samples. In addition, the recent focus on the synthesis of diversity-oriented combinatorial libraries based on natural-product-like compounds is an attempt to enhance the productivity of synthetic chemical libraries<sup>[1]</sup>.

## 1.2 Introduction of the plant(*guierasenegalensis*):

The Republic of Sudan  
Ministry of Science & Telecommunication  
**National Centre for Research**  
Medicinal & Aromatic Plants & Traditional  
Medicine Research Institute



جمهورية السودان  
وزارة العلوم والاتصالات  
**المركز القومي للبحوث**  
معهد أبحاث النباتات الطبية والعطرية  
والطب الشعبي

Date 17.1.2016

### To whom it may concern

This to certify that the plant materials were taxonomically authenticated by Yahya Sulieman Mohamed at the herbarium of Medicinal and Aromatic Plants & Traditional Medicine Research Institute (MAPTMRI) National center for Research Khartoum, Sudan, and voucher herbarium samples were deposited there for further further reference .

Botanical name: *Guiera senegalensis* J.F Gmel

Arabic name: *Ghobash*


Family : *Combretaceae*

Name : Marwan Abody & Hapeepalla Adam & Alshafee Mohamed

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Head department of Taxonomy &  
Phytochemistry



  
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Director of Medicinal and Aromatic  
Plants Research & Traditional Medicine  
Institute (MAPTMRI)

### 1.2.1 Origin and geographic distribution:-

*Guiera senegalensis* occurs in the savanna zone from Senegal east to Sudan.

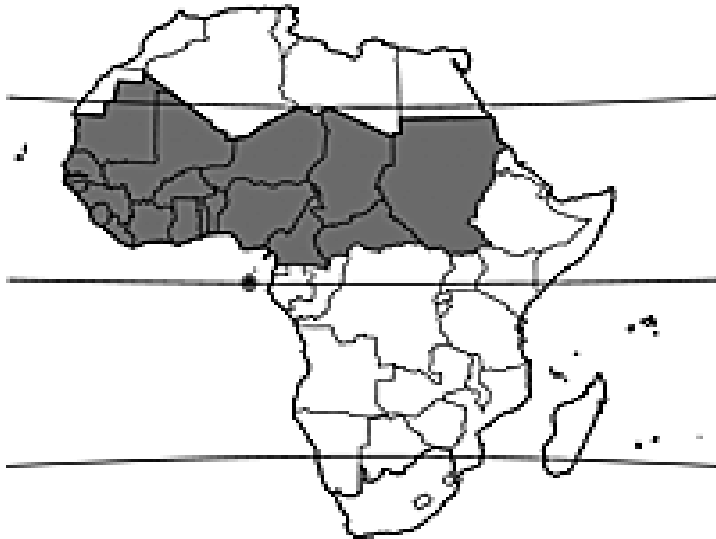


Figure 1.1: *Guiera senegalensis* distribution in Africa<sup>[2]</sup>

### 1.2.2 Uses:-

*Guiera senegalensis* is one of the most popular west African medicinal plants, and is used to treat a wide variety of diseases. Its uses are comparable to those of *Combretum micranthum* G. Don, commonly called 'kinkeliba', and the plants are often used combined, especially, to treat common colds, fever and respiratory problems. The bitter leaves are most frequently used.

A leaf decoction or infusion, sometimes combined with other species, is drunk to treat dysentery, diarrhoea, colic, gastroenteritis, beriberi, rheumatism, hypertension, eczema, epilepsy, leprosy, impotence, venereal diseases, malaria, fever, cough, colds, asthma, bronchitis and tuberculosis. It is also taken as a diuretic, as an anti-emetic in small doses and as an emetic in larger doses. Crushed leaves are mixed with tamarind pulp and eaten as a laxative and appetizer. Dried pounded leaves in food are taken by women after childbirth to increase milk flow and as general tonic and blood restorative. A leaf infusion is used to wash new-born babies. Dried leaves are mixed with tobacco and smoked to treat respiratory problems. The powdered leaves are also taken as a snuff to treat headache and sinusitis. Ground leaves, leaf powder or a leaf decoction is applied to wounds to help cicatrisation and treat skin problems, including Guinea worm, boils, burns, sores in the mouth, tumours, syphilitic sores and leprosy. A steam bath of the leaves is taken to treat tooth-ache caused by caries. A leaf infusion is also used as a mouth wash for the same purpose. Powdered or crushed leaves are added to milk and taken to treat amoebic dysentery and leprosy. Young leaves are chewed against coughs. In Sudan a leaf infusion is taken to treat diabetes.

Powdered and boiled roots are, commonly, taken to treat diarrhoea and dysentery, including amoebic dysentery and intestinal worms. A root decoction is also drunk to treat insomnia, pneumonia, tuberculosis,

haemorrhoids, poliomyelitis and gonorrhoea. A bark decoction is taken to treat colic. A fruit decoction is taken to stop hiccups and to treat rectal prolapse. The powder of roasted fruit is eaten to treat cough. A decoction of all plant parts is drunk and rubbed in to treat oedema and the bark powder is applied as a dressing. The powdered plant galls with charcoal are drunk in water as a strong diuretic in oliguria and anuria, as well as cerebral malaria. They are also, similarly, used as the leaves and roots to treat malaria, dysentery, diabetes and hypertension. The galls are used in Burkina to increase milk production in cows and to treat fowlpox infection in chickens. The leaves are fed to cows to fatten them, and to increase fertility and milk production.

Leaves and fruits, readily, eaten by ruminants, camels and horses and forms an important fodder, especially during the dry season. Dried leafy twigs are burnt in stalls or pens to repel flies and biting insects around domestic animals. Leafy branches are sometimes used for mulching.

The leaves are bitter and slimy and eaten as famine food. The gum from the stem bark is marketed in Niger as food. To ensure good fermentation of local beer crushed leaves are sometimes added in the cooking. The flowers are a good source of nectar for bees. The root is used to wash new baby naps, to render them soft and shining. The wood is used for the framework of wells, bed posts and roof lattice work and it is also, commonly, used to fence farms. It is also an important source of fuel.



The roots are split and used as chew sticks and tooth picks. The fruit yields a black dye. The leaves enter into various medico-magical preparations, e.g. to free people from evil spirits and to bring good luck<sup>[2]</sup>.

### **1.2.3 Production and international trade:-**

The dried leaves are, commonly, sold in markets throughout West Africa, for medicinal use. In Senegal, a syrup named 'nger' is prepared from the leaves and has been commercialized as a remedy for coughs<sup>[2]</sup>.

### **1.2.4 Properties:-**

From different plant parts tannins, flavonoids, alkaloids and mucilage have been isolated. Several compounds are absent or present in different quantities from plants from different provenances, indicating the presence of chemo types.

Leaves and roots contain  $\beta$ -carboline alkaloids (0.15–0.2%), and the methoxylatednaphthyl butanone guieranone A. In the roots tetrahydroharman (eleagnine) was the main alkaloid, with harman and harmalan (dihydroharman) as minor compounds. The leaves contain the alkaloids tetrahydroharman and harman, as well as guieranone A.

Tannins were present in large amounts in all plant parts, including the galls. The different plant parts showed quantitative and qualitative

differences with respect to the chemical composition of the tannins. Nine gallotannins with a quinic acid core and two condensed tannins (epicatechin and epigallocatechin gallate) have been isolated so far. The major tannin in all plant parts is 3,4,5-tri-O-galloylquinic acid. From the leaves, roots and galls a range of flavonoids was isolated, including catechin, myricitrin, several myricetin derivatives, rutin, rhamnetin, quercetin, quercetrin, kaempferol, tiliroside, apigenin and gallic acid. From the leaves the naphthopyrones 5-methyldihydroflavasperone and 5-methylflavasperone and the amino acid ascorbic acid have been isolated. Mucilage was also present in all plant parts, but mostly in the fruits. A chloroform extract of the roots exhibited a pronounced antimalarial activity against *Plasmodium falciparum* in vitro and displayed low toxicity. However, in a different experiment, petroleum ether, chloroform and methanol extracts from the leaves did not show significant in vivo antimalarial activity in mice experimentally infected with *Plasmodium berghei*, when intraperitoneally administered. Harman and tetrahydroharman showed, significant, antiplasmodial activity in vitro associated with a low cytotoxicity; harmalan was found to be less active. Guieranone A also showed significant antiplasmodial activity in vitro, but associated with a high cytotoxicity towards two cancer cell lines, human monocytes and normal skin fibroblasts. It also exhibited potent antifungal activity against *Cladosporium cucumerinum*. A methanolic leaf extract showed, significant, anti-diarrhoeal activity in

rodents. An aqueous root extract showed highly significant anti-diarrhoeal activity in castor oil-induced diarrhoea in rats. The extract also exhibited, significant, ulcer-protective properties against ethanol-induced ulceration in rats. The oral LD<sub>50</sub> values obtained were more than 5000 mg/kg in both mice and rats. In several other tests it was shown that several extracts, orally, administered did not show significant toxic effect in test animals. However, when these extracts were injected intramuscularly, they were found to be lethal within one week after administration. The condensed tannins, as well as galloylquinic acid and several flavonoids, including rhamnetin, showed, significant, antioxidant and radical scavenger activities in vitro. An extract of the total phenolic content of the leaves showed significant anti-oxidant activity in vitro in a range of tests. The root extract was less active. A crude aqueous leaf extract showed moderate central nervous system depressant effects in guinea pigs. A hydroacetic leaf extract showed significant antioxidant and anti-inflammatory activity in vitro. A methanolic leaf extract showed anti-inflammatory activity in rodents and significant dose-dependent inhibition of contraction by acetylcholine of isolated rat trachea. A crude aqueous leaf extract showed significant antitussive activity in tests with guinea pigs. The tannin 1,3,4,5-tetra-O-galloylquinic acid showed significant activity against bronchial spasms. Different leaf extracts showed, significant, antibacterial activity in vitro against a range of human pathogenic bacteria. A leaf extract

demonstrated promising trypanosidal activity against *Trypanosoma brucei brucei* intraperitoneally injected in mice. Several leaf extracts showed significant antiviral activity against Herpes simplex virus type 1 and African swine fever virus. The tannin 3,4,5-tri-O-galloylquinic acid showed moderate selective inhibition of HIV replication. A aqueous extract from the galls showed significant, in vitro, antiviral activity against fowlpox virus. A leaf extract showed a significant, in vitro, snake venom detoxifying activity when tested in mice against two common northern Nigeria snake species *Echiscarinatus* and *Najanigricolis*.

When more than 40% of the diet of sheep consisted of *Guierasenegalensis* leaves there were negative effects on digestibility, probably due to low levels of intraruminal breakdown and a high tannin content. The optimal level of browse introduction in the diet of sheep was 12.5% dry matter. In a field experiment with leaf mulch in pearl millet plots, it was shown that mulching increased millet yield with 68–94% compared to the control.

The wood is whitish or tinged red, coarse-grained, knotted and short, but very hard<sup>[2]</sup>.

### 1.2.5 Description:-

(Semi-)evergreen shrub up to 3(–5) m tall, with spindly bole or many-branched from the base; all parts covered with black glandular dots; bark

fibrous, more or less smooth to finely scaly, grey to brown, slash beige; young branches soft-hairy. Leaves (almost) opposite, simple and entire; stipules absent; petiole 2–5 mm long, short-hairy; blade oblong-elliptical, ovate to orbicular, 3–5.5 cm × 2–3 cm, base rounded to almost cordate, apex rounded or mucronate, shortly soft-hairy at both sides, with many black glandular dots, pinnately veined with 5–6(–8) pairs of lateral veins. Inflorescence an almost spherical terminal and axillary head, up to 15 mm in diameter, many-flowered; peduncle 2–3.5 cm long; involucre bracts 4(–5), up to 7 mm long, triangular, apex acute, margin white-hairy, folded backwards when flowers open, enclosing the flowers in bud. Flowers bisexual, regular, 5-merous, c. 1.5 mm in diameter; tepals 5, creamy white to yellowish, densely short-hairy; stamens 10, far exceeding the corolla; ovary inferior, becoming long spindle-shaped, 4–5-celled, style filiform. Infructescence resembling a many-legged hairy spider. Fruit linear and spindle-shaped, 3–4.5 cm long, densely covered with greyish, silky hairs, turning pinkish-red when mature, perianth and style persistent<sup>[2]</sup>.

### **1.2.6 Other botanical information:-**

Guiera comprises a single species<sup>[2]</sup>.

### **1.2.7 Growth and development:-**

*Guierasenegalensis* flowers, often, twice a year, once at the end of the rainy season and once during the dry season. It can thus be found flowering and fruiting almost all year long<sup>[2]</sup>.

### **1.2.8 Ecology:-**

*Guierasenegalensis* occurs in shrub savanna, tree savanna and fallow land, from sea-level up to 1000 m altitude. It grows in areas with (200–)400–800 mm annual rainfall. *Guierasenegalensis* occurs on all types of soil but mainly on dry sandy or degraded soils, sometimes in areas which are, temporarily, flooded. It does not tolerate heavy shading. It is colonizing degraded areas, where it can become gregarious and very abundant. It is one of the species responsible for the ‘tiger stripes bush’ pattern so typical of the bush savannah in the Sahel. It is considered an indicator of overgrazing. *Guierasenegalensis* is very drought resistant<sup>[2]</sup>.

### **1.2.9 Propagation and planting:-**

*Guierasenegalensis* is propagated by seed, stem layering and root suckers. Average 1000 seed weight is 28.4 g. Seeds are sown in pots during the dry season and transplanted into the field when the rainy season is well established. Branch layering is done by simply burying young parts of stems during the rainy season until roots grow. Roots have been observed to grow within 2 weeks after layering<sup>[2]</sup>.

### **1.2.10 Management:-**

In farmers' fields *Guierasenegalensis* is cut back to ground level before the rainy season starts. During the crop season it is cut back again, but left to sprout at the end of the rainy season. The shrubs resprout profusely from the base and help to fix the soil during the dry season. The cut branches are left in the field where they constitute a mulch, which can contribute, considerably, to increased soil fertility, water conservation and crop yield. Larger branches are usually used as firewood<sup>[2]</sup>.

### **1.2.11 Diseases and pests:-**

Stem gall infection of *Guierasenegalensis* is very common, and aphids are sometimes present, protected by ants, but other pests or diseases are rare<sup>[2]</sup>.

### **1.2.12 Harvesting:-**

The leaves, roots and galls of *Guierasenegalensis* are, extensively, harvested for their medicinal use<sup>[2]</sup>.

### **1.2.13 Yield:-**

The production of *Guierasenegalensis* in a 8 months old seedling plantation can reach 500 kg/ha leaves and 300 kg/ha wood. About 225 kg/ha can be used as firewood<sup>[2]</sup>.

### **1.2.14 Handling after harvest:-**

The plant parts harvested can be used fresh or dried for later use. When dry, the plant parts need to be stored in airtight containers in the shade<sup>[2]</sup>.

### **1.2.15 Genetic resources:-**

*Guierasenegalensis* is widespread and common, and not at risk of genetic erosion<sup>[2]</sup>.

### **1.2.16 Prospects:-**

*Guierasenegalensis* is widely used as a traditional medicinal plant. A range of phytochemical compounds have been isolated from different plant parts. Many of the uses have been subjected to some level of pharmacological screening, and tests on its antimalarial, anti-diarrhoeal, antibacterial, anti-cough, anti-inflammatory, anti-oxidant activity have been positive. Many of these tests, however, are still at a preliminary level, and need to be followed by more detailed research. Further toxicological studies are also warranted as it has been shown that most extracts when, orally, ingested seem to be relatively harmless, but when injected most extracts are toxic to varying levels. *Guiera senegalensis* is also a very important species in the crop-fallow cycle in the Sahelian zone, as well for its firewood production, and its presence should be monitored in order to prevent a decline of the species<sup>[2]</sup>.



### 1.3 Literature review:

*Guiera senegalensis* is acclaimed as a common herbal antipyretic and antimalarial among some tribal groups in northern Nigeria. Leaf extracts of the plant were thus tested for antiplasmodial, analgesic and anti-inflammatory effects in vivo. Results indicated the safe dose of extracts as 600 mg/kg body weight of mice with LD<sub>50</sub> of 1100 mg/kg bw. Only the methanolic fraction had antiplasmodial effect while ethylacetate and hexane fractions were ineffective.<sup>[3]</sup> Furthermore the methanolic extract produced a significant ( $p < 0.05$ ) suppression of up to 67.52% levels. The extracts had no prophylactic effect and high parasitaemia including mortality of sub-inoculated mice were obtained on day 14 post treatment.<sup>[3]</sup> It gave 44.83% analgesic effect but was devoid of anti-inflammatory activity. Phytochemical screening indicated the presence of alkaloids, glycosides, tannins and flavonoids<sup>[3]</sup>. The antimicrobial activities of aqueous and methanol extracts of *Guiera senegalensis* were studied against some enteropathogens by agar-well diffusion method. Both extracts inhibited the growth of *Streptococcus Pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Salmonella* spp and *Pseudomonas euroginosa*.<sup>[3]</sup> The methanol extract was more effective on *S. Pneumonia*, *E. coli*, *S. aureus*, *E. cloacae*, *Salmonella* spp, while the aqueous extract was more active on *Salmonella* spp. and *P. euroginosa*.<sup>[3]</sup> The methanol extract showed quicker curative action in, experimentally, induced diarrhoea in adult

male mice when administered once or twice at a dose of 556 mg/kg daily.<sup>[4]</sup> From this work, *G. senegalensis* root appears to be an effective measure for treating diarrhoea. Where foodpoisoning or metal poisoning are the causative agents. The phytochemical screening results of the aqueous extract, showed the presence of tannins, saponins, alkaloids, cardiac glycosides, coumarine and anthraquinones.<sup>[4]</sup>

However<sup>[4]</sup> anthraquinones were not detected in the methanol extract and cardiac glycosides were not detected in the aqueous extract. Ethno medicinal use of this plant in the treatment of diarrhoea is justified by this work and is encouraged<sup>[4]</sup>. Recent study the claimed antimicrobial activity of Sudanese medical plant, *Guiera senegalensis* leaves studies. This involved preparation and use of methanolic extract of *Guiera senegalensis* leaves. The study conducted on Guinea pig and rat with induced inhibition and slight contraction. The extract showed dose dependant inhibition on force and rate of contraction of isolated Guinea pig atrium. In isolated rat uterus preparation the extract showed slight contraction which blocked by (2 mg/mL<sup>-1</sup>) cyproheptidine<sup>[5]</sup>. The chemical composition of total alkaloids from leaves and roots of *Guiera senegalensis* was investigated. Three beta-carboline alkaloids were purified: in addition to harman and tetrahydroharman, known in roots and leaves, harmalan (dihydroharman) was isolated for the first time from roots of *Guiera senegalensis*. Guieranone A, a naphthylbutenone, was also purified from leaves and roots. The in vitro antiplasmodial

activity and the cytotoxicity of extracts and pure compounds were evaluated. Each total alkaloid extract and beta-carboline alkaloids presented an interesting antiplasmodial activity associated with a low cytotoxicity. Harmalan was less active than harman and tetrahydroharman. Guieranone A showed a strong antiplasmodial activity associated with a high cytotoxicity toward human monocytes. Its cytotoxicity was performed against two cancer cell lines and normal skin fibroblasts in order to study its anticancer potential: guieranone A presented a strong cytotoxicity against each cell strains. Finally, evaluated the potent synergistic antimalarial interaction of *Guiera senegalensis* and two plants, commonly, used in traditional remedies: *Mitragynainermis* and *Pavetta crassipes*. Three associations evaluated were additive. A synergistic effect was shown between total alkaloids extracted from leaves of *Guierasenegalensis* and those of *Mitragynainermis*. This result justified the traditional use of the plants in combination to treat malaria<sup>[6]</sup>.

## 1.4 Objective:

The main objective of this study is to identify the bioactive component responsible of healing diabetes wounds using *guiera sengalensis* plant using the following steps:

- Phytochemical analysis to confirm the presence of natural products in the plant after extracting the crude oil using soxletoperator.

- Anti-bacterial test to show the bioactive properties of the plant extract.
- Gas chromatography mass spectrometry (GC/MS) analysis to identify the components, present, in the plant extract.

# **Chapter Two**

## **Materials & Methods**

## **2. Materials & Methods**

### **2.1 Materials:**

- Guierasenegalensis leaves.
- Chloroform (99%).
- Methanol (99%).
- Methanol anhydride.
- Wagner's reagent.
- Hager's reagent.
- Alkaline reagent.
- Copper acetate.
- Hydrochloric acid.
- Lead acetate.
- Water injection.
- Micro Organism (Staphylococcus aureus ATCC6538 Passage No 4, Pseudomonas aeruginosa ATCC9027 Passage No 4)

### **2.2 Apparatus:**

- Soxhlet.

### **2.3 Instrument:**

- Gas Chromatography Mass Spectrometry (GC/MS).

Instrument information:

Detector: Mass spectrometer

Model: GC/MS-QP2010Ultra

Company: Shimadzu

Country: Japan

Column: Rtx-5MS...Length (30)...Diameter (0.25)...Thickness (0.25 $\mu$ I)

Carrier gas: Helium

## **2.4 Method:**

### **2.4.1 Sample preparation for photochemical screening:**

*Guiera senegalensis* leaves were collected from West Sudan , Northern Kordfan (UM Trakesh) and dried in shadow. 10 g of the dried leave were ground by mortar and extracted by soxhlet using chloroform, Extraction was repeated using methanol.

### **2.4.2 Phytochemical screen :**

#### **2.4.2.1 Alkaloid test:**

a) In dry and clean test tube 1 ml of the extract were added and 3 drops of dilute Hydrochloric acid then it were treated with Wagner's reagent (Iodine in Potassium Iodide). the formation of brown /reddish precipitate indicates the presence of alkaloids.

b) 1 ml of the extract were added to dry and clean test tube and 3 drops of dilute Hydrochloric acid were added then it were treated with Hager's reagent (saturated Picric acid solution). the presence of alkaloids confirmed by the formation of yellow colored precipitate.

#### **2.4.2.2 Phytosterols, libermann test, :**

2 ml of the extract few drops of chloroform ,3 drops of acetic anhydride, and 1 drop of concentrated sulphuric acid were added.The appearance of purple color shows the presence of steroid.

#### **2.4.2.3 Terpenstest, Copper actate test, :**

The extract were dissolved in water and treated with 3 drops of copper acetate solution. formation of emerald green colour indicates the presence of diterpens.

#### **2.4.2.4 Flavonoids :**

a) Alkaline Reagent test: In clean and dry test tube 1 ml, of extract, was treated with few drops of sodium hydroxide solution. formation of intense yellow color which become colorless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead Acetate test: In dry and clean test tube 1 ml, of extract was treated with, few, drops of lead acetate solution. Formation of, ayellow, precipitate indicates the presence of flavonoids.

#### **2.4.3 Anti-Bacterial test:**

##### **2.4.3.1 Preparation of Chloroform extract (Reagent 1):**

0.5 g of chloroform extracted sample by were dissolved in 2.5 ml of Methanol (Absolute) analytical grade, well, mixed and sonicated for 15 min , the volume was completed to 20 ml with water for injections ,well mixed and used.



### **2.4.3.2 Preparation of Methanol extract(Reagent 2) :**

0.396 g of methanol extracted sample were dissolved in 2.5 ml of absolute Methanol analytical grade, mixed well then sonicated for 15 min, the volume completed to 20 ml with water, for injections, well mixed, and used.

### **2.4.3.3 Methodology :**

. In a rack of 20 test tubes 1 ml of tryptone soya broth placed in each tube , tilted in screw capped bottle . At 121°C for 15 min the media were sterilized using an autoclave ,at121<sup>0</sup>C and 15bar pressure, and left Then to cool at room temperature.

Suspensions of organisms were prepared in normal saline and compared with Macfarland turbidity. Reagents (1&2) were diluted in four, separate, set, of ten test tubes, with previously prepared tryptone soya broth ( each reagent in 2 set of tubes ), suspensions of 1 ml of organisms were added in four set of tubes (each bacterium suspension in two set of different diluted Reagent). After mixing the four set were incubated at 32<sup>0</sup>C for 24 hour.

# **Chapter Three**

## **Results and Discussion**

## 3.Results & Discussion

### 3.1 Results:

Table3.1 phytochemical screening of the Chloroform extract :

Natural Product Test	Name of Reagent	Results
Alkaloids	Wagners	+
	Hagers	+
Flavonoids	Alkaline	+
	Lead Acetate	+
Terpens	Copper Acetate	+
Phytostrols	LibermannBurehards	+

**Table3.2 phytochemical screening of the Methanol extract:-**

Natural Product Test	Name of Reagent	Results
Alkaloids	Wagners	+
	Hagers	+
Flavonoids	Alkaline	+
	Lead Acetate	+
Terpens	Copper Acetate	+
Phytostrols	LibermannBurehards	+

**Table3. Anti-bacterial activity of the extracts**

Bacteria Organism	Type of extract	%Minimum Inhibitory concentration (MIC )
Staphylococcus aureus ATCC6538 Passage No 4	Chloroform	1.25%
	Methanol	2%
Pseudomonas aeruginosa ATCC9027 Passage No 4	Chloroform	2.5%
	Methanol	2%

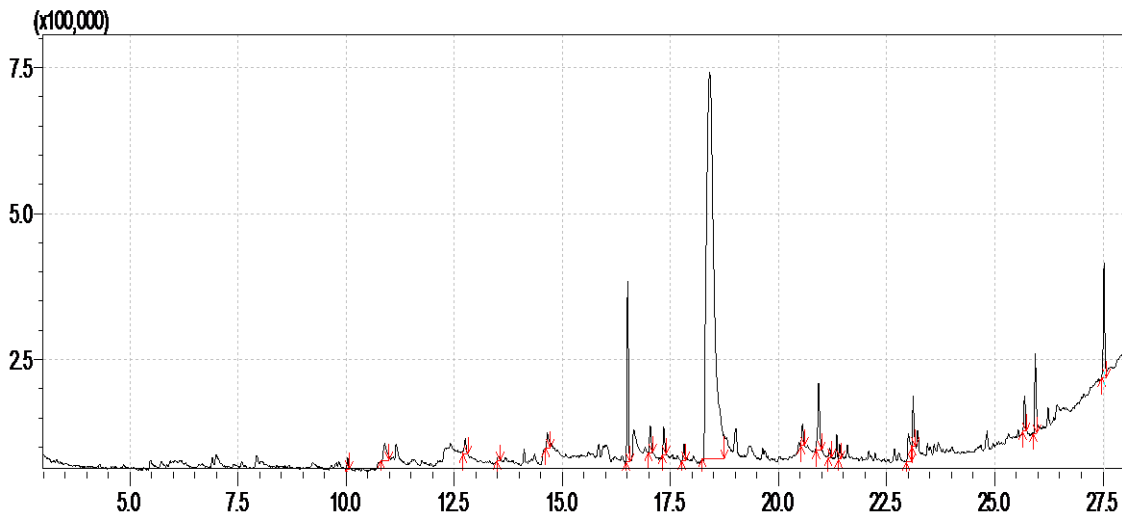
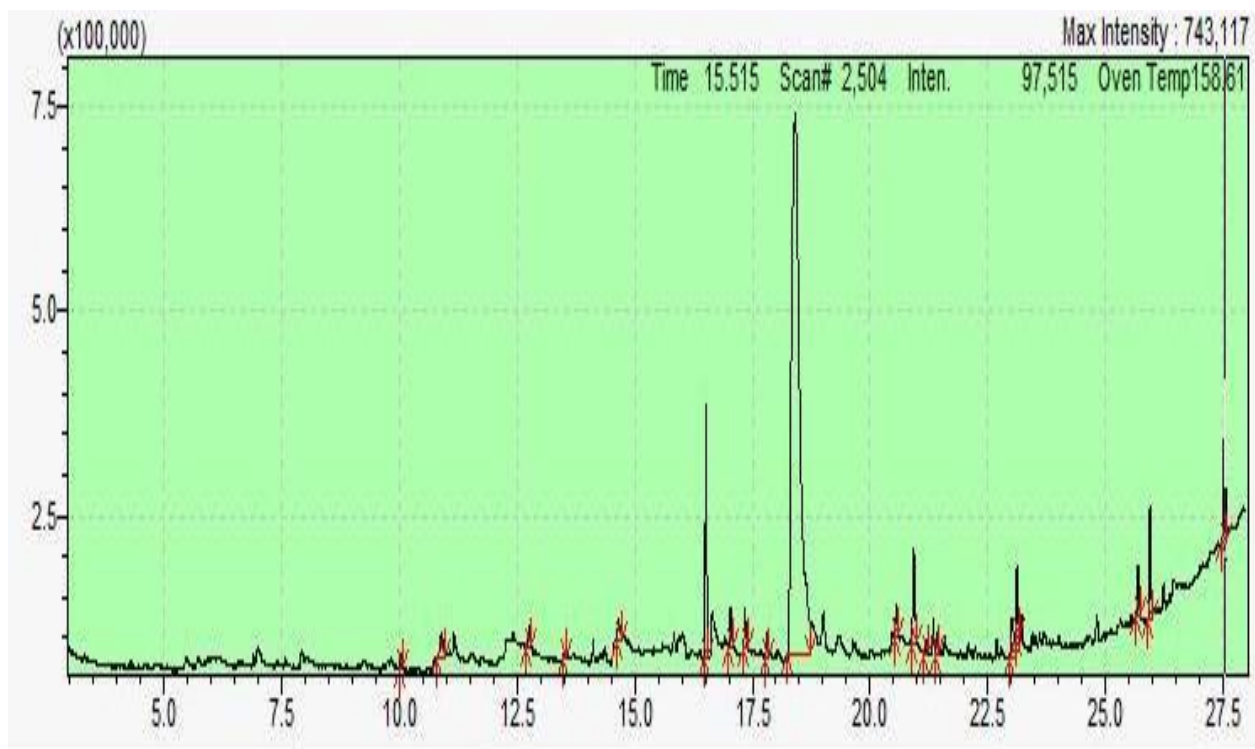


Figure3.2 GC/MS chromatogram **Guiera Senegalsis** ,dry leaves, extract.

**Table3.4 peak report TIC**

Peak	R.Time	Area	Area %	Name
1	10.035	41576	0.37	Cyclopropane,nonyl-
2	10.897	155569	1.39	Benzofuran,2,3-dihydro-
3	12.755	75651	0.68	2,Methoxy-4-vinylphenol
4	13.529	25946	0.23	Benzofuran,2,3-dihydro-
5	14.565	89836	0.80	2-(4'-Methoxyphenyl)-2-(2'-methoxyphenyl)propane
6	16.512	631889	5.66	Phenol,2,4-bis(1,1-dimethylethyl)-
7	17.034	114308	1.02	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl
8	17.345	113111	1.01	Dodecanoic acid
9	17.813	68667	0.61	Pentafluoropropionicacid,dodecyl ester
10	18.405	7985444	71.51	Quinic acid
11	20.556	90177	0.81	Tetradecanoic acid

12	20.928	354766	3.18	6-Methyl cyclodec-5-enol
13	21.180	36584	0.33	2-Cyclohexen-1-one,4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)
14	21.411	51380	0.46	Isopropyl myristate
15	23.003	229962	2.06	1H-Pyrido[3,4-b]indole,2,3,4,9-tetrahydro-1-methyl
16	23.113	186216	1.67	n-Hexadecanoic acid
17	25.688	131678	1.18	1,3Dihydrospiro[[1,3]dioxolane-2,2'-ind
18	25.938	317077	2.84	Beta-Carbolin-1-one,7-fluoro-2,3,4-trihydro
19	27.527	467561	4.19	Phenanthrene,3,9-bis (1,1-dimethylethyl)
		11167398	100.00	

**Table3.5 peak report TIC**

Peak	R.Time	Area	Area %	Name
1	17.856	3660405	13.06	Stigmasta-7,25-dien-3- ol,(3.beta.,5.alpha.)
2	18.634	3640745	12.99	17-Pentatriacontene
3	19.690	588027	2.10	Crinan,1,2-didehydro-3- methoxy-,(3.alpha.)
4	19.825	10657095	38.03	Flavone,3',4',5,6- tetrahydroxy-7-methoxy
5	20.577	393703	1.40	1,2,3,4-Butanetetrol,1- (1-phenyl-1H-pyrazol)
6	22.049	803094	2.87	Stigmasterol
7	22.551	4687101	16.72	.alpha.-Amyrin
8	22.761	3051066	10.89	Stigmast-7-en-3- ol,(3.beta.,5.alpha.,24S)-
9	22.965	543616	1.94	Fucosterol
		28024852	100.00	



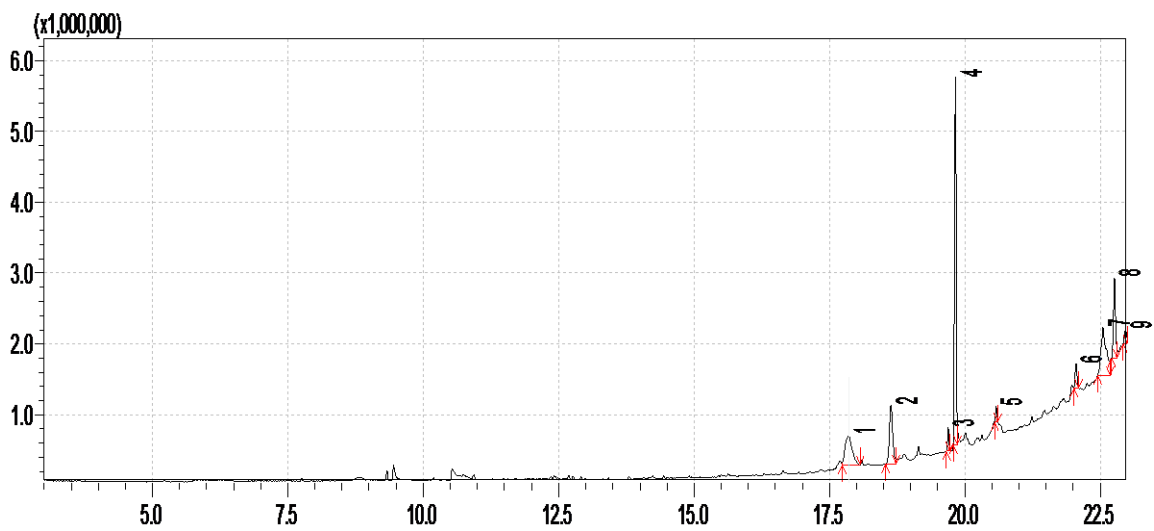


Figure3.3 Gas chromatography mass spectrometry chromatogram of the mixture extract of the *Guiera Senegensis* dry leaves.

## 3.2 Discussion:

The phytochemical screening shows the presence of natural products as the results show in Table 3.1 and 3.2. Identical quantities of alkaloids, terpenes, flavonoids and steroids were detected in the two solvents extracts which explain the high value of the use of this plant in the traditional medicine.

Antibacterial test, show significant activity against two types of bacteria, gram-positive coccal bacterium and gram-negative, rod-shaped bacterium which classify this plant as one of strong antibiotics that work in wide spectrum as shown in Table 3.3.

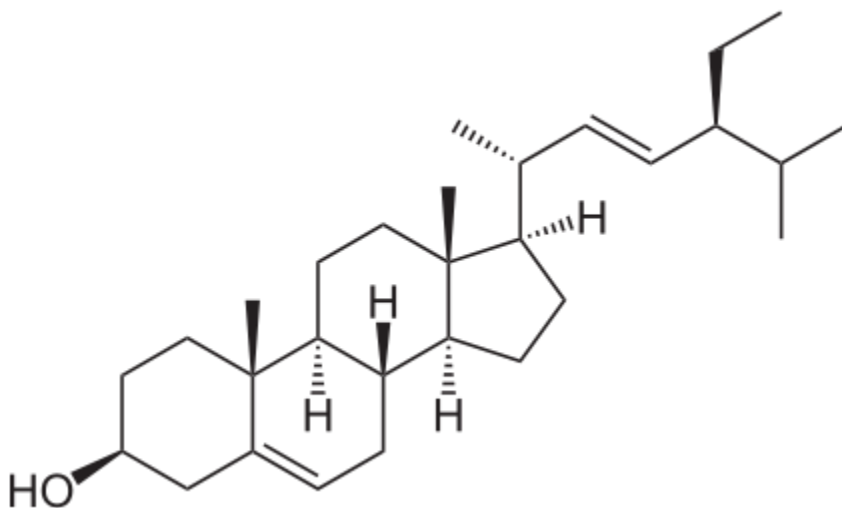
GC/MS analysis show many bioactive components as shown in Tables 3.4 and 3.5 which lead to identification of the component used of healing diabetes wound, the main objective of this study. This component is **Stigmasterol** which Upjohn company uses as the starting raw material for the synthesis of cortisone which reduces inflammations, swellings and attendant pain and at the site of injury.

One of the components of the (GC/MS) analysis is Quiniacid which is aquinine derivative used to treat malaria. This explain the antiplasmodium activity of plant extract.

These components have different activities which explain the many uses of this plant in traditional medicine.

### 3.3 Conclusion:

The bioactive component responsible for healing diabetes wound was identified as **Stigmasterol**.



### 3.4 Recommendations:

The use of traditional, herbal, medicine is recommended for the following reasons:

1. More available.
2. Less expensive
3. limited risk of contamination or expiry.
4. No side effects.

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