# *IN VITRO* ANTIASTHMATIC EVALUATION OF ETHANOL LEAF EXTRACT OF *GUIERA SENEGALENSIS* GMEL (COMBRETACEAE)

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**SPS/13/MPC/00019**

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**JUNE, 2016**

DECLARATION

I hereby declare that this work is the product of my own research efforts undertaken under the supervision of Dr. Chedi, B.A.Z. and has not been presented anywhere for the award of a degree or certificate. All sources have been duly acknowledged.

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Faruk Kassim Mandawari Date

SPS/13/MPC/00019

CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent write-up *In vitro* Antiasthmatic Evaluation of Ethanol Leaf Extract of *Guiera* s*enegalensis* by (Faruk Kassim Mandawari SPS/13/MPC/00019) were carried out under my supervision.

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APPROVAL

This dissertation has been examined and approved for the award of Master of Science in Pharmacology.

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# DEDICATION

I dedicate this work to the family of late Alhaji Nasidi Umar Mandawari and Hajiya Binta Sharubutu Koki.

# ABSTRACT

*Guiera senegalensis* is a medicinal plant that is widely used in West Africa against many illnesses. In this research, an i*n vitro* study was performed to evaluate the ethanol leaf extract of *Guiera senegalensis* (ELEGS) as antiasthmatic. The preliminary phytochemicals screening of ELEGS revealed the presence of alkaloids, anthraquinones, flavonoids, steroids, saponins and tannins. The bronchodilatory effect of ELEGS was evaluated using isolated tissues. Guinea pig trachea and ileum as well as rabbit jejunum were used to study the antihistaminergic and anticholinergic effects of the extract. ELEGS was able to significantly ($p<0.01$) reduce the tracheal smooth muscle contractions induced by histamine in a dose-dependent manner. Also, acetylcholine induced contractions of the tracheal strips were significantly reduced ($p<0.5$) by the extract. Concentration-dependent inhibitions of histamine and acetylcholine-induced contractions of guinea pig ileum by the extract were observed as well. On rabbit jejunum, ELEGS significantly ($p<0.01$) inhibited the spontaneous basal contractions as well as the acetylcholine-induced contractions. This observed effect of the extract on rabbit jejunum was significantly ($p<0.01$) inhibited by propranolol. Study on red blood cells (RBC) revealed a possible inhibition of arachidonic acid release due to the ability of ELEGS to maintain the integrity of the RBC membranes against induced hemolysis. The finding of this study is therefore an indicative of the antiasthmatic property of ELEGS possibly through the activation of $\beta_2$ adrenergic receptors and by inhibiting the release of arachidonic acid which is a precursor for the synthesis of inflammatory mediators.

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# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND TO THE STUDY

Asthma is a respiratory disease characterized by recurrent episodes of wheezing, chest tightness, cough and difficulty breathing brought about by bronchial constriction, inflammation and excessive mucus secretion due to bronchial hyper-responsiveness (GINA, 2014).

Although advancement in the treatment of asthma is on the increase, a great number of individuals have been affected with asthma. It has been estimated that about 300 million people in both developed and developing countries suffer asthma attacks worldwide (GINA, 2015). Global prevalence of asthma has been approximated to be 10% among children and 5% among adults population (Rathore *et al.,* 2011). Nigeria is not an exception and the number of its asthmatic children has been reported to be between 5.1 and 14.3% (Musa and Aliyu, 2014) while estimate for adult Nigerians having asthma has been put to 10% (Delasu, 2013).

Drugs are the major treatments given but quite a large number of patients worldwide (especially in Africa and Asia) still patronize traditional medicines as an alternative (WHO, 2013). It has been reported that 75-90% of the rural populations worldwide still depend on herbal preparations as their main source of medicines (Shettima *et al.,* 2013a). Plants contain secondary metabolites that are important sources of medicines and have been used since time immemorial to obtain cure for many diseases and great percentage of current drugs contain bioactive compounds that are derived from or modeled after plant natural products. Therefore, there is need for proper study of these

herbal remedies in order to fully exploit them. This may generate data that can be used to complement pharmaceutical drugs for the benefit of humanity. In this study, one of the most widely used plants of West Africa-*Guiera senegalensis,* has been investigated *in vitro* for its anti-asthmatic property.

## 1.2 STATEMENT OF RESEARCH PROBLEM

Prevalence of asthma has been estimated to increase by 59% worldwide in 2025 (Masoli *et al*., 2004). This can be attributed to increased industrialization and urbanization. Also, there is an issue of safety and efficacy with chronic use of current drugs. Ideal treatments should retain their efficacies whenever their need arises, but some of the current anti-asthmatic drugs, especially the $\beta_2$ agonists lost efficacy with chronic administration (Donovan *et al.,* 2014). Many toxic effects are also associated with the conventional drugs especially the corticosteroids (ICSI, 2012).

## 1.3 JUSTIFICATION

Research in traditional medicine is currently increasing worldwide and has been encouraged by the World Health Organization because of the large number of patronizers of such kind of treatment for their healthcare (WHO, 2012). There is folkloric background to the use of *Guiera senegalensis* as a medicinal plant which can be used as basis for exploitation of the plant's medicinal property. A lot of researches have been carried out on the uses of the plant and most of them turn out to give good results (Ali *et al.,* 2011). The use of the plant to treat pulmonary problems has also been documented (Sanogo, 2012). The plant could therefore be considered important in medical interventions and its potential as anti-asthmatic be exploited.

## 1.4 AIM AND OBJECTIVES

### 1.4.1 Aim

This research was aimed at evaluating the potentiality of ethanol leaf extract of *Guiera senegalensis* ELEGS as anti-asthmatic.

### 1.4.2 Objectives

The specific objectives were:

1. To study the effect of ELEGS on isolated guinea pig trachea.
2. To study the effect ELEGS on isolated guinea pig ileum.
3. To study the effect of ELEGS on isolated rabbit jejunum.
4. To study the effect of ELEGS on Red Blood Cells membrane integrity.

## 1.5 RESEARCH HYPOTHESIS

The ethanol leaf extract of *Guiera senegalensis* (ELEGS) possesses anti-asthmatic activity.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 MEDICINAL PLANTS

The use of plants for healthcare provision has been in practice since the longest recorded time in history. Asians and people of the Middle East have been known to use plants to obtain cure for several thousand years. Among the higher plants, about 70,000 species have been used in one time or another by some cultures for therapeutic purposes (WHO, 2013). These Plants that possess beneficial pharmacological effects on human's body are generally designated as medicinal plants.

The World Health Organization defines medicinal plants as plants which have been used for medical purposes at one time or another, and which, although not necessarily a product or available for marketing is the original material for herbal medicine (WHO 2015). The therapeutic properties of these plants have been attributed to the presence of natural products (phytochemicals) synthesized by these plants which are mostly secondary metabolites. Phytochemicals such as alkaloids, flavonoids, tannins, saponins, terpenoids and phenolic compounds among others are the most important of these bioactive constituents (Salihu and Usman, 2015) and they serve as important sources of modern medicine. Chemical structures derived from plants natural can be used as sources of direct therapeutic agents or models for the synthesis of new compounds. These medicinal plants also serve as taxonomic markers for the discovery of new compounds. About 40% of current drugs have been manufactured from or remodeled after natural compounds obtained from plants. Among many other drugs, as much as 60% of antibacterial and antineoplastic drugs were obtained from natural compounds which are readily available in many medicinal plants (Elhardalou, 2011).

## 2.2 *GUIERA SENEGALENSIS*

### 2.2.1 Classification

Kingdom: *Plantae*

Division: *Angiosperm*

Class: *Magnoliopsida*

Order: *Myrtales*

Family: *Combretaceae*

Genus: *Guiera*

Species: *Guiera senegalensis*

### 2.2.2 Description

The plant is a shrub that can grow to a height of 3 to 5 m according to its habitat. Its stem consists of numerous knots that send out ash-grey branches. These branches have fibrous or pubescent bark and bear opposing, short petiolated grey-green leaves that are darker on their upper surface. The leaves are opposite or sub opposite, rounded or slightly cordate at base and mucronate at the apex and are about 2 to 4 cm long by 1 to 2 cm wide. The leaves are also softly tomentos on both surfaces, with scattered black glands underneath.

Plate I: *Guiera senegalensis* in its natural habitat at Tofa Local Government Area of Kano State, Nigeria.

### 2.2.3 Distribution

The plant is widely distributed in Nigeria, Niger, Burkina Faso, Mali, The Gambia and Senegal. It is also found in Central African countries (Salihu and Usman, 2015).

### 2.2.4 Common Names

*Guiera senegalensis* has many common names. In northern Nigeria, the plant is commonly known as *Sabara* in Hausa language (Fiot *et al.,* 2006) while the Yoruba call it *Olofun* (Faisal and Habeeb, 2013).

### 2.2.5 Ethnomedicinal Uses

There is large number of available literatures that support the traditional uses of *Guiera senegalensis* as a medicinal plant in the management of many diseases. The plant is used against many health problems. For this, it is often referred to as cure-all. It is one of the plants of choice by the locals from Mauritania through West Africa down to Sudan to cure lots of ailments. Some of its traditional uses have been documented by researchers but a lot are still waiting to join the existing literature.

It is recognized as being active antitussive (Diatta *et al.,* 2007). Preparation from the roots of the plant is used to cure gastrointestinal disorders such as diarrhea and dysentery (Dambatta and Aliyu, 2011). Decoction made from the plant leaves is applied by Fulani herdsmen in Northern Nigeria to the site of snakebite (Abubakar *et al.,* 2006). The plant is traditionally considered as anticancer medication (Abubakar *et al*., 2007). Other traditional uses of the plant are for syphilis, *beriberi*, leprosy, impotence, rheumatism, diuresis and expurgation. Women in northern Nigeria use the leaves as galactagogue (Garba and Muhammad, 2008). It is also used as a general

tonic and blood restorative. The leaves are also used against respiratory diseases including asthma and bronchitis (Saraswathy *et al.,* 2014).

### 2.2.6 Some Reported Studies on the Plant

Due to the wide range of traditional uses of the plant, several studies have been conducted on it, aimed at evaluating the uses of the plant against various medical conditions. Extract from the leaves of the plant has been reported to have analgesic and anti-inflammatory as well as antibacterial effects (Mamman and Isa 2013). *In vitro* snake venom detoxifying action of the leaf extract was also reported by Abubakar *et al.,* (2006). The methanol and ethyl acetate root extracts of the plant have been reported to posses antidiarrhoeal effect (Williams *et al.,* 2009). Another study also revealed the antioxidant effect of the root extract (Sulaiman *et al.,* 2014). Gastroprotective effect of the aqueous leaf extract has also been established (Akuodor *et* al., 2013). The acute and sub-acute study on ethylacetate root extract revealed no toxic effect in mice (Shettima *et al.,* 2013b). Antifungal activity was also detected from galls of the plant (Pierre *et* al., 2013).

## 2.3 ASTHMA

### 2.3.1 Definition

Asthma is a respiratory disease characterized by recurrent episodes of difficulty breathing, cough, wheezing, and chest tightness brought about by airway resistance due to bronchial constriction, inflammation, and excessive mucus secretion; Particularly if these are frequent and recurrent; are worse at night and in the early morning; occur in response to, or are worse after, exercise or other triggers (GINA, 2014).

### 2.3.2 Types of Asthma

Atopic Asthma: This type of asthma is also referred to as extrinsic asthma and occurs as an allergic reaction due to hypersensitivity. It begins before age 6, associated with atopy, a genetic predisposition for sensitization to allergens, and an increased severity of bronchial hyper-responsiveness, which persists into adulthood.

Nonatopic Asthma: This is a recurrent airway obstruction that begins during the first two to three years of life, precipitated by upper or lower respiratory tract infection. It is not attributed to allergic reaction, exact causes are unknown. It is also known as intrinsic asthma.

### 2.3.3 Classification

Asthma has been clinically classified into four according to severity (NHLBI, 2007). These are as follows:

Mild intermittent: When the frequency of asthma attack is less than twice in a week with night time attack less than twice a month.

Mild persistent: When there is more than two attacks in a week but less than one attack daily and the occurrence of night time attack more than twice a month.

Moderate persistent: When the frequency of attack is daily and the night time attack is more than once a week.

Severe persistent: When there is continuous attack with night time attack occurring daily.

### 2.3.4 Pathophysiology of Asthma

After exposure to some specific allergens, there is development of specific antibodies known as immunoglobulin E antibodies (IgEs). Some of these IgEs circulate in the blood, but most become attached to the surface of mast cells present in nasal cavity and bronchial tissue by tightly binding to Fc$\varepsilon$RI receptors on the surface of the mast cells (Stone *et al*., 2011). By this, the mast cells are sensitized. Subsequent exposure to the same allergens results in degranulation of the mast cells to release contents of their granules into nearby tissues. The substances released are termed preformed mediators and include histamine, serotonin, heparin, bradykinin as well as enzymes like phospholipase $A_2$ ($PLA_2$), tryptase and kymase. Histamine is a potent bronchocontrictor and is responsible for the early phase reaction. It binds to the $H_1$ receptors in the airways and mediates bronchospasm. $PLA_2$ metabolizes membrane phospholipids in the airways to generate arachidonic acid which is shunted into the cyclooxygenase and lipo-oxygenase pathways to generate the mediators of the late phase. These include prostaglandins especially $PGD_2$, thromboxanes, leukotrienes, cytokines and eisonophil chemotactic factors (ECF) altogether termed as newly-formed mediators. Interactions of these cells and inflammatory mediators causes smooth muscle contraction and hypertrophy, leakage of the micro vascular walls,

oedema of the bronchial wall, neuronal activation in the airway, airway hyperresponsiveness, increased secretion of mucus leading to plugging of the airways, disruption of the airway epithelium, and ultimately causes widespread airflow limitation.

### 2.3.5 Triggers of Asthma

Asthmatic attack can be initiated by numerous triggers. These include allergens such as pollen, animal dander, dust mites as well as environmental irritants like smoke, chemical fumes, cold/warm/moist air, perfumes etc. There are also dietary triggers such as food allergens such as peanut, shell fish, soy and some food additives (GAN, 2014). Medications such as non-selective β blocker (propranolol) and NSAIDs can also trigger asthmatic attack. Viral upper-respiratory infections such as colds and flu as well as bacterial infections such as pneumonia can also trigger asthma. Others are exercise or physical exertion, stress, emotion such as laughing, fear and anger**.**

### 2.3.6 Risk Factors

These are factors that increase the predisposition of having asthma. They are not the causes per se, but can increase the chance of being affected by the disease (Sears *et al*., 2001). Numerous of such factors have been identified which include:

i. Family history of allergic conditions (genetics of asthma)

ii. Personal history of allergic rhinitis

iii. Viral respiratory illness in childhood

iv. Environment

v. Gender (mostly females)

vi. Age (children and middle aged individuals)

vii. Obesity

viii. smocking

### 2.3.7 Conventional Treatment

Over the past few decades, there have been significant scientific advances leading to improved understanding of asthma and better management. Treatment is designed to counteract the effects of autacoids and other substances implicated in the etiology of asthma. Several drugs have been used and the aim is to treat symptoms and prevent recurrence. Currently, the antiasthmatic medications have been grouped into the following.

$\beta_2$ Adrenergic Agonists: For the management of asthma, adrenergic agonists that are selective for $\beta_2$ receptors are preferred to less selective agents. These drugs are grouped into two according to their onset of action. Salbutamol, Levalbuterol, Fenoterol and Terbutaline are short-acting while Formoterol and Eformoterol are long-acting.

*Mechanism of Action*: They cause smooth muscle relaxation by binding to $\beta_2$ adrenoceptors, a GDP-bound heptameric trans-membrane G-protein coupled receptors. These receptors compose of three sub-units- alpha, beta and gamma. Activation of these receptors leads to exchange of GDP for GTP. Adenyl cyclase will then catalyze the formation of cAMP which serves as second messenger which binds

to and activate protein kinase A (cAMP-dependent kinase) which subsequently phosphorylate the target protein leading to pharmacological effect (Giovanni, 2010).

*Side Effects*: Despite the selectivity of sympathomimetics, they are not devoid of systemic adrenergic effects like tremors, arrhythmias and palpitations. They also cause hypokalemia and hyperglycaemia. Regular use of $\beta_2$ agonists is also associated with reduced sensitivity of the adrenoceptors (Harold, 2005).

Corticosteroids: These are potent inhibitors of inflammatory processes and are widely used in the treatment of asthma. They inhibit many inflammatory molecules such as cytokines, chemokines, arachidonic acid metabolites as well as adhesion molecules. Administration of these drugs inhibits bronchial inflammation and improves lung function. Examples include Fluticasone, Beclomethasone, Budenoside, Triamcinolone, Ciclesonide, Mometasone and Flunisolide all of which are aerosols. The systemic includes Betamethasone, Prednisolone, prednisone, panafcortelone and Hydrocortisone

*Mechanism of Action*: They repress the expression of pro-inflammatory proteins in the cytosol by preventing the translocation of transcription factors from the cytosol to the nucleus. Histone deacetylase (HDACs) are recruited to the site of active inflammatory gene transcription to inhibit the acetylation of core histones that are necessary for inflammatory gene transcription (Michael *et al*., 2009).

*Side Effects*: Long-term use of corticosteroids is associated with hypothalamic-pituitary-adrenal axis suppression. Inhaled steroids are associated with local effects such as hoarseness of voice, dysphonia, cough, and oral candidiasis.

Antimuscarinics: These agents have the ability to completely block acetylcholine released from apparent endings of the vagus nerves on airway smooth muscles. Although these drugs are only capable of preventing cholinergic-mediated bronchoconstriction, they can produce considerable bronchodilation. They also inhibit excessive secretion of mucus leading to near normal exhalation. There are many available antimuscarinics but very few are used in asthma because of their tendency to quickly reach the systemic circulation. Ipratropium and Tiotropium are the most widely used antimuscarinics for asthma (Ruben, 2007).

*Mechanism of Action*: The cholinergic receptor subtype responsible for bronchial smooth muscle contraction is $M_3$ receptor. These agents have the ability to block all muscarinic receptors but their affinity is more on the $M_3$ whose antagonism accounts for brochodilatory effects of the drugs.

*Side Effects*: Most side effects are tolerable and localized with dry mouth and pharyngeal irritation being the most experienced. If systemic absorption occurs, there is urinary retention, blurred vision and tachycardia.

Methylxanthines: These drugs have both bronchodilator effect and anti-inflammatory action. Use of these drugs has waned due the availability of inhaled synpathomemetics and corticosteroids. The most widely used among this class are Aminophylline and Theophylline. Theophyline also increases the availavity of histone deacetylases in epithelial cells (Barnes, 2013).

*Mechanism of Action*: They produce direct bronchodilation by their ability to inhibit several members of the phosphodiesterase family of enzymes (especially PDE4). These enzymes are responsible for cyclic nucleotides (cAMP and cGMP) hydrolysis.

Their inhibition results in higher concentrations of intracellular cAMP which is required to achieve bronchodilation. They also inhibit cell surface adenosine receptors. These receptors modulate adenylyl cyclase activity, and adenosine has been shown to cause contraction of isolated airway smooth muscle and to provoke histamine release from airway mast cells (Barnes, 2013). These effects are antagonized by theophylline, which blocks cell surface adenosine receptors.

*Side Effects*: Methylxanthines have narrow therapeutic margin and therefore requires monitoring of therapeutic levels especially in the elderly. Their side effects include gastrointestinal upset in mild toxicity and serious cardiac arrhythmias seen in high blood levels.

Mast cell stabilizers: These drugs have the potency of inhibiting the release of contractile agonists from the mast cells by making them stable. However, these drugs have no effect on airway smooth muscle tone and are ineffective in reversing bronchoconstriction (Mombeini *et al*., 2012). Therefore, they are only of value when taken prophylactically. Available drugs in this class include Cromolyn sodium, Nedocromil sodium and Ketotifen.

*Mechanism of Action*: By coating the surface of the mast cells they bring about alterations in the function of chloride channels in the cell membrane thereby inhibiting cellular activation. This confers rigidity to mast cells which prevents the cells from bursting to release their contents.

*Side Effects*: Adverse effects of cromolyn and nedocromil are minor and are localized to the sites of deposition. These include throat irritation, reversible dermatitis,

myositis, or gastroenteritis. Serious adverse effects are rare and this accounts for cromolyn's widespread use in children, especially those at ages of rapid growth.

Leukotriene modulators: Leukotrienes result from the action of 5-lipoxygenase on arachidonic acid and are synthesized by a variety of inflammatory cells in the airways, including eosinophils, mast cells, macrophages, and basophils. Leukotriene ($LTB_4$) is a potent neutrophil chemo-attractant, and $LTC_4$ and $LTD_4$ exert many effects known to occur in asthma, including bronchoconstriction, increased bronchial reactivity, mucosal oedema, and mucus hypersecretion (Ajay, 2010). Thus, drugs in this class prevent the effects of leukotrienes. Examples include Leukotriene receptor antagonists (Montelukast and Zafirlukast) and Leukotriene synthesis inhibitors (Zileuton).

*Mechanism of Action*: These drugs act by interrupting the leukotriene pathway. Zileuton inhibits 5-lipoxygenase, thereby preventing leukotriene synthesis while Montelukast and Zafirlukast Inhibit the binding of $LTD_4$ to its receptor on target tissues to prevent its action.

*Side Effects*: There is occasional liver toxicity, nausea and headache.

Anti-immunoglobulin Monoclonal Antibodies: Immunoglobulins are responsible for the initiation of cascade of reactions leading to the degranulation of mast cells. These drugs are designed to render these immunoglobulins ineffective in sensitizing mast cells. They are entirely new approach to the treatment of asthma. Example is Omalizumab

*Mechanism of Action*: Omalizumab (anti-immunoglobulin Mab) inhibits the binding of immunoglobulins to mast cells and thus preventing them from provoking mast cell degranulation.

*Side Effects*: These include joint pain, muscle pain, ear pain, dizziness, skin rash, bone fractures.

### 2.3.8 Plants with Reported Anti-asthmatic Properties

Many plants have been reported to have antiasthmatic effects and are used traditionally to relieve symptoms of asthma. Some of these plants are presented in table 1below.

**Table 1: Plants with Reported Anti-asthmatic Properties**

| Plant Name | Part Used | Reference |
|---|---|---|
| *Adhatoda schimperiana* | Leaves | Ashenafi *et al*., 2008. |
| *Aerva lanta* | Aerial Parts | Taur and Patil, 2011 |
| *Ailanthus excels* | Stem Bark | Danish *et al*., 2010 |
| *Balanites aegyptiaca* | Fruits | Patil *et al*., 2011 |
| *Bauhinia racemosa* | Leaves | Nirmal *et al*., 2011 |
| *Clerodendrum phlomidis* | Leaves | Vincent *et al*., 2013 |
| *Cordia subcordata* | Leaves | Pandi *et al*., 2011 |
| *Euphorbia hirta* | Whole Plant | Shelke *et al*., 2014 |
| *Hemidesmus indicus* | Leaves | Malviya *et al*., 2011 |
| *Labisia pumila* | Leaves | Okechukwu and Ekeuku, 2012 |
| *Leucas aspera* | Whole Plant | Limbasiya *et al*., 2012 |
| *Limonia acidissima* | Pulp | Kumar *et al*., 2014 |
| *Physalis angulata* | Leaves | Rathore *et al*., 2011 |
| *Pistacia integerrima* | Galls | Adusumalli *et al*., 2013 |
| *Scoparia dulcis* | Leaves | Ofori-Amoah and Koffuor, 2015 |
| *Tragia plukenetii* | Whole Plant | Kalaivanan and Louis, 2012 |

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 MATERIALS

### 3.1.1 Apparatus

The apparatus used were microdynamometer, organ bath, aerator, weighing balance, oven, glassware, syringes, surgical instruments, thermostat, centrifuge, spectrophotometer, refrigerator and micropipette.

### 3.1.2 Drugs

The drugs were procured locally, purported to be of analytical grades from the assigned companies. They were Acetylcholine Chloride (Sigma Chemicals, USA), Histamine Diphosphate (Sigma Chemicals, USA), Atropine Sulphate (Shandong Shenglu Pharmaceutical Co., Ltd.), Isoprenaline Hydrochloride (Sigma Chemicals, USA), Propranolol and Mepyramine Sigma (St. Louis, MO, USA).

### 3.1.3 Laboratory Animals

Adult Swiss rabbits weighing (3.0-3.5 kg) and guinea pigs (400-450 g) were obtained from the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria**.** The animals were kept at the Animal House under favourable conditions of housing and caging prior to the study. Water and feed were provided *ad libitum* according to the ethical guidelines of Ahmadu Bello University for the care and handling of animals in research.

### 3.1.4 Chemicals/Reagents

The chemicals used were Sodium Hydrogen Carbonate (BDH Chemicals Ltd Poole, England), Sodium Chloride (Johnson and Solomon Ltd. London, England), D-glucose (BDH Chemicals Ltd. Poole, England), Calcium Chloride (BDH Chemicals Ltd. Poole, England), Sulfuric Acid, Ferric Chloride, Acetic Anhydride and Ethanol.

## 3.2 METHODS

### 3.2.1 Collection, Identification and Preparation of Plant Material

Collection and Identification: Fresh leaves of *Guiera senegalensis* were obtained from Tofa Local Government Area in Kano State of Nigeria. Authentication of the plant was carried out at the herbarium of Biological Sciences Department, Bayero University Kano where a voucher number (BUKHAN0032) was assigned for reference.

Preparation of Plant Material: The plant material was cleaned, air dried under the shade and pulverized into fine powder using mortar and pestle. The powder was then macerated in 70% ethanol for two weeks after which it was filtered using cloth as a mesh and then through Whatman® Filter Paper No.1, 185 mm. The filtrate was placed in an oven at $40^0$c to concentrate it. Dark brown Ethanol Leaf Extract of *Guiera senegalensis* ELEGS was finally obtained and stored in clean container until needed for the studies.

### 3.2.2 Phytochemicals Study

The phyto-chemicals screening of ethanol leaf extract of *Guiera senegalensis* was carried out to determine the presence of secondary metabolites such as alkaloids, steroids and flavonoids as described by Tiwari *et al.,* (2011).

### 3.2.3 Histamine-induced Contractions of Guinea Pig Trachea

Healthy overnight fasted adult guinea pigs (400-450 g) were sacrificed and the tracheae removed and placed in physiological solution. The individual cartilaginous segments of the trachea were cut to form rings which were later joined by threads and mounted in 25 ml organ baths containing physiological solution maintained at $37^0$C and constantly aerated. Loads of 1g was applied and the tissues were allowed to stabilize before any studies. Effects of graded concentration of ELEGS (2, 4 and 8 mg/ml) on the tissues were studied, so also the effects of histamine (0.6 µg/ml). Effect of histamine (0.6 µg/ml) was studied alone on the tissue and then in the presence of graded concentrations of ELEGS (2, 4, and 8 mg/ml). Responses of the tissues were recorded using microdynanometer (Kulkarni, 2005).

### 3.2.4 Acetylcholine-induced Contractions of Guinea Pigs Trachea

In this experiment, the same procedure was followed as in 3.2.3 above where the method of Kulkarni, (2005) was adopted. The only dividing line was that the contractile agonist used was acetylcholine 0.4 µg/ml. Effect of ELEGS (2, 4 and 8 mg/ml) in the presence of fixed concentration of acetylcholine 0.4 µg/ml was then studied. Tissue responses were observed and recorded using microdynanometer.

### 3.2.5 Histamine-induced Contractions of Guinea Pig Ileum

Ileal strips from guinea pigs were prepared and mounted in 25 ml organ baths containing physiological solution maintained at $37^0$C and constantly aerated. Loads of 1g each was applied and the tissues were allowed to stabilize. Effects of graded doses of ELEGS (0.4, 0.8, 1.6 and 3.2 mg/ml) and that of histamine (0.04 µg/ml) on the tissues were studied separately. Effects of the graded concentrations of the extract were then studied in the presence of histamine (0.04 µg/ml) as described by Kulkarni (2005).

### 3.2.6 Acetylcholine-induced Contractions of Guinea Pig Ileum

Tissues were isolated from guinea pig ileum as in 3.2.5 above. Effects of graded doses of ELEGS (0.4, 0.8, 1.6 and 3.2 mg/ml) on the tissues as well as the effects of acetylcholine (0.04 µg/ml) were studied. Interactive studies were then performed to observe the effects of the graded concentrations of the extract in the presence of acetylcholine (0.04µg/ml). Responses of the tissues were recorded using microdynanometer (Kulkarni 2005).

### 3.2.7 Acetylcholine-induced Contractions of Rabbit Jejunum

Method of Amos *et al.,* (2000) was used to carry out these studies. Healthy overnight fasted adult rabbits (2.0 - 2.5 kg) were sacrificed humanly and the abdomen opened. Segments of the rabbit jejunum of about 2 – 3 cm long were removed and mounted in 25 ml organ baths containing physiological solution maintained at $37^0$c. Graded concentrations of ELEGS (0.8, 1.6, 3.2 and 6.4 mg/ml) were then interacted with fixed concentration of acetylcholine (0.04 µg/ml).

### 3.2.8 Action of Propranolol on the Activity of Ethanol Leaf Extract of *Guiera senegalensis* on Rabbit Jejunum

The action of propranolol on the relaxant effect of the extract was studied based on the method described by Amos *et al*., (2000), about 2 cm tissues were cut and mounted in 25 ml organ bath containing physiological solution maintained at $37^0$c. Effect of ELEGS 6.4 mg/ml was studied before and after addition of propranolol (0.02 µg/ml) using Isoprenaline as a reference standard drug.

### 3.2.9 Membrane Stabilization Assay

Bovine Red Blood Cells (RBC) were obtained from slaughter house and made into 5% suspension. 0.1 ml of 50 mM $H_2O_2$ was added to 1 ml of the RBC suspension and incubated at $37^0$c to initiate hemolysis. After 30 min, 4 ml distilled water was added to the mixture and centrifuged at 1000 rpm for 10 min. Absorbance of the supernatant was then taken at 540 nm. This procedure was repeated in the presence of the extract (0.1, 0.2, 1 and 2 mg/ml) and percentage inhibition of hemolysis was calculated (Su *et al*., 2009).

## 3.3 Data Analysis and Results Presentation

Obtained data were analyzed using one way analysis of variance (ANOVA), followed by Dunnett's post-hoc tests where values of $p \leq 0.05$ were considered statistically significant. Results were expressed as Mean ± standard error of mean (SEM) or percentages and presented in tables and charts.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 YIELD OF ETHANOL LEAF EXTRACT OF *GUIERA SENEGALENSIS*

500g of the pulverized leaves of *Guiera senegalensis* was macerated for two weeks in three litres of 70% ethanol. The mixture was stirred twice daily to facilitate the release of metabolites into the solvent. About 96g (dry weight) dark coloured ethanol leaf extract of *Guiera senegalensis* was obtained, equivalent to 19.2% w/w yield (Fig. 1).

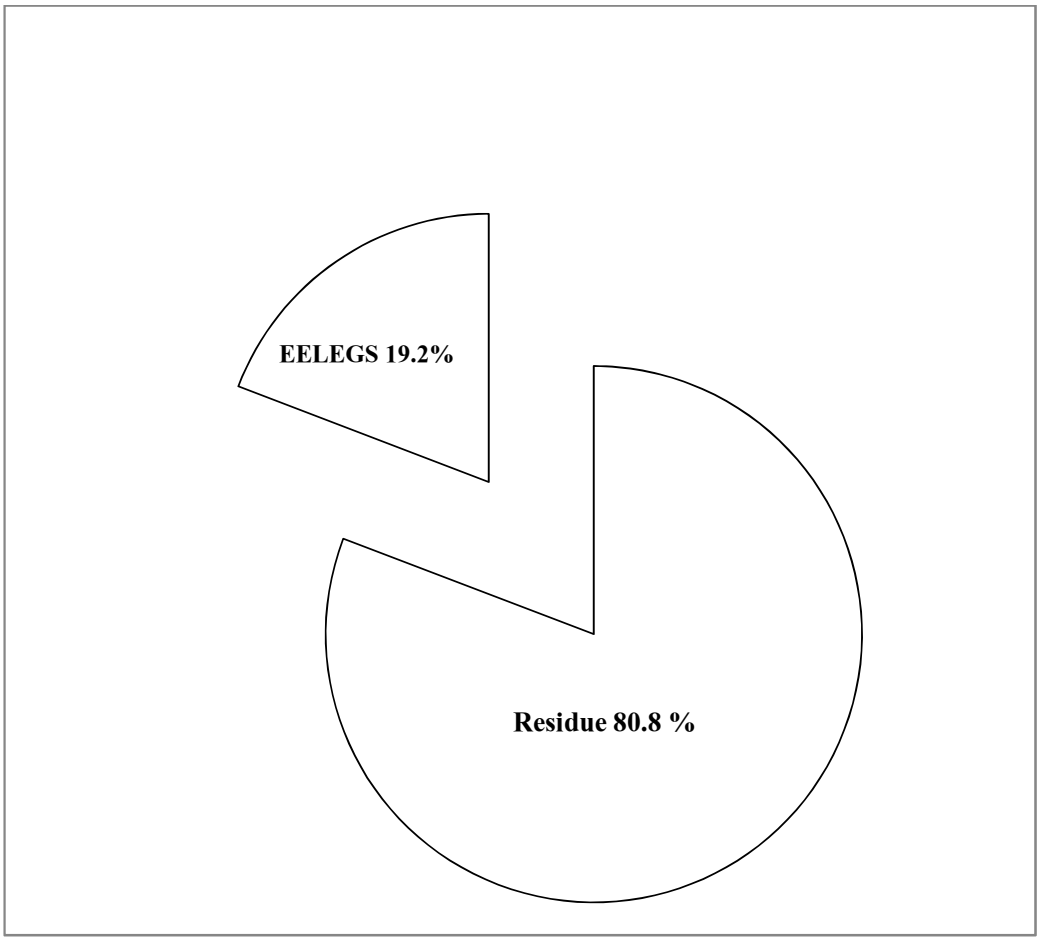


**Figure** 1**:** % Yield of ethanol leaf extract of *Guiera senegalensis*

## 4.2 PHYTOCHEMICAL CONSTITUENTS OF ETHANOL LEAF EXTRACT OF *GUIERA SENEGALENSIS*

In the preliminary qualitative phytochemical screening of ELEGS a total of nine secondary metabolites were tested for. The analyses revealed the presence of alkaloids, anthraquinones, flavonoids, saponins, steroids and tannins (Table 2).

**Table 2: Phytochemical Constituents of Ethanol Leaf Extract of *Guiera senegalensis***

| Constituent | Inference |
|---|---|
| Alkaloids | + |
| Anthraquinones | + |
| Cardiac glycosides | - |
| Flavonoids | + |
| Phlabotannins | - |
| Saponins | + |
| Steroids | + |
| Tannins | + |
| Terpenoids | - |

Key: (+) present (-) absent

## 4.3 ISOLATED TISSUES STUDIES

### 4.3.1 Effects of Ethanol Leaf Extract of *Guiera senegalensis* on Histamine-induced Contractions of Guinea Pig Trachea

The trachea was contracted using histamine (0.6 µg/ml) and graded concentrations of ELEGS (2, 4, and 8 mg/ml) produced significant (*p<0.5, **p<0.01) dose-dependent inhibition of the histamine-induced contractions. ELEGS 8 mg/ml was comparable to Isoprenaline (0.4 µg/ml) used as standard (Fig. 2).

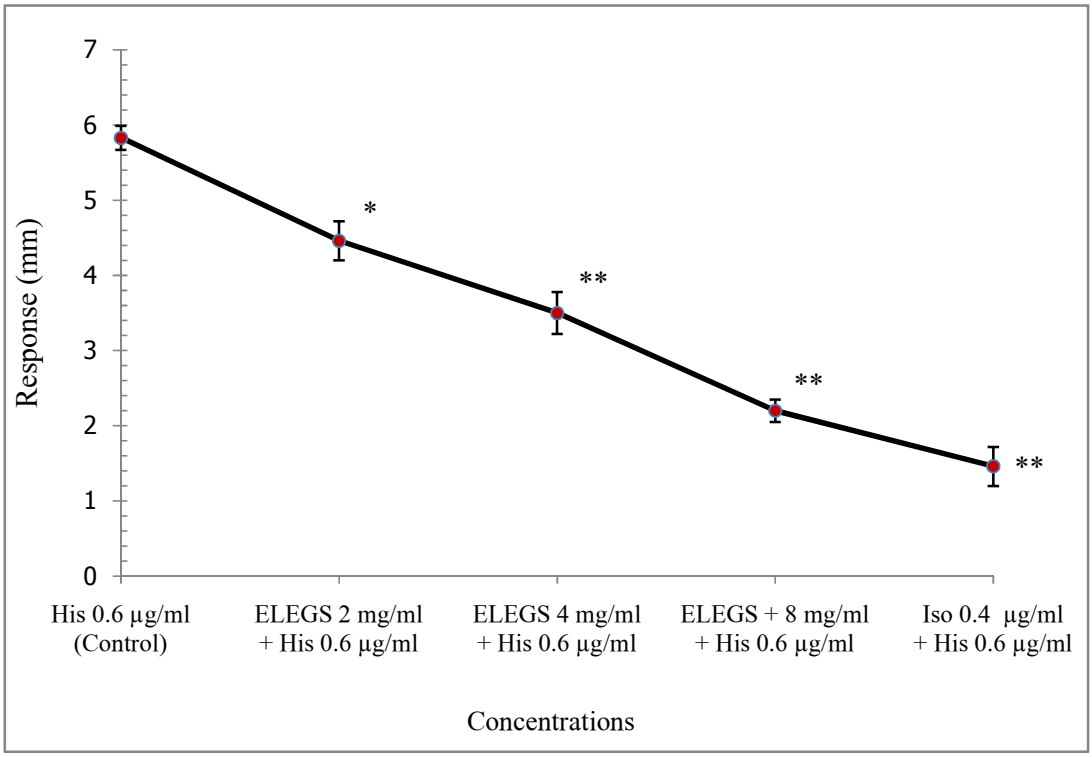


**Figure 2:** Effect of ethanol leaf extract of *Guiera senegalensis* on histamine-induced contractions of guinea pig trachea**.** Values represent mean ± SEM (n=3). *p<0.05, **p<0.01 when compared with control using one way ANOVA followed by Dunnett's test. Key: His =Histamine; Iso = Isoprenaline; ELEGS = ethanol leaf extract of *Guiera senegalensis*

### 4.3.2 Effects of Ethanol Leaf Extract of *Guiera senegalensis* on Acetylcholine-induced Contraction of Guinea Pig Trachea

Three graded doses of ELEGS (2, 4 and 8 mg/ml) were used in the presence of acetylcholine (0.4 µg/ml) on isolated guinea pig trachea. No significant inhibitions of tracheal contractions were observed with 2 and 4 mg/ml extract but 8 mg/ml produced significant (*p<0.01) inhibition of the contractions induced by acetylcholine 0.4 µg/ml which is comparable to isoprenaline 0.4 µg/ml (Fig.3).

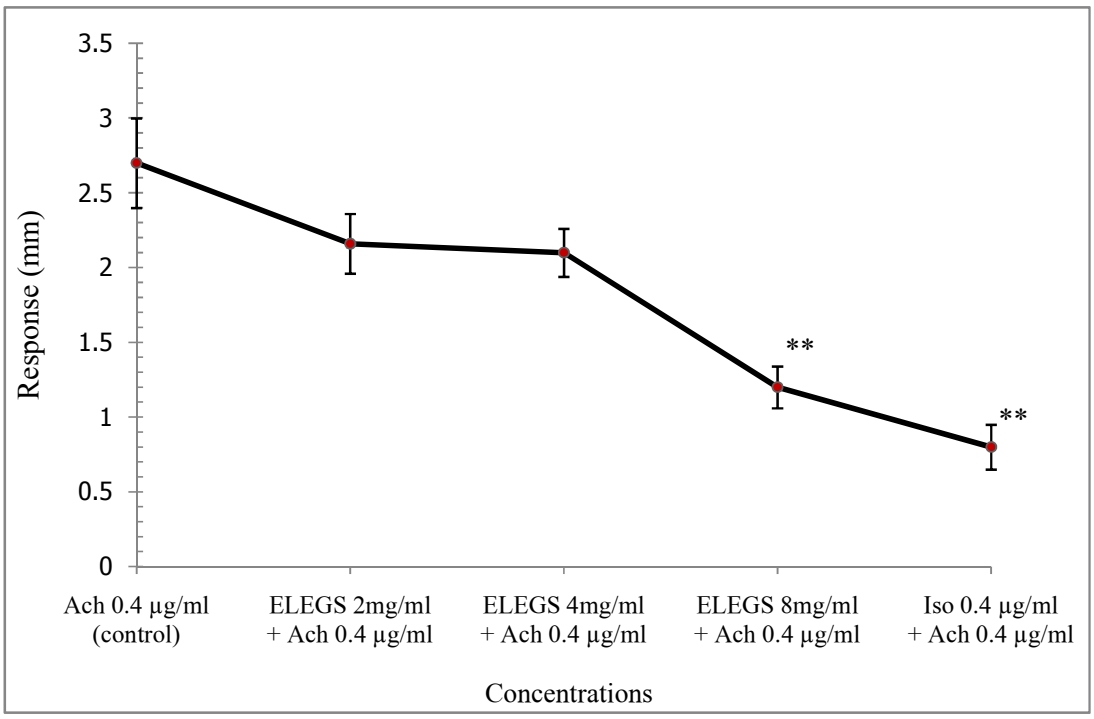


**Figure 3:** Effect of ethanol leaf extract of *Guiera senegalensis* on acetylcholine-induced contractions of guinea pig trachea. Values are expressed as mean ± standard error of the mean (n=3), *p<0.01 when compared with control using one way ANOVA followed by Dunnett's post-hock. Key: Ach =Acetylcholine; Iso = Isoprenaline; ELEGS = ethanol leaf extract of *Guiera senegalensis*

### 4.3.3 Effect of Ethanol Leaf Extract of Guiera senegalensis on Isolated Guinea Pig Ileum

ELEGS (0.4, 0.8, 1.6 and 3.2 mg/ml) produced dose-dependent relaxation of isolated guinea pig ileum. Responses were all significant (*p<0.05, **p<0.01) compared to the baseline (0 mm) which served as the control. Maximum relaxation was obtained at 3.2 mg/ml of ELEGS (Fig. 4).

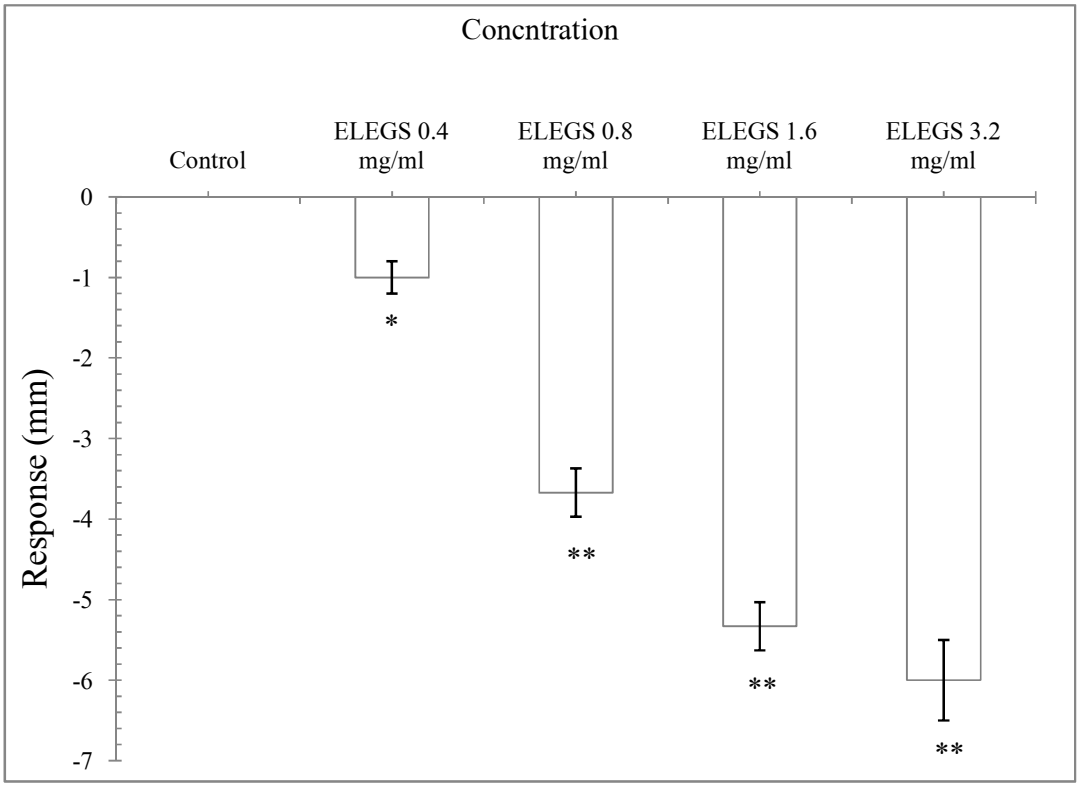


**Figure 4:** Effect of ethanol leaf extract of *Guiera senegalensis* on isolated guinea pig ileum. Values are expressed as mean ± standard error of mean (n = 3). *p<0.05, **p<0.01 when compared with control using one way ANOVA followed by Dunnett's test. Key: ELEGS = ethanol leaf extract of *Guiera senegalensis*

### 4.3.4 Effect of Ethanol Leaf Extract of *Guiera senegalensis* on Histamine-induced Contractions of Guinea Pig Ileum

Significant (**p<0.01, ***p<0.001) dose-dependent inhibition of contractions induced by histamine (0.04 µg/ml) on isolated guinea pig ileum was produced by ELEGS (0.4, 0.8 and 1.6 mg/ml). Complete inhibition followed by relaxation was observed at the concentration of 1.6mg/ml ELEGS. Mepyramine (0.04 µg/ml) was used as a standard antihistamine which also significantly inhibited histamine (0.04 µg/ml) (Fig. 5).

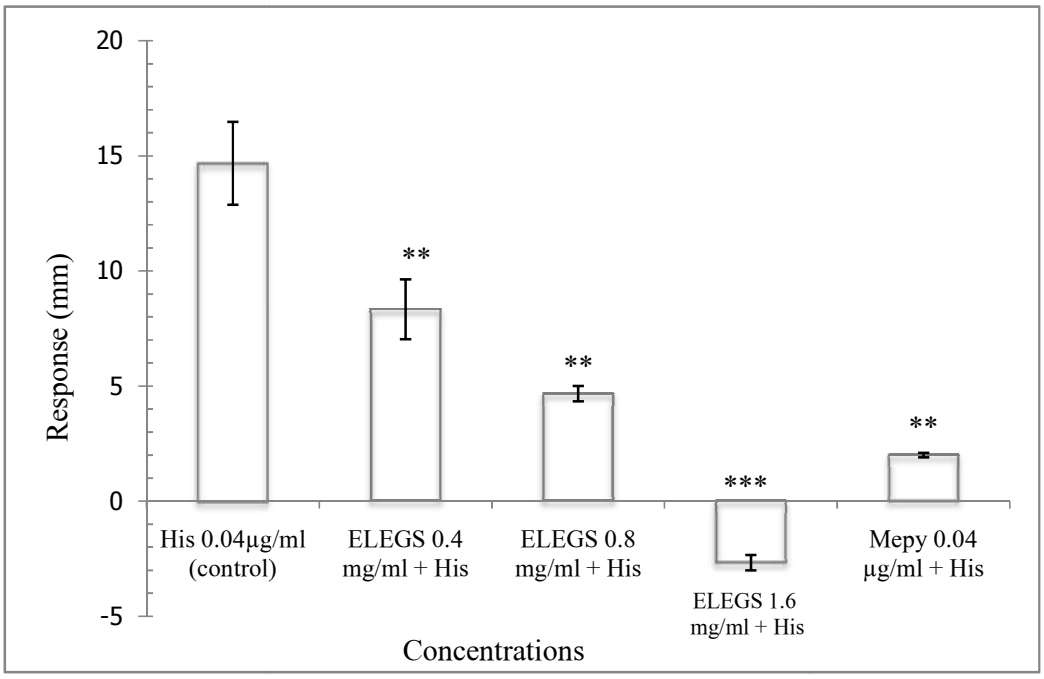


**Figure 5:** Effect of ethanol leaf extract of *Guiera senegalensis* on histamine-induced contraction of guinea pig ileum. Values are expressed as mean ± standard error of the mean (n=3). **p<0.01, ***p<0.001 when compared with control using one way ANOVA followed by Dunnett's test. Key: His = Histamine; Mep = Mepyramine; ELEGS = ethanol leaf extract of *Guiera senegalensis*

### 4.3.5 Effect of Ethanol Leaf Extract of *Guiera senegalensis* on Acetylcholine-induced Contraction of Guinea Pig Ileum

Acetylcholine-induced ileal contractions were significantly (*p<0.05, **p<0.01, ***p<0.001) inhibited by ELEGS dose-dependently. Relaxation of the ileal smooth muscles was observed at concentration of 3.2 mg/ml ELEGS. Atropine (0.04 µg/ml) was used as standard anticholinergic drug which also produced significant inhibition of the contractions (Fig. 6).

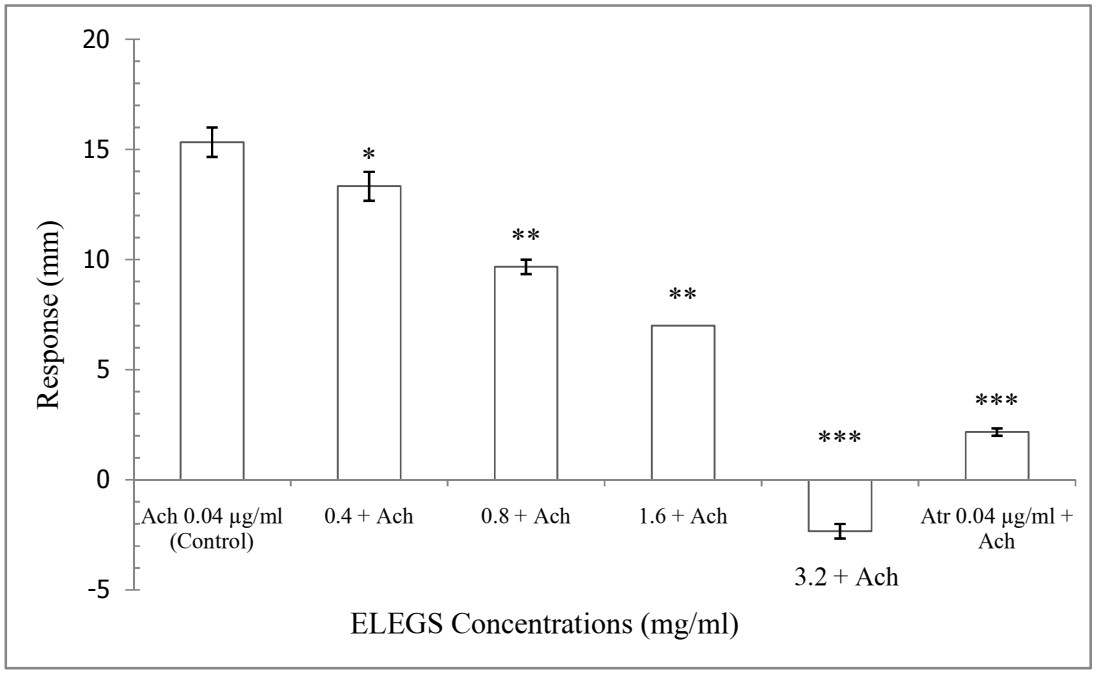


**Figure 6:** Effect of Ethanol leaf extract of *Guiera senegalensis* on acetylcholine-induced contraction of guinea pig ileum. Values are expressed as mean ± standard error of the mean (n = 3). *p<0.05, **p<0.01, ***p<0.001 compared to the control using one way ANOVA followed by Dunnett's test. Key: Ach = Acetylcholine; Atr = Atropine; ELEGS = ethanol leaf extract of *Guiera senegalensis*

### 4.3.6 Effect of Ethanol Leaf Extract of *Guiera senegalensis* on Rabbit Jejunum

Dose-dependent inhibition of the spontaneous basal contractions of isolated rabbit jejunum was observed when ELEGS was used alone in the bath. Both the amplitude and the frequency of contractions were significantly (*p<0.05, **p<0.01) reduced by grade concentrations of the extract (0.8, 1.6, 3.2 and 6.4 mg/ml) (Fig. 7).

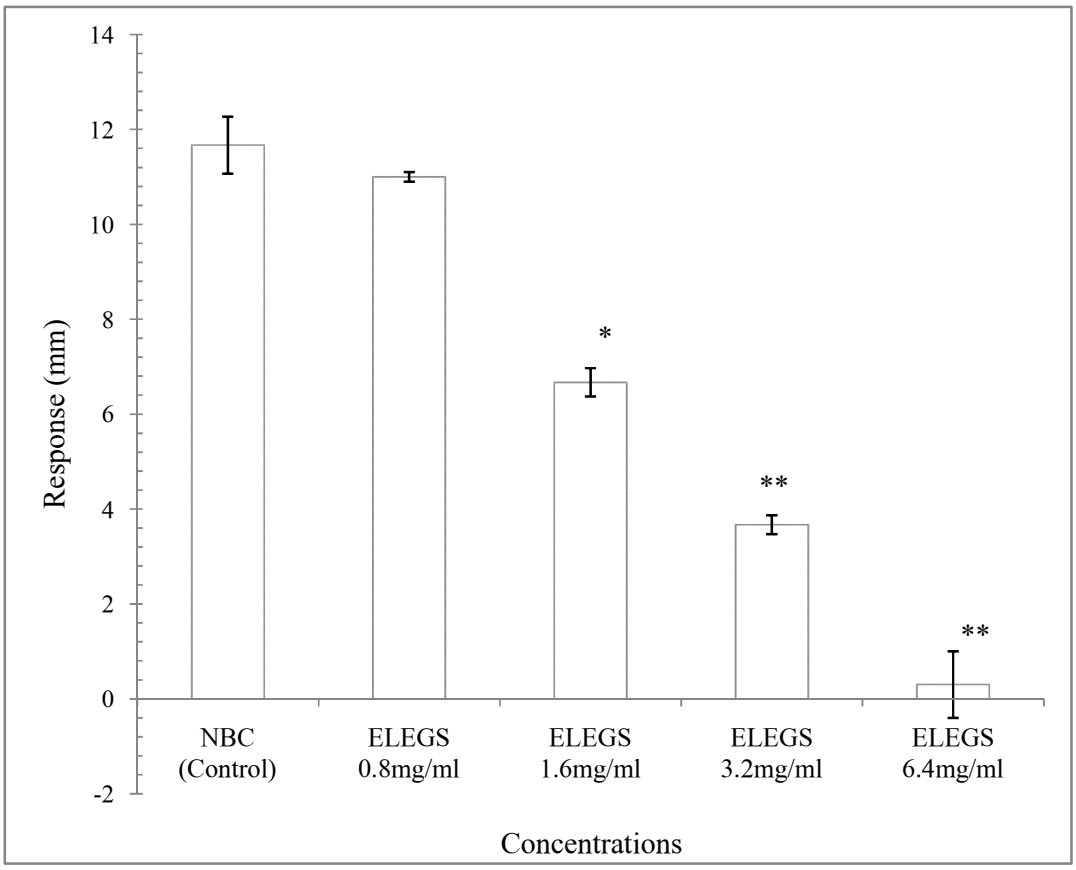


**Figure 7:** Effect of ethanol leaf extract of *Guiera senegalensis* on isolated rabbit jejunum. Values are expressed as mean ± standard error of mean (n = 3). *p<0.05, **p<0.01 compared to the control using one way ANOVA followed by Dunnett's test. Key: ELEGS = ethanol leaf extract of *Guiera senegalensis*; NBC = normal basal contractions

### 4.3.7 Effect of Ethanol Leaf Extract of *Guiera senegalensis* on Acetylcholine-induced Contractions of Rabbit Jejunum

ELEGS produced significant (**p<0.01, ***p<0.001) dose-dependent inhibition of the contractions induced by acetylcholine (0.04 µg/ml) on isolated rabbit jejunum. The extract 6.4mg/ml was comparable to atropine (0.04 µg/ml) in reducing the amplitude of the contractions but exhibited more inhibitory effect on the frequency of the contractions than atropine (Table 3).

**Table 3: Effects of ELEGS on Acetylcholine-induced Contractions of Rabbit Jejunum**

| Treatment | Responses (mm) |
|---|---|
| Ach 0.04 µg/ml (control) | 65.00 ± 4.5 |
| ELEGS 0.8 mg/ml + Ach 0.04 µg/ml | 47.33 ± 3.52** |
| ELEGS 1.6 mg/ml + Ach 0.04 µg/ml | 38.33 ± 3.33** |
| ELEGS 3.2 mg/ml + Ach 0.04 µg/ml | 30.66 ± 0.88** |
| ELEGS 6.4 mg/ml + Ach 0.04 µg/ml | 11.33 ± 0.33*** |
| Atropine 0.04 µg/ml + Ach 0.04 µg/ml | 8.40 ± 0.70*** |

Values are expressed as mean ± standard error of mean (n=3). **p<0.01, ***p<0.001 when compare to the control using one way ANOVA followed by Dunnett's test Ach = Acetylcholine; ELEGS = ethanol leaf extract of *Guiera senegalensis*

### 4.3.8 Effect of Propranolol on the Activity of Ethanol Leaf Extract of *Guiera senegalensis* on Rabbit Jejunum

The observed effect of ELELGS on rabbit jejunum after the addition of propranolol (0.02 µg/ml) was significantly different (*p<0.01) from observed effects of the extract alone. Propranolol is a nonselective β adrenoceptor blocker and so can inhibit the effect of $\beta_2$ agonists. Thus, the result suggests that ELEGS is possibly working via $\beta_2$ adrenoceptors to cause inhibition of contractions of smooth muscles (Fig. 8).

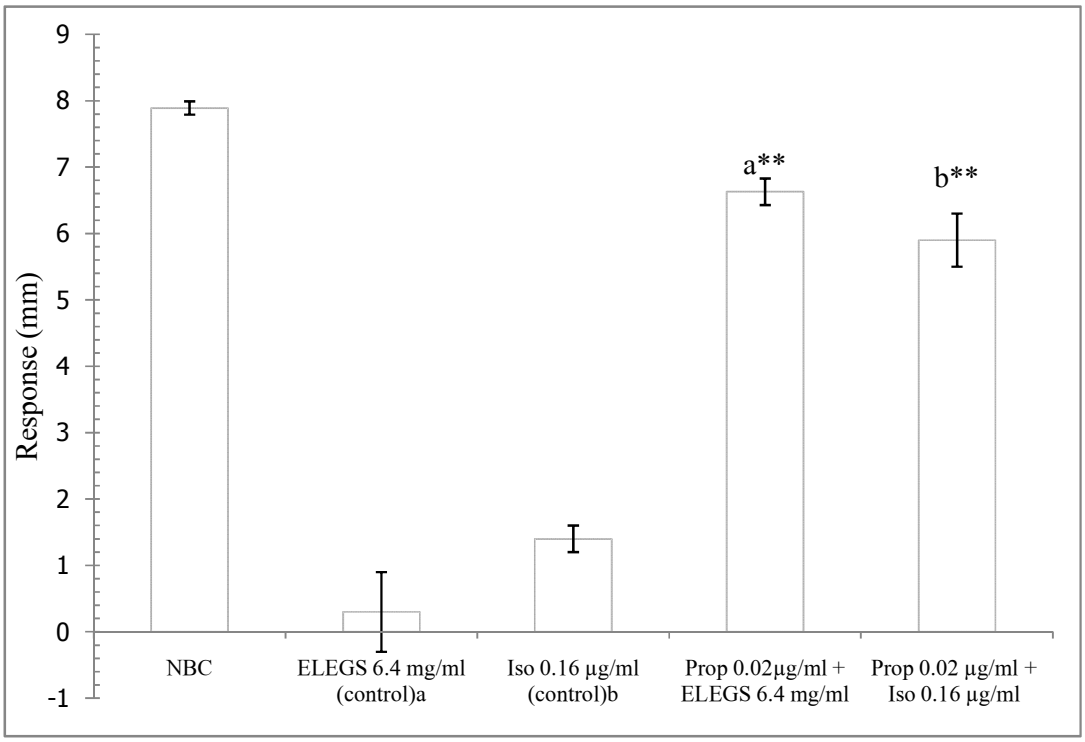


**Figure 8:** Effect of Propranolol on the Activity of Ethanol Leaf Extract of Guiera senegalensis on Rabbit Jejunum. Values are expressed as mean ± standard error of mean (n = 3). *P<0.01 when compared to the controls using unpaired t test. ELEGS = Ethanol Leaf Extract of *Guiera senegalensis;* Iso = Isoprenaline; Prop = Propranolol

## 4.3 Effects of Ethanol Leaf Extract of *Guiera senegalensis* on Membrane Stability

The membrane stabilizing effect of ELEGS was assessed using erythrocytes hemolysis inhibition assay. The extract showed significant (*p<0.05, **p<0.01) inhibition at concentrations 0.2 and 1 mg/ml respectively. Increase in the concentration of ELEGS didn't show significant inhibition of hemolyis (Table 4).

**Table 4: Effect of Ethanol Leaf Extract of *Guiera senegalensis* on Erythrocytes Membranes Stability**

| Test | Absorbance | % Inhibition |
|---|---|---|
| Control | 0.3640 ± 0.032 | - |
| 0.1 | 0.3131 ± 0.013 | 13.8 % |
| 0.2 | 0.2630 ± 0.022* | 28 % |
| 1 | 0.2247 ± 0.019** | 38.2 % |
| 2 | 0.3030 ± 0.014 | 16 % |

Values are expressed as mean ± Standard Error of Mean (n=3). *p<0.05, **p<0.01 when compared to the control using one way ANOVA followed by Dunnetts test. Absorbance was read at 540nm. ELEGS= Ethanol Leaf Extract of *Guiera senegalensis*

## 4.4 DISCUSSION

A study was conducted *in vitro* to investigate the anti-asthmatic effect of ethanol leaf extract of G*uiera senegalensis* (ELEGS). This plant has been used traditionally to treat various diseases including asthma (Sanogo, 2012). Bronchial constriction during asthma attack is primarily mediated by histamine released from degranulated mast cells and by acetylcholine which causes contractions of tracheal smooth muscles and increased mucus secretions (Brading, 2008). Inflammation of the bronchi also sets due to lipid mediators from arachidonic acid pathway generated from membrane phospholipids (Pniewska and Pawliczak, 2013). Airway smooth muscles are rich in histamine, acetylcholine and $\beta_2$ receptors (Ruben, 2007). Distribution of these receptors and their sensitivity to agonists and antagonists is similar in both humans and guinea pigs. This explains why guinea pigs are the animals of choice for antiasthmatic studies in contrast to mice and rats which do not respond to the contractile effects of histamine (Canning and Chou, 2008). Clinical interventions currently aim at maintaining normal or near normal airway smooth muscles tone mostly by the use of bronchodilators and anti-inflammatory agents (Barisone *et al*., 2010). As such, plant extracts that can mimic the orthodox drugs in reversing bronchocospasm or inflammation could possibly relieve symptoms of asthma (Patil *et al.,* 2011). Several plants have been reported to accomplish their antiasthmatic effects by their ability to inhibit contractions of bronchial muscles (Mishra *et al.,* 2014). On this basis, the antihistaminic, anticholinergic as well as the membrane stabilizing effects of ELEGS have been investigated using animals' isolated tissues.

The preliminary phytochemical analysis of ELEGS revealed the presence of alkaloids, flavonoids, saponins, steroids, anthraquinones and tannins. This concurred with the

finding of Somboro *et al.,* (2011). Similar study on the leaf extract conducted by Akuodor *et al*., (2013) also found these phytochemicals with the exception of anthraquinones. Medicinal plants are known to possess curative effects and have been used to target many diseases (Okechukwu and Ekeuku, 2012). The pharmacological effects of these plants can be attributed to the presence of the secondary metabolites in them (Kalimuthu and Prabakaran, 2013). For example, alkaloids have been known to possess antimuscarinic effects while flavonoids and tannins possess smooth muscles relaxant effect (Ghayur *et al.,* 2007). Steroids are well known for their anti-inflammatory effects (Benali *et al.,* 2012). Therefore, the observed effects of ELEGS discussed below might be due to the presence of these phytochemicals.

Histamine-induced contraction of isolated guinea pig trachea is an *in vitro* antiasthmatic model used to investigate the bronchodilatory effects of candidate compounds for asthma management (Canning and Chou, 2008). Histamine is a potent bronchoconstrictor implicated in the pathophysiology of asthma. The dominant histamine receptors in the airways are $H_1$ receptors. Activation of these receptors by histamine is responsible for the early phase bronchospasm (Roux and Ouedraogo, 2014). Effect of ELEGS on histamine induced contraction of guinea pig trachea was studied. The extract significantly reversed the tracheal contractions in a concentration-depended manner (ELEGS 2, 4 and 8 mg/ml). There was statistically significant difference between the control (histamine 0.6 µg/ml) and all the concentrations of the extract used, suggesting that the extract has bronchodilator activity. Many plants were reported to exert their antiasthmatic effects by reversing the bronchospasm induced by histamine (Mehta and Agrawal, 2008). Plants from the same family with *Guiera senegalensis* such as *Combretum molle* (Earnest, 2010). *Anogeissus leiocarpus* (Sonibare and Gbile, 2008) and T*erminalia chebula* (Anwesa *et al.,* 2013) have also

been reported to have antiasthmatic effects. ELEGS can therefore be considered as one of such plants. Inhibition of $H_1$ receptors could provide symptomatic relief in asthmatics but unfortunately, antihistamines are not used as asthma medication due to their numerous side effects therefore, the adrenergic pathway is exploited (Simon, 1999). Stimulation of these receptors leads to the formation of cAMP which then serves as a second messenger to phosphorylate Protein Kinase A leading to relaxation of the airway smooth muscles. Drugs that are $\beta_2$ specific agonists such as Salbutamol, Salmeterol and Formeterol are used (Giovanni, 2010). In the Isoprenaline, a non-selection β agonist but which is more selective to $\beta_2$ was used as a standard bronchodilator for comparison with the extract. This drug reverses bronchocontraction induced by histamine through the activation of $\beta_2$ adrenergic receptors (Tanaka *et al.,* 2003). Effect of ELEGS (8 mg/l) was comparable to isoprenaline (0.4µg/ml) in reducing the height of the control, indicating that the extract has a bronchodilatory activity.

ELEGS also significantly reduced the acetylcholine-induced contractions of isolated guinea pig trachea. Acetylcholine released from the nerve ending of the vagus inervation of the airways is also involved in the pathophysiology of asthma (Ofori-Amoah and Koffuor, 2015). The predominant cholinergic receptors in the airway are $M_3$ muscarinic receptors, activation of which causes bronchospasm. Tiotropium and ipratropium bromide are currently the available antimuscarinic agents used in the clinical management of asthma (Gosens *et al.,* 2005). ELEGS significantly reduced the acetylcholine induced contraction of isolated guinea pig trachea. The extract (8 mg/ml) significantly reduced the height of the control (acetylcholine 0.4 µg/ml) in similar manner to isoprenaline (0.4 µg/ml) thereby indicating its bronchodilatory effect. Naseri and Heidara (2006) revealed that extracts such as leaf extract of *Vitis*

*vinifera* that can inhibit acetylcholine-induced tracheal contractions could be considered to have bronchodilatory effect. Therefore, ELEGS could be considered so.

It could be observed that ELEGS reduced the contractions of the tracheal chains induced by both histamine and acetylcholine but this effect could be achieved through several mechanisms (Kumar *et al.,* 2014). The extract could block the histamine and acetylcholine at their receptors or antagonized them via the adrenergic pathway by activating $\beta_2$ receptors (Holgate, 1999). This prompted additional study to determine the antihistaminic and anticholinergic effects of the extract in isolated guinea pig ileum.

Models for *in vitro* antiasthmatic studies use guinea pig ileum as adjunct to trachea because of their similarity in receptor distribution and also for its greater sensitivity to histamine and acetylcholine (Vincent *et al.,* 2011). Guinea pig ileum has similar receptor distribution with the trachea thereby making it a good tissue for studies involving histamine and acetylcholine (Canning and Chou, 2008). ELEGS showed significant inhibition of contractions induced by histamine in guinea pig ileum. The inhibition was concentration-dependent. At the 1.6 mg/ml, ELEGS completely attenuated the spasmogenic effect of histamine (0.04 µg/ml) and even caused further relaxation of the ileum below the baseline. Mepyramine, a known antihistamine was used as a reference standard drug for comparison with the extract. At concentration of 0.04 µg/ml, this antagonist also abolished the contractile effect of histamine (0.04 µg/ml). Increase in concentration of mepyramine did not produce any relaxation of the ileum as observed with the extract. Rathore *et al.,* (2011) reported that methanol extract of *Physalis angulata* which is also used as antiasthmatic inhibited the effect of histamine on guinea pig ileum as seen with ELEGS.

The result obtained from the acetylcholine-induced contraction of guinea pig ileum revealed a similar finding as obtained in histamine-induced contractions. ELEGS (3.2 mg/ml) totally abolished the contraction induced by acetylcholine (0.04 µg/ml) and also caused further relaxation of the ileum below the baseline. Atropine, a standard antimuscarinic, when used at concentration of (0.04 µg/ml) did not produce relaxation of the ileum below the baseline but significantly inhibited the effect of same concentration of acetylcholine. The study also showed that the extract exhibited smooth muscle relaxing effect. Relaxation of ileal muscles can be mediated either through α or $\beta_2$ adrenoceptor stimulation and many plants were reported to cause ileal relaxation through activation of one of these receptors (Mali and Dhake, 2011).

Sequel to this, further study on isolated rabbit jejunum was performed. Rabbit jejunum has excellent adrenergic receptors distribution (Sewell and Broadley, 2012). ELEGS completely abolished the pendular movement of the isolated rabbit jejunum. It also significantly inhibited acetylcholine-induced contractions of the jejunum in concentration-dependent manner. Atropine, when used as a standard antimuscarinic drug for comparison with the extract, also inhibited the contractions induced by acetylcholine but did not abolish the pendular contractions of the rabbit jejunum. This suggested that the extract might have inhibited the basal contractions via different mechanisms other than being muscarinic antagonists because plant extracts with similar effects were observed to act through either $\alpha_1$ or $\beta_2$ receptors (Boye, 2010). Propranolol is a nonselective β adrenoceptor blocker that binds to all β receptors and inhibits their activity. Thus, the extract was used in the presence and absence of this blocker. It was observed that ELEGS (6.4 mg/ml) failed to inhibit the rhythmic contractions of rabbit jejunum incubated with propranolol (0.02 µg/ml) compared to its effects alone on the tissue. This suggested that the observed effect of the extract is

possibly mediated via stimulation of $\beta_2$ adrenergic receptor because according to Amos *et al*., (1998), relaxant effects of plant extracts can be inhibited by propranolol if the extract is acting through $\beta_2$ adrenergic receptor. Effect of isoprenaline (0.16 µg/ml), which relaxed the rabbit jejunum, was also abolished by propranolol. This suggests that ELEGS inhibits smooth muscle contractions by activating $\beta_2$ adrenergic receptors in the airways. Plants such as *Adhatoda schimperiana* (Ashenafi *et al.,* 2008), *Albizzia lebbek* (Spatulla, 2011) and *Picrorhiza Kurroa* (Sehgal *et al.,* 2013), have also been reported to exert their antiasthmatic effects by activation of airway $\beta_2$ adrenergic receptors.

The membrane stabilization property was assessed using erythrocytes hemolysis inhibition assay by studying the possible inhibition of arachidonic acid derivatives which are also implicated in the pathophysiology of asthma (Barnes, 2006). The arachidonic acid pathway generates mediators of the late phase of asthma such as leucotrienes and prostaglandins. Therefore arachidonic acid release can be an important step in the control of inflammatory mediators (Anita and Bowle, 2009). ELEGS was able to protect the membranes of erythrocytes from possible damage. This suggests that the extract could possibly inhibit generation of lipid mediators of inflammation. It has been reported that plants extracts that can hinder the lysis of membrane phospholipids could serve as potential inhibitors of eicosanoid synthesis may be helpful to asthmatics (Middleton and Theoharides, 2008). Similar activity has also been reported of *Anchomanes difformis* (Idhu and Oghale, 2016), *Helicanthus elastica* (Arun and Chandrakant, 2010) as well as *Balanites aegyptiaca* (Patil *et al.,* 2011).

# CHAPTER FIVE

# SUMMARY, CONCLUSION AND RECOMMENDATION

## 5.1 SUMMARY

The preliminary phytochemical studies of Ethanol Leaf Extract of *Guiera senegalensis* (ELEGS) revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, steroids and tannins. Graded concentrations of ELEGS 2, 4 and 8 mg/ml produced significant (*$p<0.05$, **$p<0.01$) dose-dependent inhibition of contractions induced by histamine 0.6µg/ml on guinea pig trachea. Acetylcholine 0.4 µg/ml was also significantly (**$p<0.01$) inhibited by 8 mg/ml extract on the trachea. The inhibitory effects of the extract in both studies were comparable to that observed with 0.4 µg/ml isoprenaline. Similarly, ELEGS 0.4, 0.8 and 1.6 mg/ml significantly (**$p<0.01$, ***$p<0.001$) inhibited contractions induced by histamine 0.04 µg/ml on guinea pig ileum while 0.4, 0.8, 1.6 and 3.2 mg/ml produced significant (**$p<0.01$, ***$p<0.001$) inhibition of acetylcholine 0.4µg/ml on the ileum. In rabbit jejunum, contractions induced by acetylcholine 0.04 µg/ml were significantly inhibited by ELEGS 0.8, 1.6, 3.2 and 6.4 mg/ml. Propranolol (0.02µg/ml) was used in the presence of the extract 6.4 mg/ml and the extract failed to produce any effect on rabbit jejunum contrary to prior observations in which it inhibited the spontaneous basal contractions when use alone. Red blood cells membranes stability was also enhanced by the extract 0.2 and 1mg/ml indicating its possible inhibition of synthesis of lipid mediators.

## 5.2 CONCLUSION

The ethanol leaf extract of *Guiera senegalensis* was found to exhibit bronchodilatory effect by inhibiting the contractions induced by histamine and acetylcholine on isolated guinea pig trachea. According to this finding, the inhibition is probably mediated through

the activation of $\beta_2$ adrenergic receptors as observed when the extract failed to cause relaxation of rabbit jejunum pretreated with propranolol. This could therefore serve as a basis that explains the use of this part of the plant in the traditional management of asthma.

## 5.3 RECOMMENDATIONS

Since the study was conducted *in vitro*, little knowledge is known about the long term toxicity or otherwise of the plant extract because previous studies have only reported the safety of the leaf extract after acute toxicity study. It is therefore recommended that safety profile of the leaf extract be established through sub-acute and chronic toxicity studies. The *in vivo* antiasthmatic study on the plant extract should also be performed. Moreover, isolation, characterization and structure elucidation of the bioactive components responsible for the observed antiasthmatic effects should be performed.

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**GLOSSARY**

| | |
|---|---|
| □ | Less than |
| - | Negative |
| % | Percentage |
| + | Positive |
| ± | Plus/minus |
| µg/ml | Microgram per mill |
| ANOVA | Analysis of Variance |
| BUKHAN | Bayero University Kano Herbarium Accession Number |
| $Ca^{2+}$ | Calcium ion |
| cAMP | Cyclic Adenosine Monophosphate |
| Fig. | Figure |
| g | Gram(s) |
| $H_2SO_4$ | Tetraoxo Sulphate (VI) Acid |
| HCl | Hydrochloric Acid |
| Ltd | Limited |
| Mg/ml | Milligram per milliliter |
| ml | Milliliters |
| mM | Millimolar |
| ºC | Degree Celsius |
| W.H.O | World Health Organisation |

**APPENDIX**

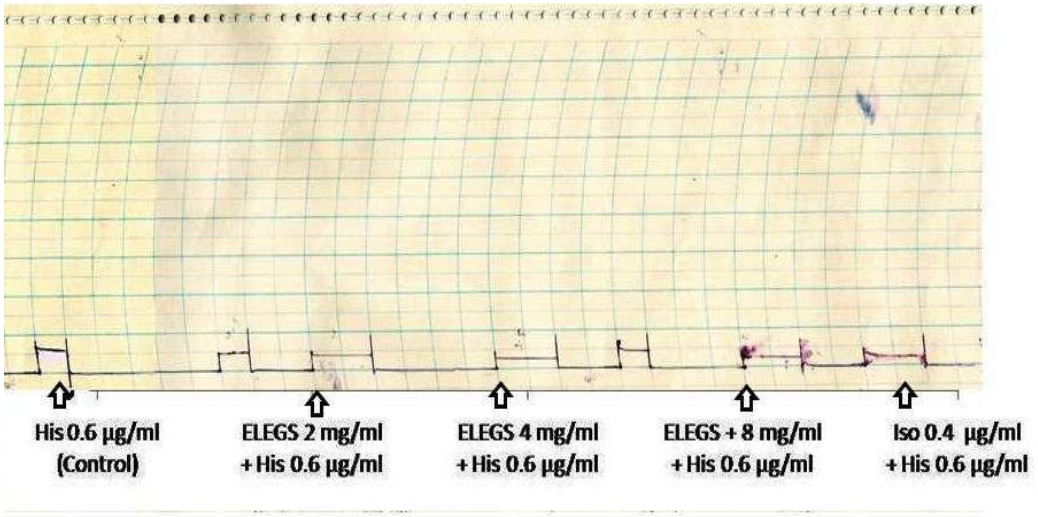


PLATE II: Effects of ethanol leaf extract of *Guiera senegalensis* on histamine-induced contractions of guinea pig trachea

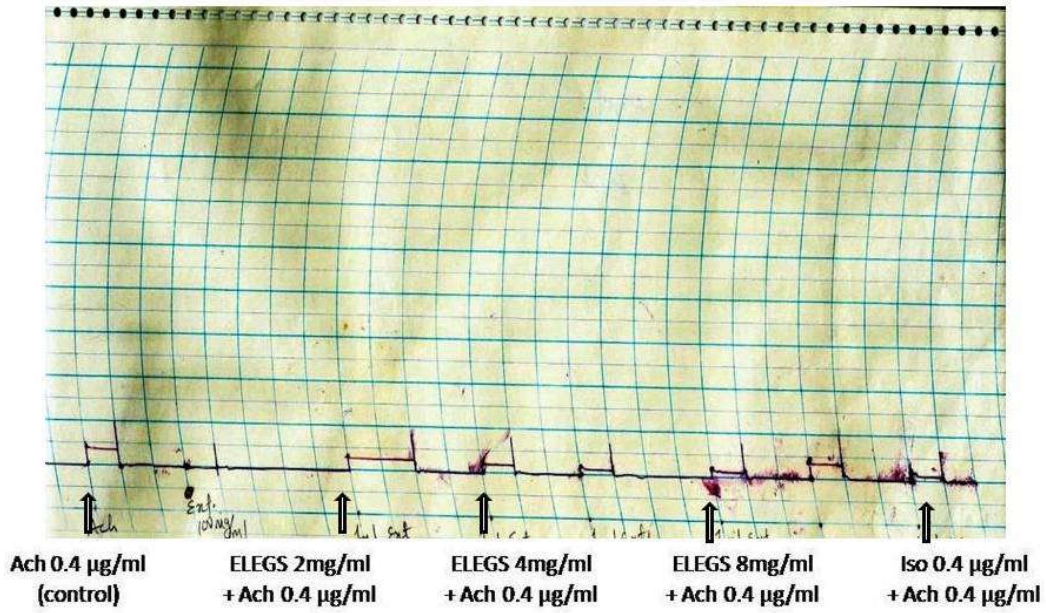


PLATE III: Effects of ethanol leaf extract of *Guiera senegalensis* on acetylcholine-induced contractions of guinea pig trachea.

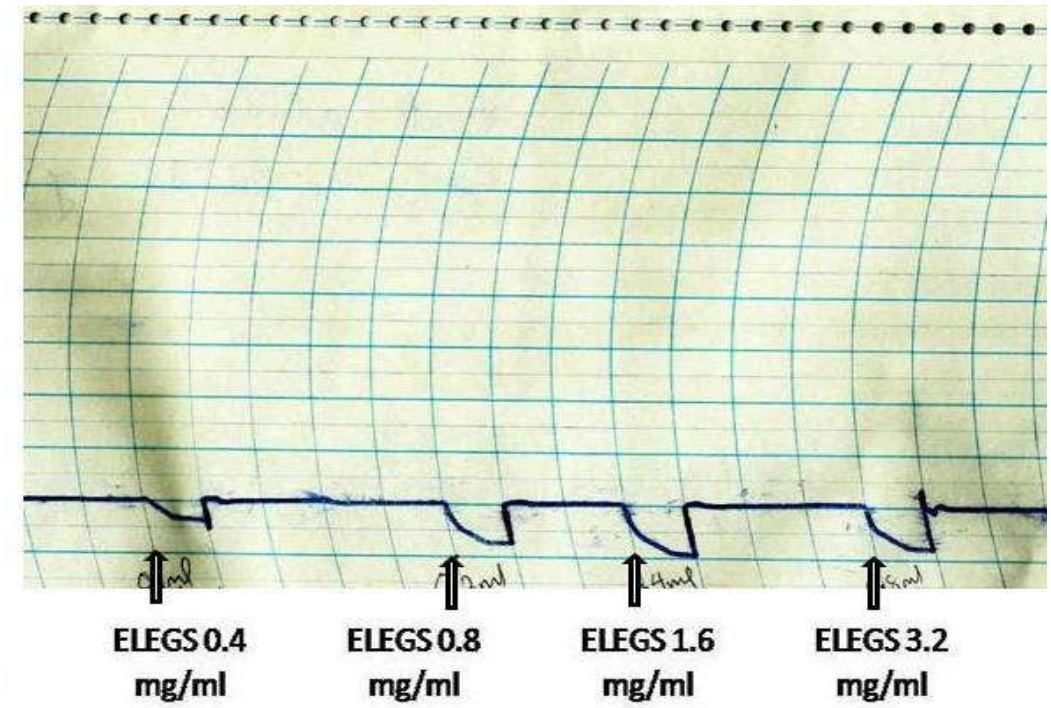


PLATE IV: Effect of ethanol leaf extract of *Guiera senegalensis* on guinea pig ileum.

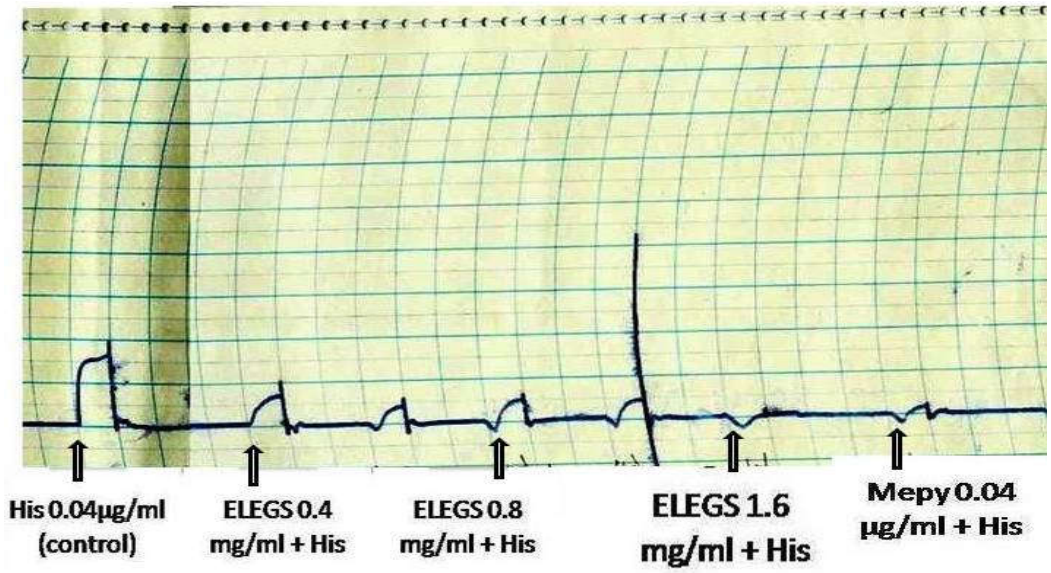


PLATE V: Effects of ethanol leaf extract of *Guiera senegalensis* on histamine-induced contractions of guinea pig ileum.

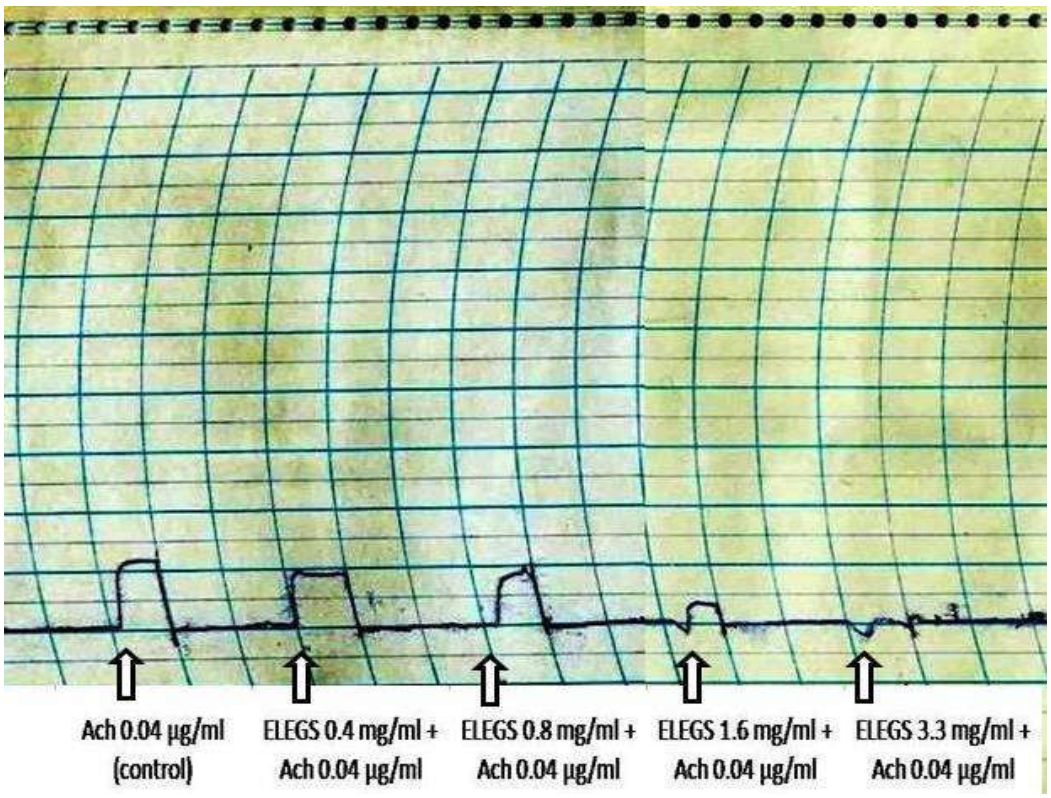


PLATE VI: Effects of ethanol leaf extract of *Guiera senegalensis* on acetylcholine-induced contractions of guinea pig ileum.

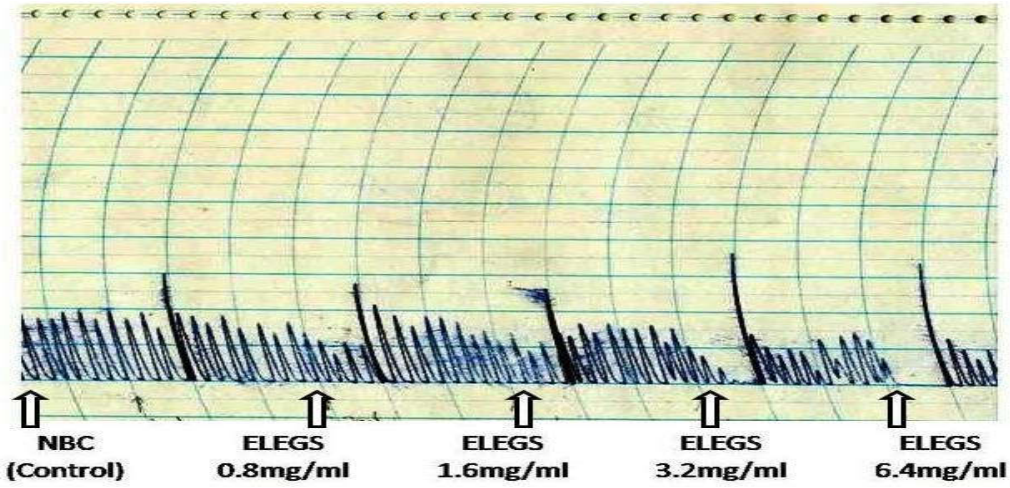


PLATE VII: Effects of ethanol leaf extract of *Guiera senegalensis* on rabbit jejunum.

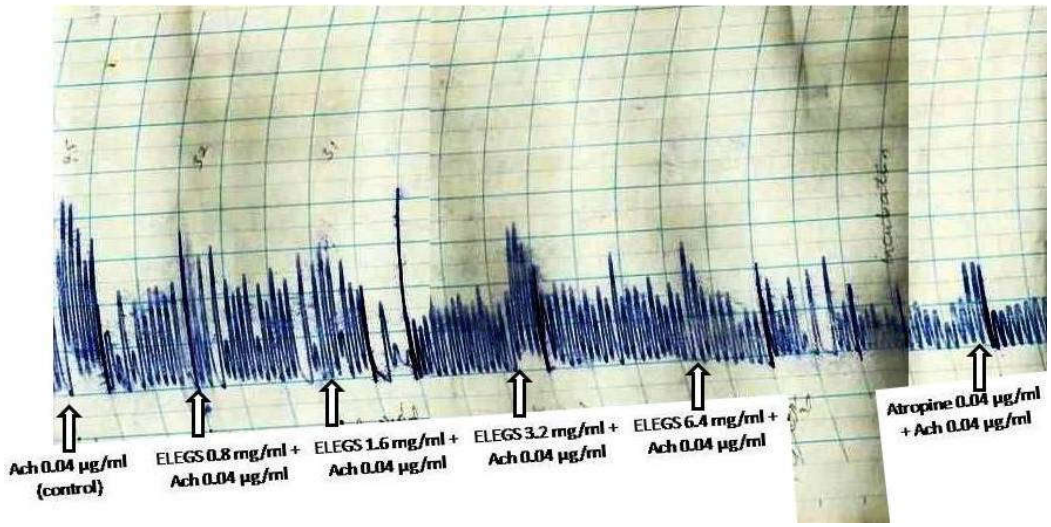


PLATE VIII: Effects of ethanol leaf extract of *Guiera senegalensis* on acetylcholine-induced contractions of rabbit jejunum.

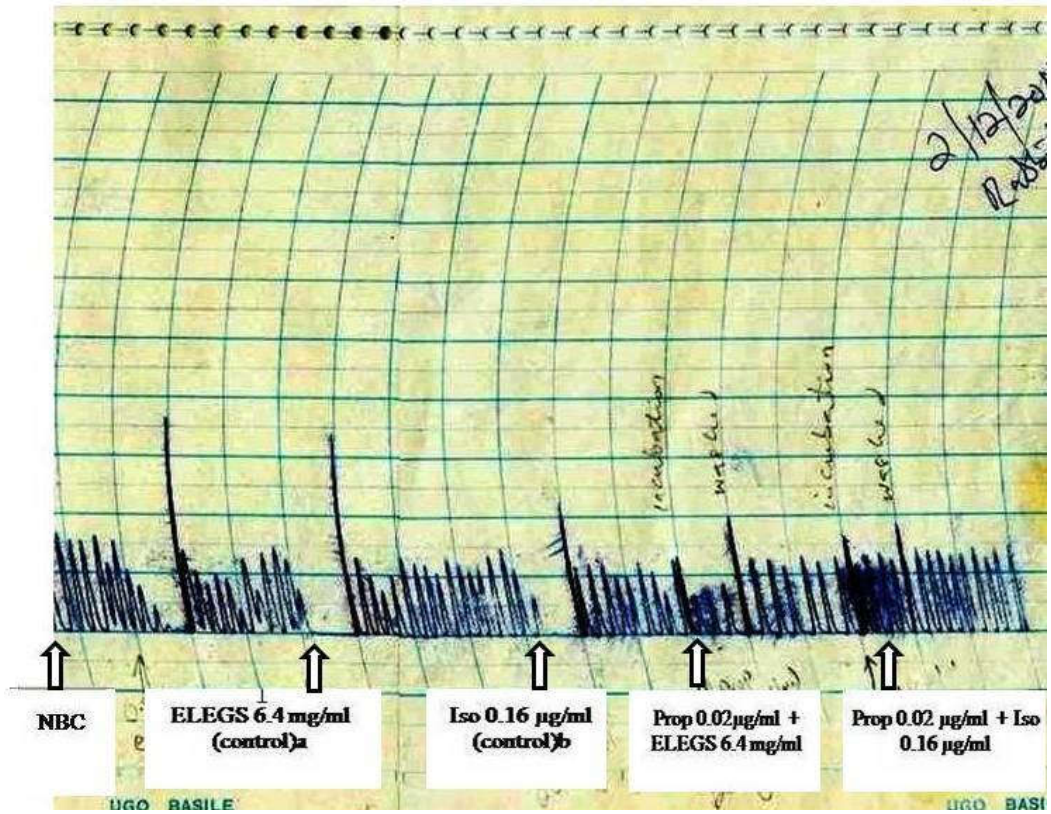


PLATE IX: Effects of propranolol on the activity of ethanol leaf extract of *Guiera senegalensis* on rabbit jejunum.