# Phytochemical and Biological Investigation of *Senna alata* leaves

A thesis report submitted to the department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the degree of B. Pharm

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**Declaration by the Candidate**

I, Maria Afrin (ID:2011-3-70-004), hereby declare that the dissertation entitled **"Phytochemical And Biological Investifgation Of *Senna alata* Leaves"** , submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy is a genuine & authentic thesis work carried out by me during Fall 2014-Spring 2015 under the supervision and guidance of Tirtha Nandi, Lecturer, Department of Pharmacy, East West University.

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**Certificate by the Invigilator**

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## ABSTRACT

The crude methanolic extracts derived from the ***Senna alata*** leaves was screened in vitro for possible phytochemical and biological. Crude plant powders were extracted sequentially with methanol and the dried extracts obtained demonstrated the presence of significant pharmacological activity on diabetes, microorganisms responsible for disease. The objective of this study is to characterize the functional compounds that were extracted and separated from leaves of ***Senna alata*** and were carried out using different methods using Thin Layer Chromatography ( TLC), Vacuum Liquid Chromatography (VLC), Column Chromatography. Under phytochemical analysis, antioxidant test & Chemical screening was done. The antioxidant property found in crude methanolic extracts derived from the ***Senna alata*** leaves was very good.

***S. alata*** leaves have also been found to contain anthraquinones and anthracene derivatives of rhein, emodol, aloe-emodin, sennosides A and B, 4,5- dihydroxy-1-hydroxymethylanthrone and 4,5-dihydroxy-2- hydroxymethylanthrone.Phytochemical screening of the leaves and roots of ***S. alata*** revealed the presence of alkaloids, carbohydrates, tannins, saponins, phenols, flavonoids, anthraquinones and cardiac glycosidess. Amongst the secondary metabolites are steroids, flavonoids, anthraquinones, anthrones, and a few less common compounds such as ellagitannin, naphthalene, phenolic acid, purine, and xanthone.Of special interest are compounds such as kaempferol glycosides and anthraquinones, already proven to have antimicrobial properties.

## RATIONALE AND OBJECTIVE OF THE WORK

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such insects, fungi etc. Plants have been used for health and medical purposes for several thousands of years. According to world health organization, The number of higher plant species on earth is about 250 000 and It is estimated that 35 000 to 70 000 species have, at one time or another, been used in some cultures for medicinal purposes. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs. Herbal medicines are often used to provide first-line and basic health service, both to people living in remote areas where it is the only available health service, and to people living in poor areas where it offers the only affordable remedy. Even in areas where modern medicine is available, the interest on herbal medicines and their utilization have been increasing rapidly in recent years.

Bangladesh is also a major country where people use a high percentage of medicinal plants for various therapeutic activities. Use of volatile and penetrating plant extracts in therapeutic applications for psychological and physical well being was in practice from ancient times. Bangladesh is gifted by extraordinary natural resources which continuously help us in many ways. One of the most beneficial natural resources is the plant resource which provides us with food, shelter and medicine. According to the World Health Organization more than 80% of the world population in developing countries depends on plant-based medicines for basic healthcare needs.

Fabaceae, also called Leguminosae, pea family of flowering plants (angiosperms), within the order Fabales. Fabaceae, which is the third largest family among the angiosperms after Orchidaceae (orchid family) and Asteraceae (aster family), consists of more than 700 genera and

about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. *Senna alata* plant (family of Fabaceae) is grown in several tropical countries. It produces a large shrub with very large once-compound leaves consisting of 8-14 pairs of leaflets and the very large leaflets (5-17 cm long and 2-5 cm wide) have entire margins and rounded tips. Its golden yellow flowers are borne in dense elongated clusters (30-60 cm long) near the tips of the branches, these flowers are interspersed with yellow or orange floral bracts. Its elongated pods (15-25 cm long) are somewhat four-angled and have papery wings. *Senna alata* plant has been used to skin problems, arthritis, HBP (high blood pressure), and laxative or purgative, boils, wound, eye, urinary and gastrointestinal tract infections, diarrhoea and scarlet fever. Recent reports have credited the use of *S. alata* in the successful treatment of haemorroids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes.

*Senna alata* leaves has several medicinally important phytochemical constituents including, mineral elements: k, Zn, Cd, Na ,Mg ,Fe ,Ca. And the vitamin elements are β-Carotene (IU), Vitamin C (mg/L), Vitamin E (IU). Vitamin C anthraquinones and anthracene derivatives of rhein, emodol, aloe-emodin, sennosides A and B, 4,5- dihydroxy-1-hydroxymethylanthrone and 4,5-dihydroxy-2- hydroxymethylanthrone, alkaloids, carbohydrates, tannins, saponins, phenols, flavonoids, anthraquinones and cardiac glycosidess, Amongst the secondary metabolites are steroids, flavonoids, anthraquinones, anthrones, and a few less common compounds such as ellagitannin, naphthalene, phenolic acid, purine, and xanthone etc.

The aim of this research project was to carry out the characterization of the functional molecules present in the Different extract of leaves of the *Senna alata* and investigate their biological activities.

# Chapter 1: Introduction

## 1.1 General Introduction :

Plants extract or pure compounds or standardized extracts those are natural product which provides unlimited opportunities for new drug discoveries because of the unmatchedavailability of chemical diversity (Sasidharan et al,. 2011). According to the WHO, "A medicinal plants is any plant which, in one or more of its organs, contains substances that can be used fort herapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." When a plant is designated as "medicinal", it is implied that the said plant is used as a drugor therapeuticagent or an active ingredient ofmedicinal preparation(Behera,2006).Thetherapeutic treatment of disease with the use of herbs began long ago. Methods of folk healing throughout the world commonly used herbs as part of their tradition. The practice of using herbs to treat diseases is very common among many non-developed societies. It issometimes more easier to get than purchasing expensive modern pharmaceuticals.Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic aswell as infectious diseases. Due to the development of adverse effects and microbial resistance tothe chemically synthesized drugs, men turned to ethnopharmacognosy. They found literallythousands of phytochemicals from plants as safe and broadly effective alternatives with lessadverse effect. Many beneficial biological activity such as anticancer,antimicrobial, antioxidant,antidiarrheal, analgesic and wound healing activity were reported. In many cases the peopleclaim the good benefit of certain natural or herbal products(Sasidharan et.al,. 2011).

## 1.2 Phytochemistry:

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites—compounds which are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs—examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. Toxic plants even have use in pharmaceutical development.

Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs.

Alkaloids are a class of chemical compounds containing a nitrogen ring. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the antihypertension agent reserpine; the cholinomimeric galatamine; the spasmolysis agent atropine; the vasodilator vincamine; the anti-arhythmia compound quinidine; the anti-asthma therapeutic ephedrine; and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.

Polyphenols (also known as phenolics) are compounds contain phenol rings. The anthocyanins that give grapes their purple color, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics.

Glycosides are molecules in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by a herbivore.

Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, which are often strong smelling and thus may have had a protective function. They are the major components of resin, and of turpentine produced from resin. (The name "terpene" is derived from the word "turpentine"). Terpenes are major biosynthetic building blocks within nearly every living creature. Steroids, for example, are derivatives of the triterpene squalene. When terpenes are modified chemically, such as by oxidation or rearrangement of the

carbon skeleton, the resulting compounds are generally referred to as terpenoids. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives. Vitamin A is an example of a terpene. The fragrance of rose and lavender is due to monoterpenes. The carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes.

A consortium of plant molecular researchers at Washington State University, the Donald Danforth Plant Science Center, the National Center for Genome Resources, and the University of Illinois at Chicago began an NIH-sponsored study of over thirty medicinal plant species late 2009. The initial work, to develop a sequence reference for the transcriptome of each, has led to the development of the Medicinal Plant Transcriptomics Database.

### 1.3 Medicinal Plant

"Medicinal Plants are plants that provide people with medicines - to prevent disease, maintain health or cure ailments." In one form or another, they benefit virtually everyone on Earth. No exact definition of a Medicinal Plant is possible. There are related issues, such as for nutrition, toiletry, bodily care,incense and ritual healing.

#### 1.3.1 History of medicinal plants:

The use of plants as medicine predates written human history. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds. The use of herbs and spices in cuisine developed in part as a response to the threat of food-borne pathogens. Studies show that in tropical climates where pathogens are the most abundant, recipes are the most highly spiced. Further, the spices with the most potent antimicrobial activity tend to be selected. In all cultures vegetables are spiced less than meat, presumably because they are more resistant to spoilage. Angiosperms (flowering plants) were the original source of most plant medicines.(Sherman et al, 2001)

Many of the common weeds that populate human settlements, such as nettle, dandelion and chickweed, have medicinal properties.

A large amount of archaeological evidence exists which indicates that humans were using medicinal plants during the Paleolithic, approximately 60,000 years ago. Furthermore, animals such as non-human primates, monarch butterflies and sheep are also known to ingest medicinal plants to treat illness. Plant samples gathered from prehistoric burial sites are an example of the evidence supporting the claim that Paleolithic peoples had knowledge of herbal medicine. For instance, a 60 000-year-old Neanderthal burial site, "Shanidar IV", in northern Iraq has yielded

large amounts of pollen from 8 plant species, 7 of which are used now as herbal remedies.(Edward et al, 1986)

The deliberate placement of flowers has been challenged. Paul Pettitt has stated that the "deliberate placement of flowers has now been convincingly eliminated", noting that "A recent examination of the microfauna from the strata into which the grave was cut suggests that the pollen was deposited by the burrowing rodent Meriones tersicus, which is common in the Shanidar microfauna and whose burrowing activity can be observed today". Also medicinal herbs were found in the personal effects of Ötzi the Iceman, whose body was frozen in the Ötztal Alps for more than 5,000 years. These herbs appear to have been used to treat the parasites found in his intestines. (Solecki and Ralph, 1975)

Ancient time: In the written record, the study of herbs dates back over 5,000 years to the Sumerians, who created clay tablets with lists of hundreds of medicinal plants (such as myrrh and opium). In 1500 B.C., the Ancient Egyptians wrote the Ebers Papyrus, which contains information on over 850 plant medicines, including garlic, juniper, cannabis, castor bean, aloe, and mandrake.

In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 1900 BC. Earliest Sanskrit writings such as the Rig Veda, and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system. Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BC. The Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources.

The mythological Chinese emperor Shennong is said to have written the first Chinese pharmacopoeia, the "Shennong Ben Cao Jing". The "Shennong Ben Cao Jing" lists 365 medicinal plants and their uses - including Ephedra (the shrub that introduced the drug ephedrine to modern medicine), hemp, and chaulmoogra (one of the first effective treatments for leprosy). Succeeding generations augmented on the Shennong Bencao Jing, as in the Yaoxing Lun (Treatise on the Nature of Medicinal Herbs), a 7th-century Tang Dynasty treatise on herbal medicine.

The earliest known Greek herbals were those of Diocles of Carystus, written during the 3rd century B.C, and one by Krateuas from the 1st century B.C. Only a few fragments of these works have survived intact, but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals. Greek and Roman medicinal practices, as preserved in the writings of Hippocrates (e.g. De herbis et curis) and - especially - Galen (e.g. Therapeutics), provided the pattern for later western medicine. Sometime between 50 and 68 A.D., a Greek physician known as Pedanius Dioscorides wrote Περὶ ὕλης ἰατρικῆς (commonly known by its Latin title De Materia Medica), a compendium of more than 600 plants, 35 animal products, and ninety minerals. De Materia Medica remained the authoritative reference of herbalism into the 17th century. Similarly important for herbalists and botanists of later centuries was Theophrastus'

Historia Plantarum, written in the 4th century BC, which was the first systematization of the botanical world. (Sumner and Judith, 2000)

### 1.3.2 Contribution of medicinal plants

All culture from ancient timk6es to the present day has used plants as sources of medicine. Today the majority of the world's population continues to rely on medicinal plants for the health care needs. Cultural global demand for herbal product is vast and growing. The habitants that supports medicinal plant are being rapidly degraded or destroyed for instance by over grazing or conversion toagricultural land. Many plants synthesize substance that is useful to the maintenance of health in human and other animals. These include aromatic substance, most of which are phenols or their oxygen substituted derivatives such as tannins. Many are secondary metabolites .of which at least 12000 have been isolated –a number estimated to be less than 10% of the total. In many cases, substances such as alkaloids serve as plant defense mechanism against predation by microorganism, insects, and herbivores. Many of the herbs and species used byhumans to season food yield useful medicinal compounds. (Falco et al., 2003)

Plants up regulate and down regulate their biochemick2al paths in response to the local mix of herbivores, pollinators and microorganisms. The chemical profile of a single plant may vary over time as it reacts to changing conditions. It is the secondary metabolites and pigments that can have therapeutic actions in humansand which can be refined to produce drugs. Some examples are given below

**Table 1.1:** The list of the medicinal substance derived from plants.

| Drugs /chemical Name | Action /Clinical use | Plant source |
|---|---|---|
| Atropine | Antichlolinergic | *Atropa belladonna* |
| Arecoline | antihelmintic | *Areca catechu* |
| Caffeine | CNS stimulant | *Camellia sinensis* |
| Camphor | Rubifacient | Cinnamonum acemphore |
| Codeine | Analgesic, Sedative | *Papaver somniferum* linn. |
| Digitoxin,Digoxin | Cardiotonic | Digitalis purpurea Lin. Digitailis lanata |
| Ergotametrine,Ergotamine, Ergotoxin | Vasoconstrictor,ergotamine, | Claviceps purpurea. Tul |
| Hyosine Hyosinamine | Parasympatholytic,Mydriatic,anti spasmodic | Datura , hyocyamus, scopolia , |

### 1.3.3 Medicinal plant users :

### 1.3.4 Reasons behind popularity of plant medicine

It is estimated by the World Health Organization that approximately 75-80% of the world's population uses plant medicines either in part or entirely. For many this is out of necessity, since many cannot afford the high costs of pharmaceutical drugs. Growing numbers of American health care consumers are turning to plant medicines for many reasons - low cost and seeking natural alternatives with fewer side effects are commonly cited.

### 1.3.5Historic use:

Based upon the traditions of Europeans, Chinese, Egyptians, American Indians, and other cultures, Information on plant medicines handed down over the centuries. Currently anthropologists and other scientists are investigating newly discovered cultures (visits with shamans in Amazonia and Belize, for example), to determine what plants are being used - this "ethnobotany" has introduced a number of new compounds into pharmaceutical research (Joy et al., 1998)

### 1.3.6 Extrapolation

Extrapolation can be referred to the process by which a biological test is conducted thinking that some activity in vitro (in the test tube or in the lab) implies major activity in humans. The reasoning involved in predicting a conclusion or making a logical judgment on the basis of circumstantial evidence and prior conclusions rather than on the basis of direct observation.

### 1.3.7 Phytomedicinal prospecting

Screening plants for biological activity. Huge projects are currently underway by such organizations as INBio, Costa Rica's National Institute of Biodiversity. INBio is cataloging all species of plants and animals in the country – estimated to be around 500,000! To complete this ambitious task they train "community taxonomists" to identify plants and animals (Christopher et al., 2008).

### 1.3.8 Current Legal Status in the US

US currently have an "open market" for anything labeled as a "nutritional supplement". This is both good and bad - it means "caveat emptor" - let thebuyer beware! Unfortunately the buyer may occasionally be gambling with their life if they are not making knowledgeable choices. Since "nutritional supplements" are not sold as a treatment for a specific problem, they buyer must be informed. The bad aspect of this open market approach is that even products with proven health benefits may not be marketed for that use unless the producer has gone through the FDA protocols for determining safety and efficacy. The best example, perhaps, of this restriction is that it would be absolutely illegal to sell prune juice as a treatment for constipation. While no one would argue that it is safe and effective, no one has gone through the rigorous FDA approval process. A current controversy surrounds the sale of products containing natural sources of ephedrine. Ephedrine is an adrenaline-like compound found in some plants, especially ephedra species such as Ma Huang and Mormon Tea. This stimulant is found in "thermogenic" weight loss products and also sold as a "legal amphetamine" under such names as "Herbal Ecstasy". A number of people who have taken excessive amounts of this drug have died. The FDA is

currently mulling over the idea of taking it off the market. Manufacturers are hurrying to change the labels and provide warnings in an effort to prevent FDA action.

### 1.3.9 Current Industry Problems

Is the "natural" product you are buying really what it says it is??? This problem has long plagued the industry. When some dried root or leaf products sell for $80 per pound, the temptation to add a little extra something along the way goes from the harvesters to the middlemen to the retailers - all of whom are paid by the pound.

There have been studies of retail ginseng, for example, which show numerous purchased products with no ginseng content whatsoever! Perhaps these products rely on the power of suggestion! Looking at this more innocently, a peasant in the Amazon may not know one plant from a similar-looking cousin. The industry is establishing standards in an effort to improve its credibility – some products, such as ginkgo, are now standardized and offer some uniformity.

## 1.4 Description Of the plant :

Senna alata, the candle bush, is an important medicinal tree, as well as an ornamental flowering plant in the subfamily Caesalpinioideae. It also known as a candelabra bush, empress candle plant, ringworm tree, or candletree. A remarkable species of Senna, it was sometimes separated in its own genus, Herpetica.S. alata is native to Mexico, and can be found in diverse habitats. In the tropics, it grows up to an altitude of 1,200 m. It is an invasive species in Austronesia. In Sri Lanka, it is used as an ingredient in Sinhala traditional medicine.The shrub stands 3–4 m tall, with leaves 50–80 cm long. The inflorescence looks like a yellow candle. The fruit, shaped like a straight pod, is up to 25 cm long. Its seeds are distributed by water or animals. The leaves close in the dark. The seed pods are nearly straight, dark brown or nearly black, about 15 cm long, and 15 mm wide. On both sides of the pods is a wing that runs the length of the pod. Pods contain 50 to 60 flattened, triangular seeds.This species is easy to grow from the seed. They may either be sown directly or started in a nursery (Dr Hans Martin & Bindanda, 2008)

### 1.4.1 Taxonomy :

Senna (Leguminosae) is a large, widespread genus that includes species with enantiostylous, asymmetric flowers and species with extra floral nectaries. Clarification of phylogenetic relationships within Senna based on parsimony analyses of three chloroplast regions (rpS16,

rpL16, and matK) provides new insights on the evolution of floral symmetry and extrafloral nectaries. Senna displays a high diversity of habits, including herbs, shrubs, treelets, tall trees, and lianas,and has successfully colonized a wide range of habitats in different climates and latitudes. Of the approximately 350 species currently ascribed to the genus, 80% occur on the American continent, while most of the remaining members are found in tropical Africa, Madagascar, and Australia, with only a few species in southeastern Asia and some on the Pacific Islands .

**1.4.2 Some common name** :

Alcapulco, candelabra bush, candelabra plant, candle bush, candlebush, candlestick senna, Christmas candle, Christmas-candle, emperor's candlesticks, empress candle plant, empress-candle-plant, empress candleplant, golden candelabra tree, golden-candle senna, ringworm bush, ringworm plant, ringworm senna, ringworm shrub, ringwormbush, ringwormshrub, Roman candle tree, seven golden candles, seven golden candlesticks, stick senna, yellow top weed

**1.4..3 BOARD OF TAXONOMICAL CLASSIFICATION :**

| | |
|---|---|
| Kingdom | plantes, Planta, Vegetal, plants |
| Subkingdom | Viridiplantae |
| Infrakingdom | Streptophyta – land plants |
| Superdivision | Embryophyta |
| Division | Tracheophyta vascular plants,tracheophytes |

| | |
|---|---|
| Subdivision | Spermatophytina – spermatophy tes, seed plants, phanérogames |
| Class | Magnoliopsida |
| Superorder | Rosanae |
| Order | Fabales |
| Family | Fabaceae – peas, legumes |
| Genus | Senna Mill. |
| Species | Senna alata (L.) Roxb. – emperor's candlesticks |

## 1.5 ORIGIN AND DISTRIBUTION :

Senna alata is from the bean family and originally found in tropical regions of Africa, Southeast Asia, the Pacific Islands and America. It can reach a height of up to 30 feet in its native habitat, although 5 to 8 feet is more typical in a backyard garden, with a spread approximately half that width. An aggressive grower, especially in areas with a high water table, Senna alata often forms thickets through natural propagation. For this reason, the shrub is a good choice for borders. As a specimen plant, it could also be grown in outdoor containers or tubs (Bonnie Singleton, 2015**)**
A widespread species with a scattered distribution throughout northern and eastern Australia. It is most common in the coastal and sub-coastal parts of the Northern Territory and northern Queensland. Less common along the central and southern coasts of Queensland and also recorded in north-western Western Australia (Bostock and Holland, 2007)
Also naturalised in tropical Africa, tropical Asia, Papua New Guinea, Mexico, south-eastern USA (i.e. Florida), the Caribbean and on several Pacific islands (i.e. the Cook Islands, Fiji, Guam, Palau, Tonga, Western Samoa and Hawaii) (Bostock and Holland, 2007)

## 1.6 REQUIREMENTS FOR CULTIVATION

Plant seeds about three quarters of an inch deep in a well-drained soil and humus mixture with a pH range of 5.5 to 6.5. Find an area with full sun for the seedlings' permanent home and feed with a balanced fertilizer after planting and then once a month during the growing season. Senna alata plants are drought-tolerant, but they will still benefit from being watered regularly and given a layer of mulch during the hottest summer months. As young plants develop, pinch new growth to increase the number of future flower spikes, and prune mature plants back in spring to improve flowering ( Bonnie Singleton, 2015)

**LIGHT:**Christmas candle performs best in full sun.

**MOISTURE:** Normal garden soils and moisture suit this tropical shrub quite well. Mature plants are drought resistant.

**HARDINESS:** USDA Zones 10 - 11. Christmas candle is a tropical shrub that dies as soon as temperatures get near freezing. But in Zones 7,8 and 9 you can grow it as an annual. Just start from seed along with your peppers and tomatoes each spring. It will still get 6-10 ft (2-3 m) tall and begin blooming in October.

**PROPAGATION:** Christmas candle is easy to start from seed, and you can expect volunteer seedlings to emerge under last year's plants in late spring when soil temperatures warm. However, we recommend starting seeds indoors several weeks before the last frost to give the plants a head start on the season (FLORIDA PLANT ENCYCLOPEDIA, 2015)

### 1.6.1 SOIL REQUIREMENTS :

Plant seeds about three quarters of an inch deep in a well-drained soil and humus mixture with a pH range of 5.5 to 6.5. Find an area with full sun for the seedlings' permanent home and feed with a balanced fertilizer after planting and then once a month during the growing season. Senna alata plants are drought-tolerant, but they will still benefit from being watered regularly and given a layer of mulch during the hottest summer months. As young plants develop, pinch new growth to increase the number of future flower spikes, and prune mature plants back in spring to improve flowering (Bonnie Singleton, 2015)

### 1.6.2 CONSIDRATION :

All parts of the Senna alata plant are poisonous if swallowed and should be kept away from children or pets. Because this shrub can become invasive under certain conditions, some areas have banned the introduction of the plant or seeds into the region. This is less of a problem in the U.S. than in other places, such as some areas of Australia.Caution should be taken when adding Senna alata to garden and keep any eye on where it goes to prevent its invasion into natural habitats (Bonnie Singleton, 2015).

## 1.7 Plant Information :

### 1.7.1 Stems and leaves

The thick, pithy stems are upright (i.e. erect or ascending) and occasionally branched. The once-compound (i.e. pinnate) leaves are alternately arranged along the stems and very large (45-80 cm long and 12-25 cm wide). They are borne on stalks (i.e. petioles) 2-4 cm long and have 8-14 pairs of large leaflets. The individual leaflets (5-17 cm long and 2-5.5 cm wide) are either oblong, oval (i.e. elliptic) or egg-shaped in outline (i.e. ovate) and have entire margins. They are finely hairy (i.e. pubescent) and have rounded or slightly notched tips (i.e. obtuse, retuse or emarginate apices) ( Navie, 2004 )


Figure 1.1 : Senna Alata Leaves

### 1.7.2 Flowers

The golden yellow or orange flowers are borne in elongated clusters (15-60 cm long) at the tips of the stems or in the upper leaf forks (i.e. interminal or axillary racemes). These clusters are borne on hairy stalks (i.e. pubescent peduncles) 15-30 cm long and contain numerous (20-40) densely crowded flowers. The individual flowers (2-3 cm across) are borne on short stalks (i.e. pedicels) 5-8 mm long. They are initially held within dark yellow or orange coloured bracts, but these fall off as the flowers open (i.e. they are caducous). Each flower has five sepals (9-15 mm long and 8 mm wide), five bright yellow petals (up to 20 mm long and 12 mm wide) and two stamens with relatively large elongated anthers(11-12 mm long). There are also eight small filaments (2-4 mm long) that do not have any anthers, or only have rudimentary anthers (i.e.staminodes), and an elongated ovary topped with a style and stigma.

Flowering occurs mainly during late autumn, winter and spring (i.e. from May to November) **(**Navie, 2004).

Flower

Flower

Figure 1.2 : Flower of Senna Alata

**1.7.3 Fruit :**

The large and elongated pods (12-25 cm long and 8-20 mm in wide) turn dark brown to black in colour as they mature and contain numerous (about 50) seeds. These pods are somewhat four-angled in cross-section and have papery wings (about 6 mm wide) along these angles. The flattened (i.e. compressed) seeds are dark brown, dark grey or black in colour (4-5 mm in size) and dull in appearance (Navie, 2004).

Fruite


Fruite

Figure : Fruites Of Senna Alata

**using Information**

1.Senna alata is most commonly used in a tisane for cooling or as a laxative. As a bath for itching, crush kasialata and kaka betje leaves (Senna bicapsularis) in water and bathe with the liquid. One herbalist uses kasialata, three to five leaves, with china (Exostema sanctae-luciae), an inch of the bark, when "the blood is dirty, have boils on the skin, have pus." kasialata leaves are boiled and the water used to wash the face or if something "comes up on the skin" A bath made of these leaves makes the skin "come shiny."

2. Leaves or sap are used to treat fungal infections such as ringworm. They contain a fungicide, chrysophanic acid. Because of its anti-fungal properties, it is a common ingredient in soaps, shampoos and lotions in the Philippines. The effectiveness of this plant against skin diseases is confirmed by modern scientific studies.

3. Other chemicals contained in the plant includes saponin which acts as a laxative and expels intestinal parasites. In Africa, the boiled leaves are used to treat high-blood pressure. In South America, besides skin diseases, it is also used to treat a wide range of ailments from stomach problems, fever, asthma to snake bite and venereal diseases (syphilis, gonorrhoea)."

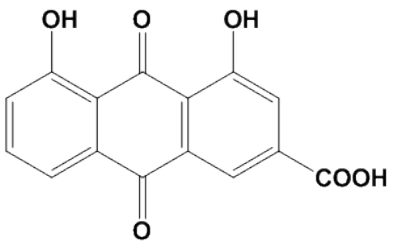
4. *S. alata* is often called the ringworm bush because of its very effective fungicidal properties, for treating ringworm and other fungal infections of the skin. The leaves are ground in a mortar to obtain a kind of "green cotton wool". This is mixed with the same amount of vegetable oil and rubbed on the affected area two or three times a day. A fresh preparation is made every day.Its active ingredients include the yellow chrysophanic acid.

5. Its laxative effect, due to its anthraquinone content, is also well proven.

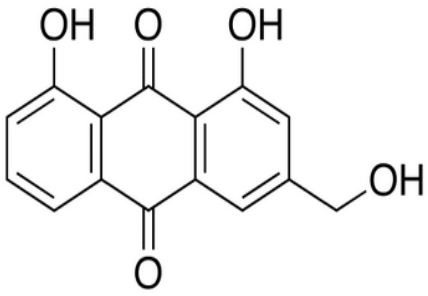
# Chapter 2: Literaturer Rivew

## 2.1 CHEMICAL CONSTITUENTS

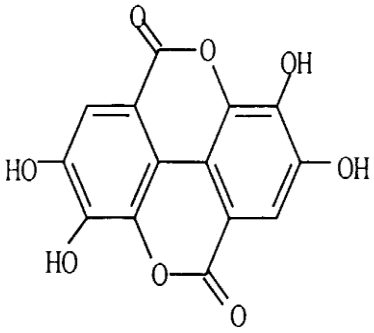
Some chemical compounds are found in the leaves of *Senna alata*



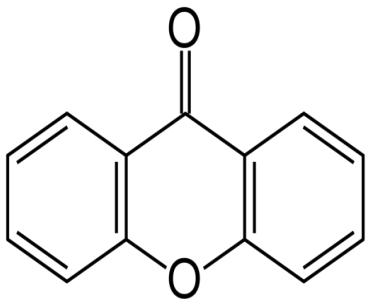

Rhein



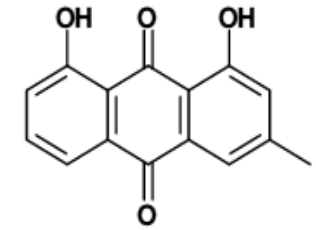

**emodol**



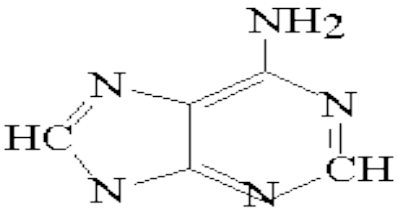

**ellagitannin**




**xanthone**

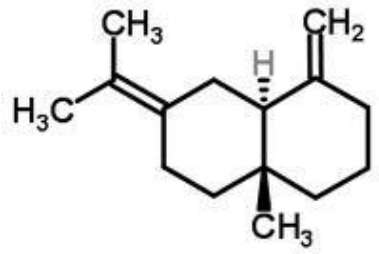



Chrysophanic Acid

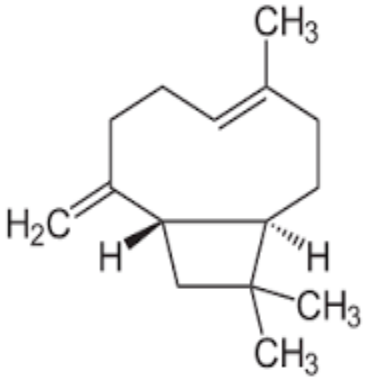



adenine

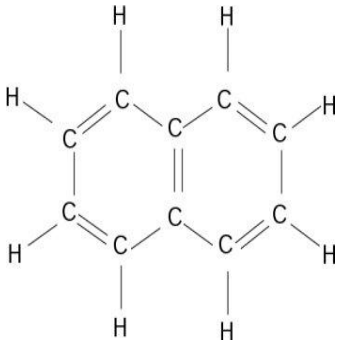
Figure : Some chemical constitution of *Senna alata* leaves



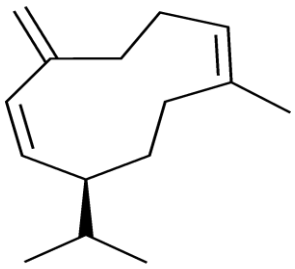

**Caryophyllen**




**Selinene**

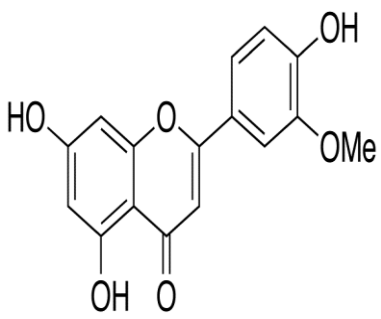


**Naphthalene**

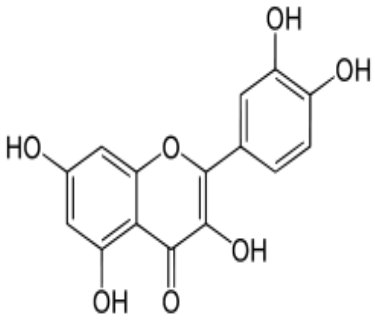


**Germacrene D**

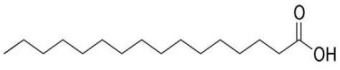
Figure : Some chemical constitution of *Senna alata* leaves




**Chrysoeriol**




**Quercetin**




**Palmitic acid**


**n-triacontanol**

Figure : Some chemical constitution of *Senna alata* leaves

**2.1.1 Uses**

2.1.2**Role in the habitat**

It is the food plant of some butterflies. The plant recruits ant bodyguards against these caterpillars. It has "extrafloral nectaries" near the base of the leaves, that produce sweet nectar to attract ants. As a short-lived plant that grows commonly in wastelands which are damp and on flood plains, it helps to colonise these areas and pave the way for regeneration of growth (Ivan Polunin, 1987).

**Anti-infective**

The methanolic extract of Senna alata leaves have been evaluated on various kinds of microrganism. And the anti-microbial effect was determined by disc diffusion method. The extract exhibit more antifungal than antimicrobial properties.

**Laxative or purgative**

The main medicinal uses of *Senna alata* are as a laxative or purgative and in the treatment of skin problems. For laxative purposes usually a decoction of the leaves is drunk, and less often the flowers, roots or the stem are used.

**Treatment of skin**

Skin problems treated with Senna alata include ringworm, favus and other mycoses, impetigo, syphilis sores, psoriasis, herpes, chronic lichen planus, scabies, rash and itching. Skin problems are most often treated by applying leaf sap or by rubbing fresh leaves on the skin.

**Others uses**

become a weed in pastures; it is not eaten by livestock and is reported to be poisonous, especially for goatss Other ailments treated in tropical Africa with *Senna alata* include stomach pain during pregnancy, dysentery, haemorrhoids, blood in the urine (schistosomiasis, gonorrhoea), convulsions, heart failure, oedema, jaundice, headache, hernia, one-sided weakness or paralysis. A strong decoction made of dried leaves is used as an abortifacient. In veterinary medicine too, a range of skin problems in livestock is treated with leaf decoctions. Such decoctions are also used against external parasites such as mites and ticks (Protabase , 2015).The seeds are a source of gum. The young pods are eaten as a vegetable, but only in small quantities. Toasted leaves are sometimes used as a coffee substitute. *Senna alata* can. The bark is used as fish poison and for tanning leather. The roots and the bark are reported to be used for tattooing. Senna alata is widely appreciated as a garden ornamental and bee forage (Protabase , 2015)

## 2.2 **NUTRITIONAL FACTS**

Table 1.1 : Mineral composition of Senna alata Linn leaf (Abdulwaliyu et al, 2013:p.4)

| **Elements** | **Leaf (mg/100g)** |
|---|---|
| k | 779.20 |

| | |
|---|---|
| Zn | 0.55 |
| Cd | 0.61 |
| Na | 0.53 |
| Mg | 142.80 |
| Fe | 42.35 |
| Ca | 158.38 |

Table1.2 : Antinutrient composition of *Senna alata* Linn leaf (Abdulwaliyu et al, 2013:p.4)

| **Antinutrient** | **Leaf** |
|---|---|
| Alkaloid (%) | 6.75±0.70 |
| Saponin (%) | 2.00±0.01 |
| Oxalate (mg/100g) | 8.03±0.06 |

Table 1.3: Proximate analysis of *Senna alata* Linn leaf (Abdulwaliyu et al, 2013:p.3 )

| **i. Parameters** | **b. Leaf (g/100g)** |
|---|---|
| Moisture | 4.49±0.50 |
| Ash | 9.53±0.06 |

| | |
|---|---|
| Crude fibre | 15.73±0.03 |
| Crude protein | 18.23±0.13 |
| Crude lipid | 3.91±0.01 |
| Carbohydrate | 47.73±0.01 |
| Food energy value | 298.61±0.40 (Kcal/100g) |

# Chapter 3: Materials

The following materials were used during the course of phytochemical study:

**3.1 Solvents:**

- Petroleum ether
- Methanol
- Chloroform
- Acetic acid
- Ethyl acetate
- Toluene
- Acetone
- Benzene
- Dichloromethane

**3.2 Glassware:**

- Thin layer chromatography (TLC) tank
- TLC plates, size in cm (20 x 20), (20 x 5)
- Precoated TLC plates
- Quick fit flasks
- Capillary tube
- Micropipette 1000 microliter

**3.3 Silica gel:**

- TLC grade (PF-254)
- Column grade (60-120 mesh)

**3.4 Spray Reagent:**

- Vanillin-$H_2SO_4$

**3.5 Filter aids:**

- Filter paper (Whatman no: 1)
- Cloth
- Cotton pad
- 1000 ml Beakers

**3.6 Equipment:**

- Rotary evaporator
- Grinding Machine
- Electronic balance
- Distill water maker

UV Light

**Figures of Equipment:**

**3.6.1 Rotary Evaporator**

**3.6.2 Grinding Machine:**

### 3.6.3 Electronic balance

### 3.6.4 Distill water make

# Chapter 4: Methods

### 4.1 Selection of plants

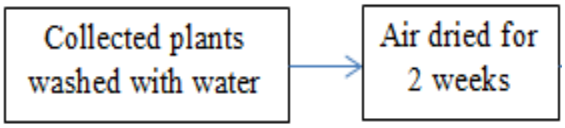
Fresh plants of *Senna Alata* were selected for biological investigation.

### 4.2 Collection of the plant part

For this present investigation the plants of *Senna Alata leaves* was collected from Jhalokathi . The specimen of plant was taxonomically identified at the Bangladesh National Herbarium.

### 4.3 Drying of the plant part

The collected plants were washed with water and unwanted materials were discarded. Collected plants were air and sun dried for 14 days.

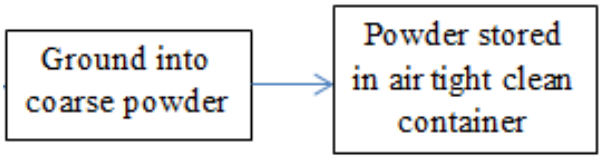



**4.4 Storage and Preservation of plant part**

Most plant parts from desired plants have undergone a period of storage before they were finally used for research purpose in the laboratory. During this period many undesirable changes may occur in the plant parts if they were not properly stored and preserved against the reabsorption of moisture, oxidation, excessive heat, humidity, direct sunlight, growth of molds and bacteria and infestation by insects and rodents. Proper storage and preservation of plant parts are thus are very important factors in maintaining a high degree of quality in them. All efforts towards proper storage should be geared to protect the drugs from all the above deteriorating factors and agents.

**4.5 Grinding of the plant parts**

The dried small pieces of plant parts were grinded into small fine particles by a grinder machine from. The powder was stored in an air tight container and kept in a cool dark and place until analysis commenced




**4.6 MACERATION OF DRIED POWDERED SAMPLE :**

**Principle**

The choice of extraction procedure depends on the nature of the plant material and the components to be isolated. The principle of solid-liquid extraction is that when a solid material comes in contact with a solvent, the soluble components in the solid material move to the solvent (Zarai, 2011). Thus, solvent extraction of plant material results in the mass transfer of soluble active principle (medicinal ingredient) to the solvent, and this takes place in a concentration gradient.The rate of mass transfer decreases as the concentration of active principle in the solvent increases, until equilibrium is reached, i.e., the concentration of active principle in the solid material and the solvent are the same. Thereafter, there will no longer be a mass transfer of the active principle from plant material to the solvent. Since mass transfer of the active principle also depends on its solubility in the solvent, heating the solvent can enhances the mass transfer.Moreover, if the solvent in equilibrium with the plant material is replaced with fresh solvent, the concentration gradient is changed

**4.6.1 Procedure**

After getting the sample as dried powdered, the sample (1690Gram) was then soaked in 6080 ml of methanol for 5 days, the process is known as maceration technique. A glass made jar with plastic cover was taken and washed thoroughly with ethanol and dried. Then the dried powder sample was taken in the jar. After that methanol (6080ml) was poured into the jar up to 1-inch height above the sample surface as it can sufficiently cover the sample surface. The plastic cover with aluminum foil was closed properly to resist the entrance of air into the jar. This process was performed for 5 days. the jar was shaked in several times during the process to get better extraction.

**4.6.2 FILTRATION OF THE EXTRACT**

After the extraction process the plant extracts was filtered with sterilized cotton filter. The cotton was rinsed with ethanol and fitted in a funnel. The filtrate was collected in a beaker. Then again it was filtered and this time What man's filter was used for getting more clear extract which would be useful making the sample more concentrate in Rotary Evaporation Technique. Then the filtrate was taken into a volumetric flask and covered with aluminum foil paper and was prepared for rotary evaporation.

Figure 3.4: Extract obtained after fractionation by methanolic extract

## 4.7 SAMPLE CONCENTRATION BY ROTARY EVAPORATION TECHNIQUE

### 4.7.1 Principle :

Rotary evaporation is the process of reducing the volume of a solvent by distributing it as a thin film across the interior of a vessel at elevated temperature and reduced pressure. This promotes the rapid removal of excess solvent from less volatile samples. Most rotary evaporators have four major components: heat bath, rotor, condenser, and solvent trap. Additionally an aspirator or vacuum pump needs to be attached, as well as a bump trap and round bottom flask containing the sample to be concentrated.

- A motor unit that rotates the evaporation flask or vial containing the user's sample.

- A vapor duct that is the axis for sample rotation, and is a vacuum-tight conduit for the vapor being drawn off of the sample.
- A vacuum system , to substantially reduce the pressure within the evaporator system.
- A heated fluid bath (generally water) to heat the sample.
- A condenser with either a coil passing coolant, or a "cold finger" into which coolant mixtures such as dry ice and acetone are placed.
- A condensate-collecting flask at the bottom of the condenser, to catch the distilling solvent after it re-condenses.
- A mechanical or motorized mechanism to quickly lift the evaporation flask from the heating bath.

The vacuum system used with rotary evaporators can be as simple as a water aspirator with a trap immersed in a cold bath (for non-toxic solvents), or as complex as a regulated mechanical vacuum pump with refrigerated trap. Glassware used in the vapor stream and condenser can be simple or complex, depending upon the goals of the evaporation, and any propensities the dissolved compounds might give to the mixture (e.g., to foam or "bump").( Harwood,et al ,1989; Craig, L. C.; Gregory, J. D.; Hausmann, W,1950).

*Figure* 3.5.1: Rotary Evaporato

Figure 3.5.1: Rotary machine of east west university

### 4.8 Affecting Factors

There are following factors, omission of one of the following factor may interfere the sample concentration procedure and thus which may interfere the phytochemical investigation. Remove the flask from the heat bath.

- Opening the stopcock.
- Heating the rotor.
- Turning off the vacumm/aspirator.
- Disconnecting the flask.
- Dropping flask in heat bath

#### 4.8.1 Procedure

After the filtration process two parts were obtained namely 'residue part' and 'filtered part or filtrate'. The filtrate part, which contains the substance soluble in methanol, was putted into a 1000 ml round bottom flask (BOROSOL), and then the flask was placed in a rotary evaporator. The evaporation was done at 45 degree Celsius temperature. The number of rotation per minute was selected as 30 RPM. The pressure of the vacuum pumper machine (Biometra) was 6 bar. The water flow through the distillation chamber was also provided in a satisfactory flow rate. When the evaporation seemed to be satisfactory, then the methanolic extract was collected in a 100 mi beaker. The evaporator flask was rinsed by diethyl ether, Then the beaker was covered with aluminum foil paper and kept on the water bath for 60 minutes and the temperature of water bath maintained as 50' C. Finally the concentrated methanolic extract was found and stored in the laboratory refrigerator from which the extract was used for many chemical investigation.

## 4.9 SAMPLE CONCENTRATION BY VACUUM LIQUID CHROMATOGRAPHY(VLC) TECHNIQUE :

### 4.9.1 principle :

Chromatographic purification is an integrated part of organic synthesis. The Dry Column Vacuum Chromatography presented here, has excellent resolving power, is easily applied to large scale chromatography (up to 100 g) and is fast. Furthermore, the technique is economical and environmentally friendly due to significant reductions in solvent and the amount of silica used. Therefore, it is an excellent alternative to the commonly used Flash Column Chromatography for purification in organic synthesis.

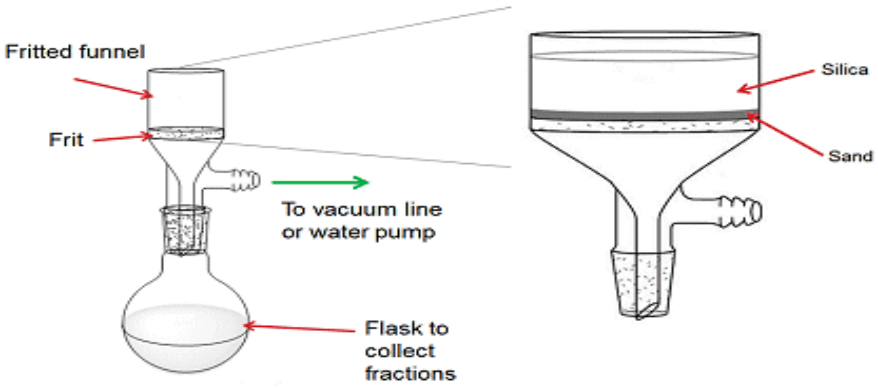



*Figure* 3.6.1: Vacuum Liquid Chromatography

### 4.9.2 Apparatus

- VLC chamber.
- Filter paper

### 4.9.3 Reagents

- Silica gel
- Hexane methanol
- Cyclohexane
- Chloroform
- Dichloromethane.
- N-butanol
- Ethanol

**4.9.4 Procedure:**

The 500gm Methanol extract of *Senna alata* was further exploited in an attempt to isolate the active principle which exhibited the antibacterial activity. In the isolation procedure, different fractions were obtained by using vacuum liquid chromatography apparatus **.** A sintered glass Buckner funnel attached to a vacuum line was packed with TLC grade silica gel. The silica gel was compressed under vacuum in order to achieve a uniform layer in order to get a better separation. The methanol extract was added to the amount (200 mg) of silica gel in order to make a smooth paste. n-Hexane, dichloromethane, n-butanol, Ethyl Acetate and methanol were used as mobile phase in different ratios of increasing polarity from hexane to ethanol. The mixture to be separated according to the polarity of solvents. Each fraction was collected in a separate 100ml beaker. The fractions were monitored by thin layer chromatography. The most active fractions having the similar thin layer chromatography profile were pooled together.

**4.9.5 Drying of extract :**

Using rotary evaporator, the methanolic extract of plant was evaporated at 55-60 degree Celsius temperature and a rotation speed of 160-180 rpm for 1 month. After this drying process, a slurry concentration were obtained, which were kept in small 50 ml beakers for further drying. During transfer to the beaker the extracts were rinsed by acetone.

### 4.9.6 Separation of oil part :

The crude extract was kept untouched for several days. And after this, an oil layer was formed on the upper surface of the extract. The oil was then separated from the upper surface of the extract through decantation. The oil portion separated from extract. the extract portion remained after decantation is termed as crude extract by which further analysis was commenced.

### 4.9.7 Chemicals and other reagents :

Ferric chloride, Sodium carbonate, deionized water, Gallic acid, Sodium nitrite, Aluminum chloride, Sodium hydroxide, Hydrogen peroxide, Normal saline, Wagner's reagent, Hydrochloric acid, Glacial acetic acid, Ammonia, Phoshomolybdic acid, Acetic anhydride, Alcoholic ferric chloride, 5-aqua copper sulphate, Sodium potassium tartrate, DPPH (2,2-diphenyl-1-picrylhydrazyl), Sulfuric acid, Folin reagent, Ciocalteu reagent, prolein amino acid (protein), 1-butanol, glacial acetic acid, Ninhydrine solution, Glucose, Galactose, Maltose, Lactose, Acetone, Phosphate buffer, Anisaldehyde, L-Ascorbic acid, potassium ferricyanide,Ttrichloro acetic acid (TCA)

### 4.9.8 Solvents for experiments :

Dichloromethane, Benzene, Ammonium hydroxide, Formic acid, Dimethylsulfoxide (DMSO), Acetone, Chloroform, Distilled water, Ethanol, Methanol, Diethyl ether, Acetic acid, n-Hexane, Ethyl acetate

## 4.10 Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC

functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC is to obtain well defined, well separated spots.They all have a stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates.A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

As the solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates. (The plate itself contains a fluorescent dye which glows everywhere except where an organic compound is on the plate.).

### 4.10.1 Retention Factor :

After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent.

The Rf formula is

Rf = distance traveled by sample / distance traveled by solvent

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions, the compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value.

Rf values and reproducibility can be affected by a number of different factors such as layer thickness, moisture on the TLC plate, vessel saturation, temperature, depth of mobile phase, nature of the TLC plate, sample size, and solvent parameters. These effects normally cause an increase in Rf values. However, in the case of layer thickness, the Rf value would decrease because the mobile phase moves slower up the plate.

If it is desired to express positions relative to the position of another substance, x, the Rx (relative retention value) can be calculated:

Rx=distance of compound from origin / distance of compound x from origin

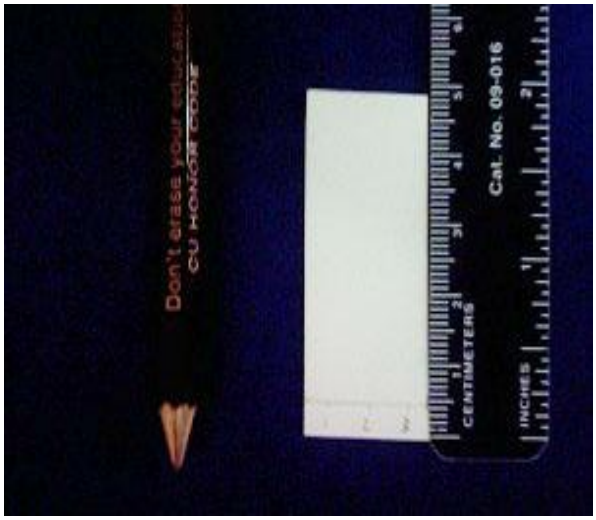
Rx can be greater than 1.

**4.10.2 Materials & reagents :**

1. TLC plate
2. TLC tank
3. Cutter
4. Scale
5. Pencil
6. Solvents & reagents
   - ✓ Methanol
   - ✓ Ethanol
   - ✓ Ethyl acetate
   - ✓ Benzene
   - ✓ Diluted sulfuric acid
7. Hot plate

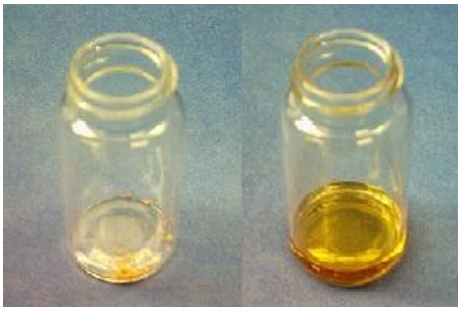
**4.10.3 Test TLC procedure :**

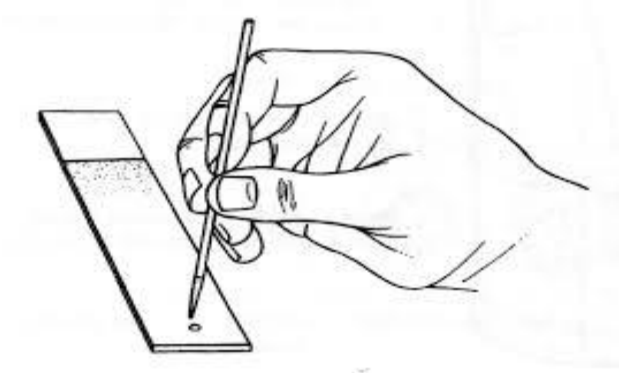
1. Each large TLC sheet (made up of metal & coated with silica) was cut horizontally into plates which are 5 cm tall by 1.5cm in widths. Handle the plate was carefully handled so that the coating of adsorbent was not damaged.




2. 0.5 cm from the bottom of the plate was measured. Using a pencil, draw a line was drawn across the plate at the 0.5 cm mark. This is the origin: on which the extract was spotted. The name of the samples spotted on the plate was lightly marked under the line.

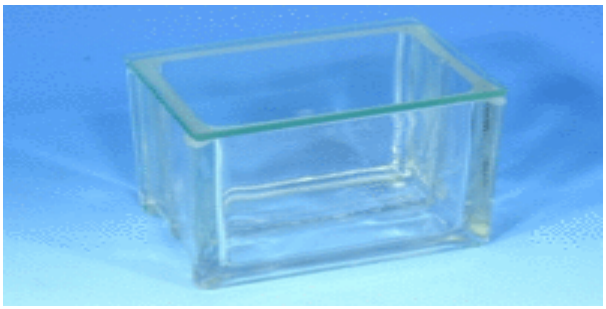
3. ~1 mg of the hard extract was dissolved in 1 ml of methanol in a watch glass to make the solution of the extract.

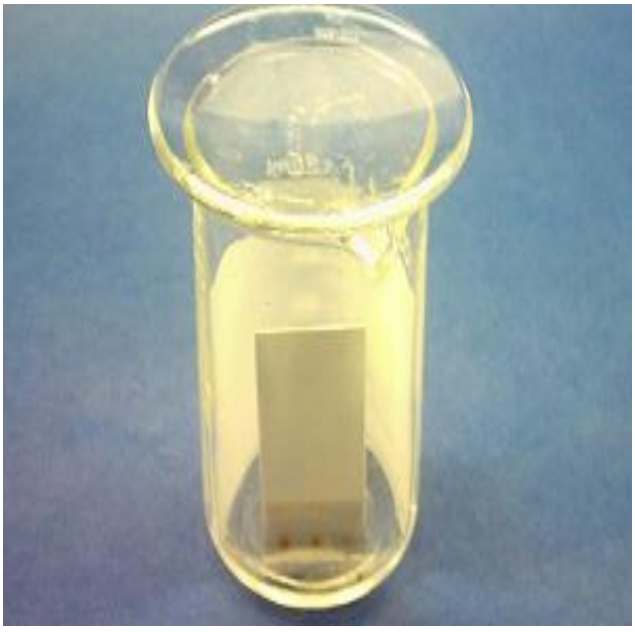




4. The prepared extract solution was then spotted on TLC plate

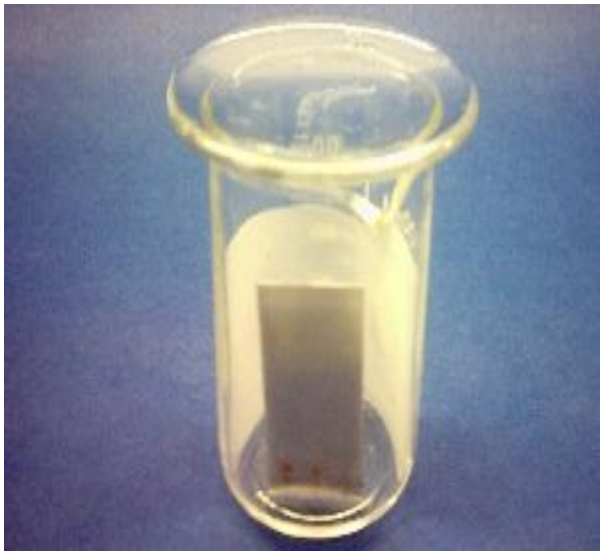
5. Measured solvents were taken in TLC tank

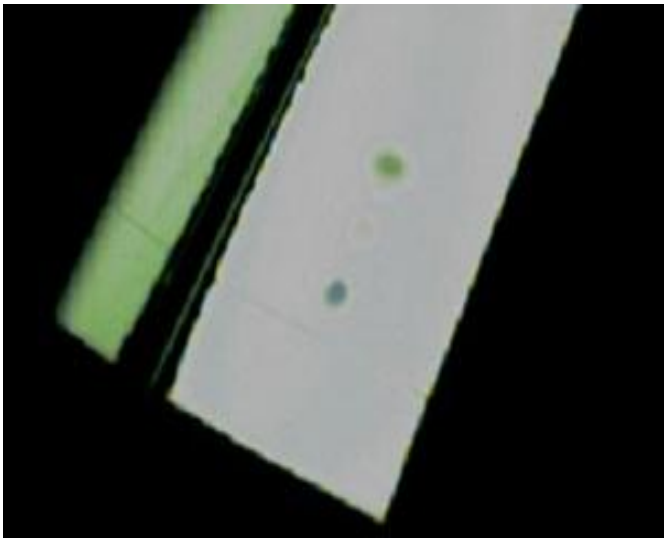




6. The prepared TLC plate was placed in the tank in such a way so that the solvent front remains under the drawn line, the tank was covered with the lid, and left undisturbed on the bench top. The solvent rose up the TLC plate by capillary action. Make sure the solvent does not cover the spot

7. The plate was developed until the solvent is about half a centimeter below the top of the plate. The plate was removed from the beaker and the solvent front was immediately marked with a pencil. The plate was allowed to dry.





8. The plate was then kept under UV lamp to observe any band if appeared.

9. The TLC plate was then sprayed by sulfuric acid in fume hood.


10. The plate was then heated on hot plate so that the band color can be appeared clearly.

11. Then Rf valued by measuring the distance traveled by the sample and the distance travled by the solvent in the TLC plate.

Test TLC was performed for the crude extract of *Senna Alata* on the basis of trial & error for several times by changing the ratio of solvents used as mobile phase.

Different solvents used as mobile phase and results based on this in test TLC

Table : The composition of various solvent systems for TLC

| | | |
|---|---|---|
| Benzene 9ml | Chloroform 5ml | Ethyl acetate 8ml |
| Ethanol 1ml | Ethyl acetate 4ml | Ethanol 1.2ml |
| AlOH 0.1ml | Formic acid 1ml | Water 0.8ml |

**4.10.4 Preparative Thin Layer Chromatography:**

**4.10.4.1 Apparatus and reagents:**

- Ethanol

- Ethyl acetate
- Chloroform
- Spatula
- Silica gel
- Distilled Water
- Glass rod
- Preparative TLC tank
- Test TLC plates
- Test TLC tank
- Sulfuric acid
- Heater
- Forcep
- UV spectrometer

**4.10.4.2 TLC plate preparation:**

TLC plates had prepared by Silica gel and distilled water. 500 gm of silica gel was mixed with distilled water of a suitable amount to make a suitable paste that could be made as a layer on a square shaped glass plate. The paste was made and its viscosity was checked by making a sample plate. Then the paste was smoothly applied on the glass plates to make a suitable TLC plate. Then the plates were kept for 24 hour to let them dry.

Fig: Preparative TLC plate

**4.10.4.3 Sample Introduction:**

When the TLC plates were completely dried, sample was introduced on side the plate by using micropipette. A few microgram of the sample extract was diluted by ethanol in a small beaker then the diluted sample was introduced by the micropipette. After this, the plate was dried for few minutes.

**4.10.5 Tank preparation:**

Fig: preparative TLC tank

The Tank for Preparative TLC is a larger one then the test TLC tank. The tank was cleaned well first then dried by electrical drier. The TLC reagents Ethanol 350 ml was introduced into the tank and the closed it for few moments to saturate the internal environment by the reagent.

#### 4.10.5.1 Operation:

The prepared TLC plate was introduced to the tank very carefully that must not touch the wall of the tank and the reagent must not cross the sample line. Then the tank closed by the closure and waited to run the reagent through the plate. When the reagent reaches the top, it came out from the tank and let it for drying.

#### 4.10.5.2 Observation:

After drying the plate it was observed under UV light at Wavelength 257nm and bands were observed. After that the plates were placed into the Fume hood for charring with $H_2SO_4$. Then the three bands became clearer.

### 4.11 Band collection:

The bands were collected by a clean Spatula into a small beaker. Then the contents of the beaker were dissolved in chloroform and ethyl acetate and kept for one day. Then the contents were filtered by filter paper and kept the filtrate for 3 days to make that contents dried and to remove solvents. Then the dried content was dissolved by acetic acid and chloroform for test TLC purpose. Then the sample was introduced onto test TLC plate and a test TLC was done with Ethanol.

**Result:** The test TLC showed a single band for each band in preparative TLC.

(1 ) (2) (3)

Figure 5.2: (1)TLC plate Under UV light,(2)TLC plate after charring with $H_2SO_4$, (4) TLC plate after application of DPPH.

**4.11.1 Flow Chart:**

Cleaning preparative TLC plate and Tank

↓

Drying

↓

Silica gel mixing with distilled water

↓

Silica Gel Paste introduce onto the plate

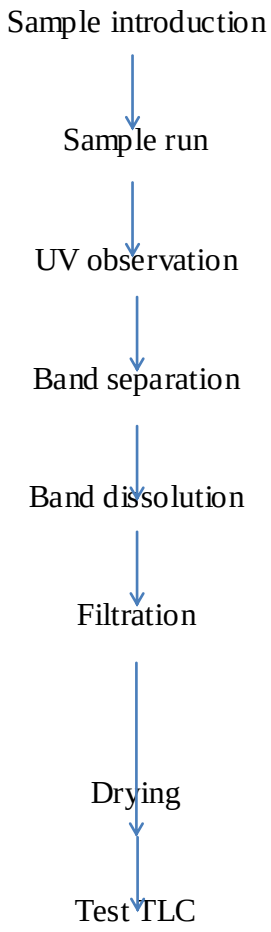
↓

Smooth surface making by glass rod

↓

Drying for 24 hours

↓




**4.12 Discussion:**

Result of preparative TLC said that the sample contain some different compounds which gave the bands. The bands were for a single compound for each band as the test TLC for each band showed only a single band.

**4.13 Antimicrobial Activity by disc diffussion method :**

**4.13.1Principal :**

The disk diffusion susceptibility method is simple and well-standardized. Bacterial inoculumsare applied to the surface of a large agar plate. Antibiotic discs and disc of test materials areplaced on the inoculated agar surface. Plates are incubated for 16–24hr at 35°C prior to determination of results. The test materials having antimicrobial property inhibit microbialgrowth in the media surrounding the discs and thereby yield a clear, distinct area defined as zoneof inhibition. The zones of growth inhibition are measured to the nearest millimeter around eachof the antibiotic disks. The diameter of the zone is related to the susceptibility of the isolate andto the diffusion rate of the drug through the agar medium (Barry, 1976).

**4.13.2 Apparatus And Reagent :**

Table 3.2: Apparatus and reagents for antimicrobial test

| | |
|---|---|
| Filter paper discs | Screw cap test tubes |
| Petri dishes | Nose mask and Hand gloves |
| Inoculating loop | Laminar air flow hood |
| Sterile cotton | Autoclave |
| Sterile forceps | Incubator |
| Spirit burner | Ethanol |
| Micropipette | Nutrient Agar Medium |

**4.13.3 Test Sample of Senna Alata :**

**4.13.4Test Organism :**

The bacterial strains used for the experiment were collected as pure cultures from the East WestUniversity microbiology laboratory. Both gram positive and gram-negative organisms were taken for the test and they are listed in the following table.

| **type of bacteria** | **Name of bacteria** |
|---|---|

| | |
|---|---|
| Gram +ve | Streptococcus aureaus<br>Bacillus subtilis<br>Bacillus ceresus<br>Sarcina lutea<br>*Streptococcus pyrogeny* |
| Gram –ve | Escherichia coli<br>Salmonella paratyphi<br>Vibrio parahaemolyticus<br>Shigella dysenteriae<br>Pseudomonas<br>Vibriomimicus<br>Shigella Boydii<br>Kleb Seilla |
| Fungi | *Candida albicans*<br>*Aspergillus niger*<br>*Bacillus megaterium* |

**4.13.5 Procedure :**

**4.13.6 Preparation of the medium :**

To prepare required volume of this medium, 5.6gm of agar medium was taken in a bottle with a cap and distilled water was added to it to make 200ml volume. The contents were then autoclaved to make a clear solution.

**Figure : Autoclave mechine**

### 4.13.7 Sterilization Procedure :

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 1210C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

**Figure : Laminar hood**

### 4.13.8 Preparation of the test plate :

The test organisms were transferred from the subculture to petridish containing about 10 ml of melted and sterilized agar medium. The bacterial and fungal suspension was taken by a loop mixed with normal saline with the help of vortex machine. Then a sterilized cotton bud was taken and dipped into the bacterial suspension. Then the bacterial sample is applied to the petridish with the help of this cotton bud.

### 4.13.9 Preparation of the disc :

Three types of discs were used for antimicrobial screening

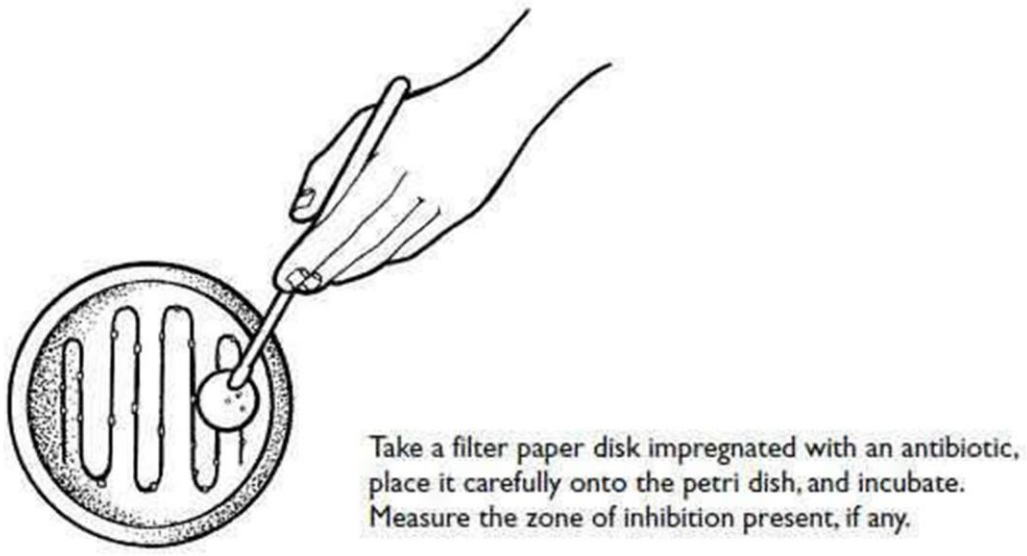



Figure : Preparation of filter paper discs

**Standard Discs**: These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, ciprofloxacin (30µg/disc) disc was used as the reference.

**Blank Discs**: These were used as negative controls which ensure that the residual solvent (left over the discs even after air-drying) and the filter paper were not active themselves.

**Sample Discs**: These discs were soaked with solutions of test samples of known concentration, dried and used to determine the anti-activity of the samples.

### 4.13.10 Preparation of test sample :

Measured amount of test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical filter paper discs were taken ina blank petridish under the laminar hood. Then discs were soaked with solutions of test samplesand dried.

**4.13.10.1 Application of test sample :**

Standard ciprofloxacin discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. Methanol discs were used asnegative controls which ensure that the residual solvents (left over the discs even after airdrying)and the filter paper were not active themselves.

**4.13.10.2 Diffusion and incubation**

The sample discs, the standard antibiotic discs and the control discs were placed gently on thepreviously marked zones in the agar plates pre-inoculated with test bacteria. The plates were thekept in a refrigerator at 4°C for about 24 hours upside down to allow sufficient diffusion of thematerials from the discs to thsurrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours

Figure : Incubator

The antimicrobial potency of the test agents are measured by their activity to prevent the growth

**4.13.10.3 Determination of Antmicrobial activity by measuring the zone of inhibition :**

of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

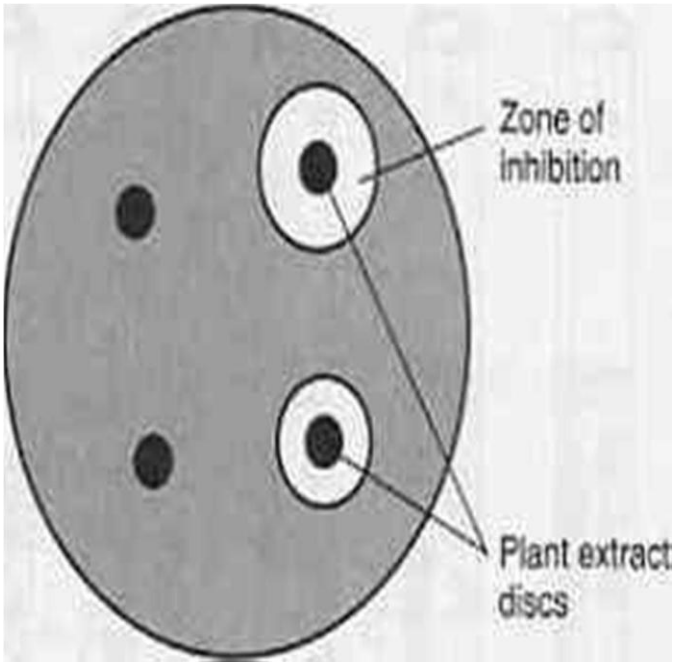



Figure : clear zone of inhibition

**4.14 Test of Antidiabetics :**

**4.14.1 Introduction :**

is a chronic condition associated with abnormally high levels of sugar (glucose) in the blood. Insulin produced by the pancreas lowers blood glucose. Absence or insufficient production of insulin causes diabetes. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications.Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma(Kitabchi et al, 2009) Serious ($IC_{50}$, $63.75 \pm 12.81$ µg/ml), was fractionated. The α-glucosidase inhibitory effect of the crude extract The methanol extract of leaves of *S. alata*, which showed potent α-glucosidase inhibitory activity was far better than the standard clinically used drug, acarbose ($IC_{50}$, $107.31 \pm 12.31$ µg/ml). A subsequent fractionation of the crude extract was made using solvents of ascending polarity (petroleum ether, chloroform, ethyl acetate, *n*-butanol and water). The ethyl acetate ($IC_{50}$, $2.95 \pm 0.47$ µg/ml) and *n*-butanol ($IC_{50}$, $25.80 \pm 2.01$ µg/ml) fractions which contained predominantly kaempferol ($56.7 \pm 7.7$ µM) and kaempferol 3-

*O*-gentiobioside (50.0 ± 8.5 μM), respectively, displayed the highest carbohydrate enzyme inhibitory effect. One of the possible antidiabetic mechanisms of action of *S. alata* is by inhibiting carbohydrate digestion. This is the first report on α-glucosidase activity of kaempferol 3-*O*-gentiobioside.( George et al, 2013) Antidiabetic components of *Cassia alata* leaves: Identification through α-glucosidase inhibition studies).
long-term complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes ( *WHO*, 2013).

There are three main types of diabetes mellitus:

- Type 1 DM results from the pancreas' failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown ( *WHO*, 2013).
- Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly ( *WHO*,2013). As the disease progresses a lack of insulin may also develop. This form was previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The primary cause is excessive body weight and not enough exercise ( *WHO*, 2013).
- Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood sugar level ( *WHO*, 2013).

Prevention and treatment involve a healthy diet, physical exercise, not using tobacco and being a normal body weight. Blood pressure control and proper foot care are also important for people with the disease. Type 1 diabetes must be managed with insulin injections ( *WHO*,2013).Type 2 diabetes may be treated with medications with or without insulin. Insulin and some oral medications can cause low. Weight loss surgery in those with obesity is sometimes an effective measure in those with type 2 DM. Gestational diabetes usually resolves after the birth of the baby ( Cash, 2014).As of 2014, an estimated 387 million people have diabetes worldwide, with type 2 diabetes making up about 90% of the cases.(Shi et al, 2014) This represents 8.3% of the adult population (Shiet al, 2014) with equal rates in both women and men. From 2012 to 2014, diabetes is estimated to have resulted in 1.5 to 4.9 million deaths each year *World Health Organization*,2013) (Diabetes at least doubles a person's risk of death ( *WHO*, 2013) .The number of people with diabetes is expected to rise to 592 million by 2035. The global economic

cost of diabetes in 2014 was estimated to be $612 billion USD ( International Diabetes Federation, 2013) In the United States, diabetes cost $245 billion in 2012 (American Diabetes, Association ,2013)

The investigation of antidiabetic agents of plant origin which are used in traditional medicine is of great importance. The seed kernel of Mangifera indica is one such herbal source which is mentioned in Ayurvedic literature for treating Diabetes mellitus. The kernel is astringent, antihelmintic, stimulant, anti-inflammatory, antibacterial, antifungal, anti-pasmodic, anti-scorbutic and is administered in asthma, diabetes, nasal bleeding, diarrhea and ulcers (Jain, 2011). Similarly, *Senna alata* leaves possess numerous medicinal properties such as antibacterial, antioxidant, antidiabetic, anti-inflammatory, anti-diuretic, immunomodulatory and have been useful in the treatment of skin diseases, convulsions, constipation.Non-enzymatic reaction between reducing sugar and free amino group of proteins, also known as Maillard reaction, leads to the formation of glycated protein termed Amadori product. Further rearrangement, oxidation and reduction of the Amadori product result in the formation of several advanced glycation endproducts (ages) such as pentosidine, carboxymethyllysine, crossline and pyralline. Some of these products can react with a free amino group nearby and form cross linking between proteins (Ulrich and Cerami, 2001). The cross linked protein, e.g. Cross linked collagen, are postulated to confer pathological conditions found in patients with diabetes and aging, such as arterial stiffness and decreased myocardial compliance, resulting from the loss of collagen elasticity (Aronson, 2003). Thus, agents that inhibit the formation of ages are purported to have therapeutic potentials in patients with diabetes and age-related diseases. The oxidation process is believed to play an important role in ages formation. Further oxidation of Amadori product leads to the formation of intermediate carbonyl compounds that can react with the nearby lysine or arginine residues to form protein crosslink and ages. The reactive carbonyl compounds may also be generated from the metal ion-catalyzed autooxidation of glucose (Voziyan et al, 2003).

### 4.14.2 **Principle:**

Advanced glycation end products (AGEs) are modifications of proteins or lipids that become non enzymatically glycated and oxidized after contact with aldose sugars (Schmidt AM,et

al,1994;SinghR,et al, 2001). Early glycation and oxidation processes result in the formation of Schiff bases and Amadori products. Further glycation of proteins and lipids causes molecular rearrangements that lead to the generation of AGEs. (Schmidt AM,et al,1994). AGEs may fluoresce, produce reactive oxygen species (ROS), bind to specific cell surface receptors, and form cross-links. (Schmidt et al, 1994; Brownlee, Vlassara and,Cerami, 1985) AGEs form in vivo in hyperglycemic environments and during aging and contribute to the pathophysiology of vascular disease in diabetes.( Schmidt et al, 1985).This review summarizes AGE formation and biochemistry, cellular receptors for AGE, AGE-induced effects on extracellular and intracellular functions, and developing AGE therapies.AGEs accumulate in the vessel wall, where they may perturb cell structure and function. AGEs have been implicated in both the micro vascular and macro vascular complications of diabetes. As reviewed by Brownlee (Brownlee, 1995) AGEs may modify the extracellular matrix (ECM); modify the action of hormones, cytokines, and free radicals via engagement of cell surface receptors; and impact the function of intracellular proteins. Glycation is the key molecular basis of several diabetic complications like diabetes retinopathy, nephropathy, neuropathy and some cardiovascular diseases. This is a non enzymatic reaction between amino groups of proteins and carbonyl groups of reducing sugars forming florescent, insoluble Advanced Glycation End Products that accumulate on loprinciplglycation lived proteins thus compromising the physiological functions.A large number of studies have focused on the factors involved in the pathogenesis of diabetic complications, most seeking effective therapies, but the exact cellular or molecular basis of these complications has not yet been fully elucidated. Hyperglycemia is still considered the principal cause of diabetes complications. Its deleterious effects are attributable, among other things, to the formation of sugar-derived substances called advanced glycation end products (AGEs). AGEs form at a constant but slow rate in the normal body, starting in early embryonic development, and accumulate with time. However, their formation is markedly accelerated in diabetes because of the increased availability of glucose (Melpomeni et al, 2003)Various studies have shown that diabetes mellitus is associated with an increased production of free radicals leading to oxidative stress. Thus disturbed balance between radical formation and radical neutralization leads to oxidative damage of cell components such as proteins, lipids and nucleic acids. Oxidation plays an important role in the formation of Advanced Glycation End Products and the Plants derived

agents with the antiglycation and antioxidant activities are highly important in preventing diabetic complication.

### 4.14.3 Procedure:

Antidiabetic activity of leaves of *Senna alata* Were investigated by glucose uptake in yeast cell. Yeast cells were prepared according to the method of Yeast cells (Kotowaroo et.al, 2006). Briefly, commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 50% (v/v) suspension was prepared in distilled water. Various concentrations of Isolated constituents (1 mg) after VLC were added to 1 ml of glucose solution (10 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Metronidazole was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula-Increase in glucose uptake (%) = Abssample – Abscontrol X 100 Abssample Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates (Kabir et al, 2011).

## 4.15 ANTI OXIDANT TESTS :-

### 4.15.1 Principle

DPPH is a common abbreviation for an organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free-radical molecules. DPPH has two major applications, both in laboratory research: one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay ( Om P. Sharma & Tej K.Bhat,2009),and another is a standard of the position and intensity of electron paramagnetic resonance signals.DPPH is a well-known radical and a trap

("scavenger") for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm or in the EPR signal of the DPPH ( Mark and Alger, 1997). As a stable and well-characterized solid radical source, DPPH is the traditional and perhaps the most popular standard of the position (g-marker) and intensity of electron paramagnetic resonance (EPR) signals – the number of radicals for a freshly prepared sample can be determined by weighing and the EPR splitting factor for DPPH is calibrated at g = 2.0036. DPPH signal is convenient by that it is normally concentrated in a single line, whose intensity increases linearly with the square root of microwave power in the wider power range. The dilute nature of the DPPH radicals (one unpaired spin per 41 atoms) results in a relatively small line deprecated (1.5–4.7 Gauss). The line deprecated may however increase if solvent molecules remain in the crystal and if measurements are performed with a high-frequency EPR setup (~200 GHz), where the slight g-anisotropy of DPPH becomes detectable ( Davies , 2000)

**4.15.1 Apparatus :**

- Test tube
- Racker
- Beaker
- Uv-spectrophotometer
- Spatula
- Analytical balance

**4.15.2 Reagents**

- DPPH
- L-ascorbic acid
- Methanol
- Water

**4.15.3 Procedure :**

**4.15.3.1 Sample Preparation :**

- The methanolic extract of the *Senna alata leaves* both husk and tegmen of different fraction were taken in test tubes to prepare different concentrations.
- 1µg/ml sample was taken in test tubes, and prepared 10 ml sample solution with 9 ml water. Then each sample was diluted ino 1ml, 2ml, 3ml, 4ml and volume adjusted to 4ml with water in all the test tubes.

4.15.3.2 **Standard Preparation :**

- 250 mg Ceevit Tablet (Square) was crushed in mortar and pestle.
- 20 ml distilled water was added and the solution was filtered.
- It was then diluted by 10 times (2 ml of the filtered solution was taken and 18 ml water added).
- The solution was taken in 5 test tubes to prepare 5 different concentrations.
- 1ml, 2ml, 2ml,and 4ml solution were taken in 4 different test tubes and the volume adjusted to 4 ml with water in all the test tubes.

4.15.3.3 **Blank Preparation**

Blank was prepared by adding 1 ml methanol in a test tube and volume adjusted with 9 ml water. Blank was made in same way of the sample.

- After preparation of sample and blank preparation 100 µl DPPH solution was added in dark and left for half an hour. After that UV absorbance was measured in UV machine at 517 nm.
- After 30 min at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the

equation, DPPH antiradical scavenging capacity (%) = [1 – (Abof sample – Abof blank)/Abof control] × 100.

# Chapter 5: Result and Discussion

**Result :**

**5.1 Antioxident Result :**

Table:Antioxidant activity of Ascorbic Acid

| sample name | concentration µg/ml | Absorbance 1st time | Absorbance 2nd time | % of inhibition |
|---|---|---|---|---|
| | 500 | 0.036 | 0.168 | ±9.11 |
| Ascorbic Acid | 250 | 0.044 | 0.199 | ±12.55 |
| | 125 | 0.055 | 0.219 | ±22.35 |
| | 62.5 | 0.034 | 0.235 | ±8.47 |
| | 31.25 | 0.056 | 0.246 | ±17.60 |

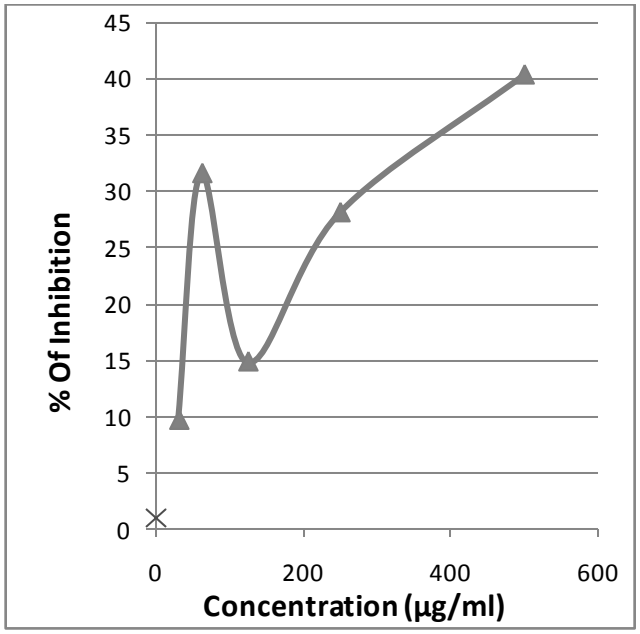



Figure : % Free radical scavenging activity of Ascorbic Acid

Table : .Antioxidant activity of Methanol fraction of methanolic extract of *Senna alata leaves*

| sample name | concentration µg/ml | Absorbance 1st time | Absorbance 2nd time | % of inhibition |
|---|---|---|---|---|
| | 500 | 0.065 | 0.125 | ±29.11654 |
| | 250 | 0.053 | 0.162 | ±32.51418 |
| Methanol – Methanol Fraction | 125 | 0.054 | 0.145 | ±37.61525 |
| | 62.5 | 0.058 | 0.153 | ±41.01502 |
| | 31.25 | 0.041 | 0.135 | ±22.98663 |

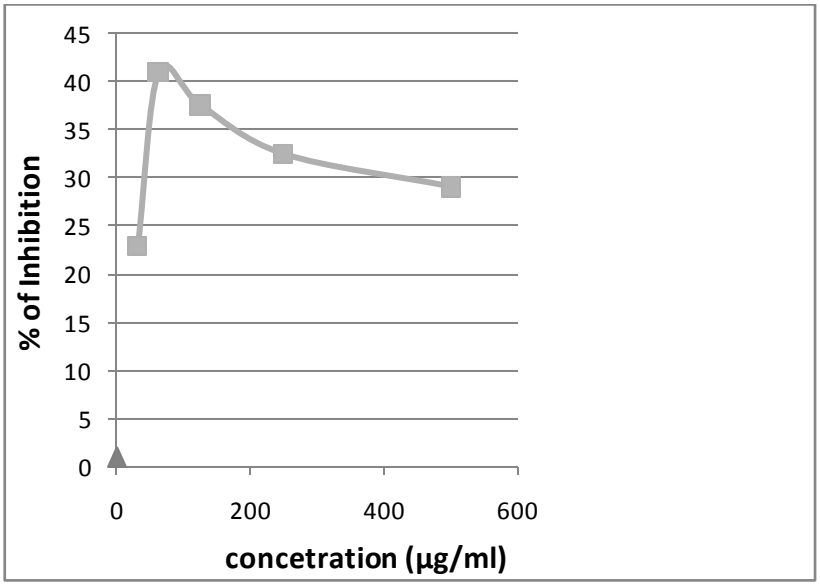



Figure : % Free radical scavenging activity Methanol Fraction of Methanolic Extract

Table :Antioxidant activity of DCM fraction of methanolic extract of *Senna alata leaves*

| sample name | concentration µg/ml | Absorbance 1st time | Absorbance 2nd time | % of inhibition |
|---|---|---|---|---|
| | 500 | 1.212 | 0.108 | ±1548.33 |
| Methanol-DCM fraction | 250 | 0.204 | 0.115 | ±238.94 |
| | 125 | 0.140 | 0.419 | ±87.88 |
| | 62.5 | 0.127 | 0.191 | ±122.03 |
| | 31.25 | 0.058 | 0.258 | ±17.52 |

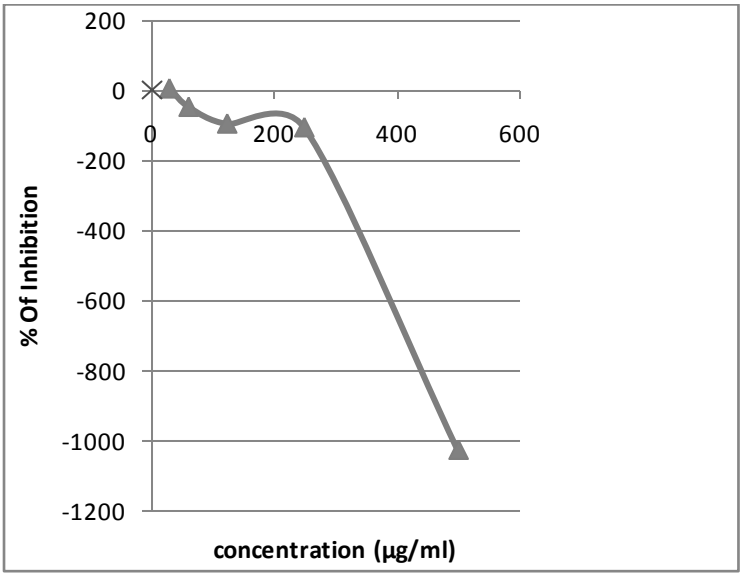



Fig 4.5.7 : % Free radical scavenging activity of DCM fraction Methanolic extract of *Senna alata leaves*

Table:Antioxidant activity of Ethyl Acetate fraction of methanolic extract of *Senna alata leaves*

| sample name | concentration µg/ml | Absorbance 1st time | Absorbance 2nd time | % of inhibition |
|---|---|---|---|---|
| | 500 | 0.232 | 0.155 | ±266.32 |
| | 250 | 0.093 | 0.580 | ±9.12 |
| Methanol-Ethyl Acetate Fraction | 125 | 0.070 | 0.292 | ±610.91 |
| | 62.5 | 0.059 | 0.230 | ±25.08 |
| | 31.25 | 0.067 | 0.167 | ±49.55 |

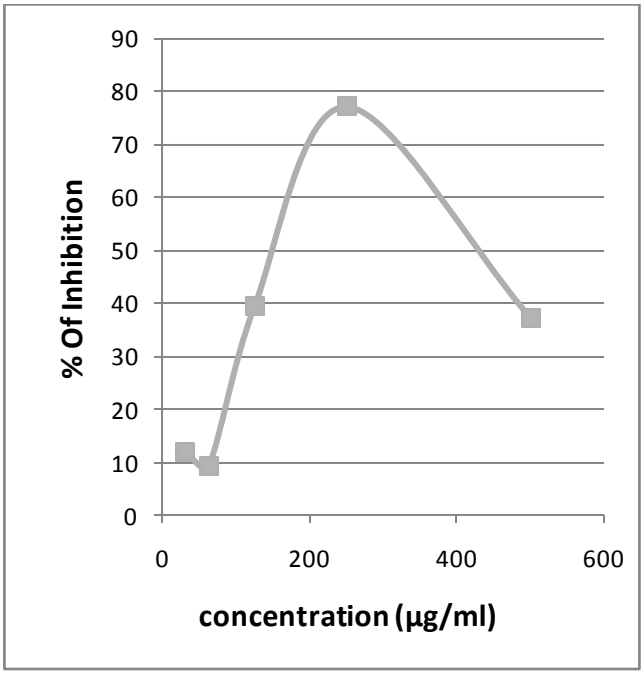



Figure: % Free radical scavenging activity of Ethyl Acetate fraction of Methanolic extract *Senna alata leaves*

Table 4.4.6:Antioxidant activity of Methanol fraction of DCM extract of *Senna alata leaves*

| sample name | concentration µg/ml | Absorbance 1st time | Absorbance 2nd time | % of inhibition |
|---|---|---|---|---|
| | 500 | 0.183 | 0 .128 | ±208.78 |
| | 250 | 0.129 | 0.208 | ±120.11 |
| DCM-Methanol Fraction | 125 | 0.074 | 0.248 | ±40.51 |
| | 62.5 | 0.070 | 0.248 | ±35.32 |
| | 31.25 | 0.050 | 0.306 | ±3.60 |

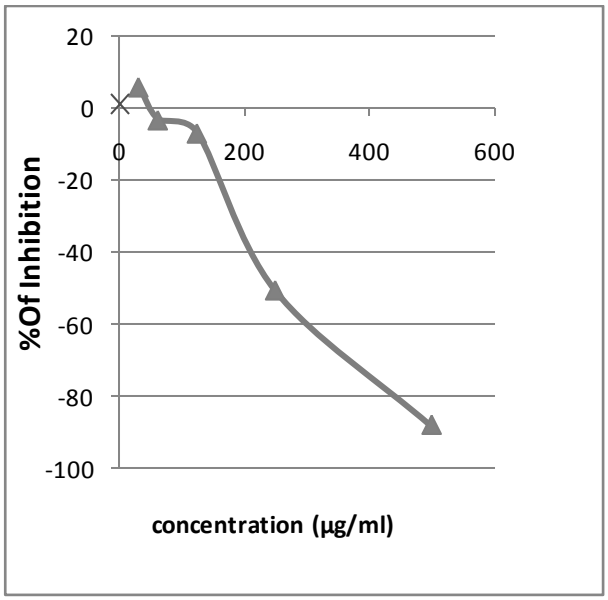



Fig 4.5.7 : % Free radical scavenging activity of Methanol fraction of *DCM extract of Senna alata leaves*

Table:Antioxidant activity of n- hexan fraction of DCM extract of *Senna alata leaves*

| sample name | concentration µg/ml | Absorbance 1st time | Absorbance 2nd time | % of inhibition |
|---|---|---|---|---|
| | 500 | 0.322 | 0.118 | ±391.37 |
| | 250 | 0.170 | 0.127 | ±192.14 |
| DCM- n Hexan Fraction | 125 | 0.104 | 0.122 | ±107.63 |
| | 62.5 | 0.077 | 0.180 | ±59.62 |
| | 31.25 | 0.079 | 0.233 | ±50.35 |

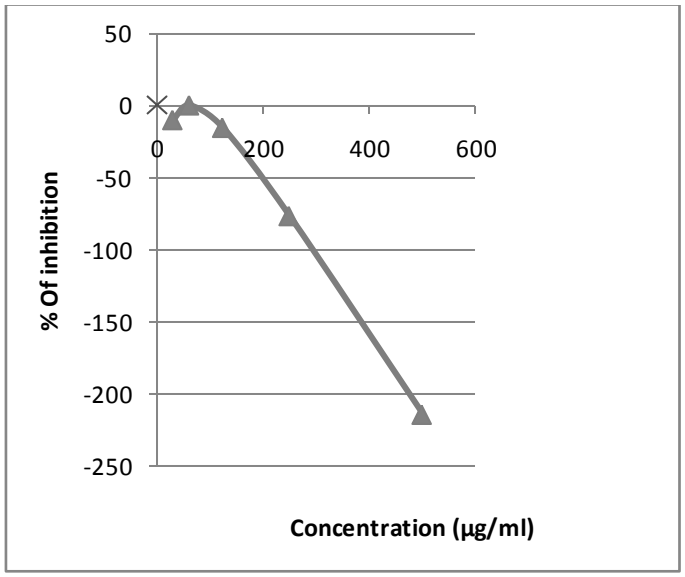



Figure : % Free radical scavenging activity of n-Haxen fraction of *DCM extract ofSenna alata leaves*

Table 4.4.6:Antioxidant activity of DCM fraction of DCM extract of *Senna alata leaves*

| **Sample Name** | **Concentration µg/ml** | **Absorbance 1st Time** | **Absorbance 2nd time** | **% of Inhibition** |
|---|---|---|---|---|
| | 500 | 0.421 | 0187. | ±445.97 |
| | 250 | 0.411 | 0.200 | ±436.58 |
| DCM- DCM fraction | 125 | 0.107 | 0.265 | ±56.70 |
| | 62.5 | 0.327 | 0.739 | ±448.20 |
| | 31.25 | 0.054 | 0.304 | ±2.03 |

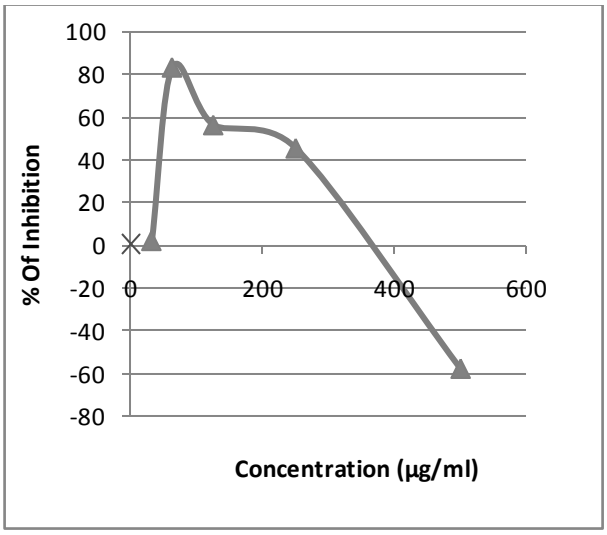



Fig : % Free radical scavenging activity of DCM fraction of *DCM extract of Senna alata leaves*

Table :Antioxidant activity of Ethyl Acetate fraction of DCM extract of *Senna alata leaves*

| Sample Name | Concentration µg/ml | Absorbance 1st Time | Absorbance 2nd time | % of Inhibition |
|---|---|---|---|---|
| | 500 | 0.321 | 0.172 | ±377.99 |
| | 250 | 0.293 | 0.258 | ±296.46 |
| DCM-Ethyl Acetate Fraction | 125 | 0.241 | 0.791 | ±348.26 |
| | 62.5 | 0.124 | 0.438 | ±117.47 |
| | 31.25 | 0.08 | 0.245 | ±50.268 |

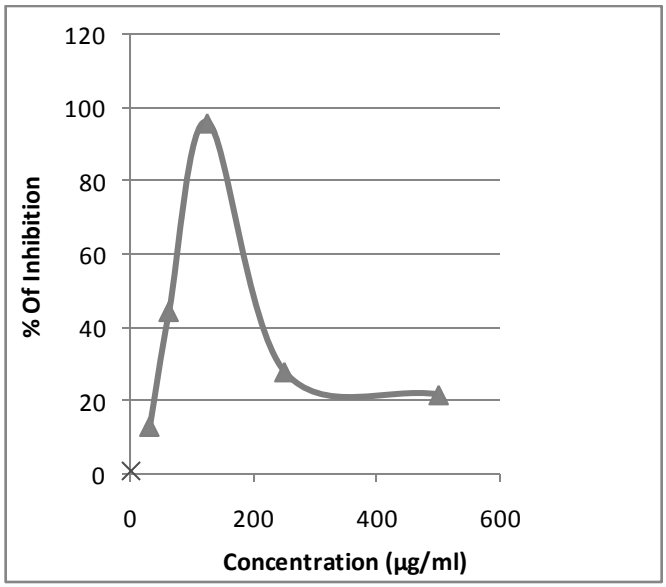



Figure : % Free radical scavenging activity of Ethyl Acetate fraction of DCM extract *Senna alata leaves*

Table:Antioxidant activity of DCM fraction of Ethyl Acetate extract of *Senna alata leaves*

| **Sample Name** | **Concentration µg/ml** | **Absorbance 1st Time** | **Absorbance 2nd time** | **% of Inhibition** |
|---|---|---|---|---|
| | 500 | 0.156 | 0.117 | ±87.16 |
| | 250 | 0.152 | 0.201 | ±25.72 |

| | | | | |
|---|---|---|---|---|
| Ethyl Acetate-DCM fraction | 125 | 0.124 | 0.330 | ±549.35 |
| | 62.5 | 0.109 | 0.346 | ±77.42 |
| | 31.25 | 0.094 | 0.321 | ±52.36 |

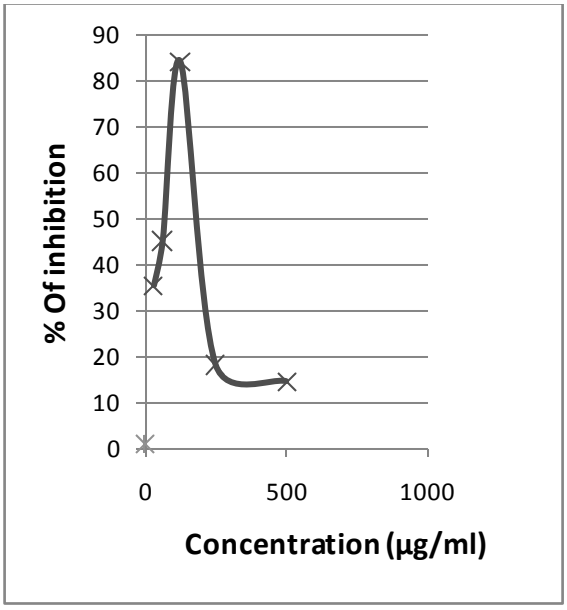



Figure : % Free radical scavenging activity of DCM fraction of ethyl acetate extract *Senna alata leaves*

Table:Antioxidant activity of Ethyl Acetate fraction of Ethyl Acetate extract of *Senna alata leaves*

| Sample Name | Concentration µg/ml | Absorbance 1st Time | Absorbance 2nd time | % of Inhibition |
|---|---|---|---|---|
| | 500 | 0.589 | 0.219 | ±671.77 |
| | 250 | 0.413 | 0.184 | ±435.59 |
| Ethyl Acetate-Ethyl Acetate fraction | 125 | 0.282 | 0.318 | ±294.72 |
| | 62.5 | 0.176 | 0.132 | ±198.81 |
| | 31.25 | 0.189 | 0.253 | ±160.40 |

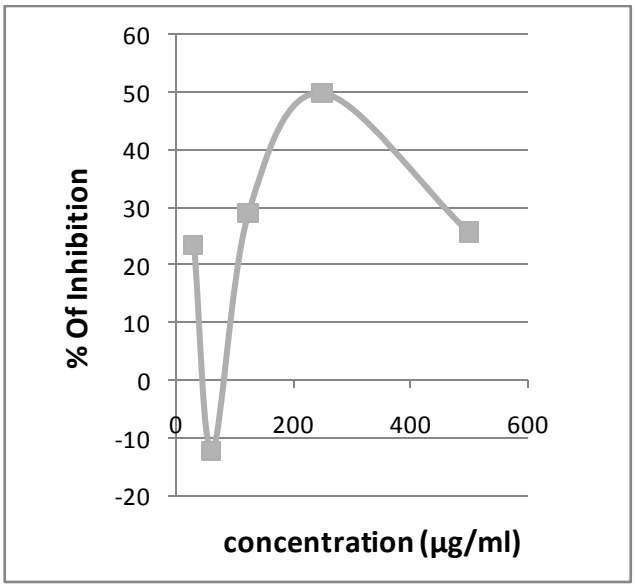



Figure : % Free radical scavenging activity of Ethyl Acetate fraction of Ethyl Acetate extract *Senna alata*

Table:Antioxidant activity of n- hexan fraction of Ethyl Acetate extract of *Senna alata leaves*

| **Sample Name** | **Concentration µg/ml** | **Absorbance 1st Time** | **Absorbance 2nd time** | **% of Inhibition** |
|---|---|---|---|---|
| | 500 | 0.243 | 0.231 | ±260.90 |
| | 250 | 0.166 | 0.185 | ±115.35 |
| Ethyl Acetate- n hexan | 125 | 0 .141 | 0.345 | ±118.71 |
| | 0.62.5 | 0.191 | 0.249 | ±162.15 |

| | | | | |
|---|---|---|---|---|
| | 31.25 | 0.189 | 0.293 | ±16.26 |

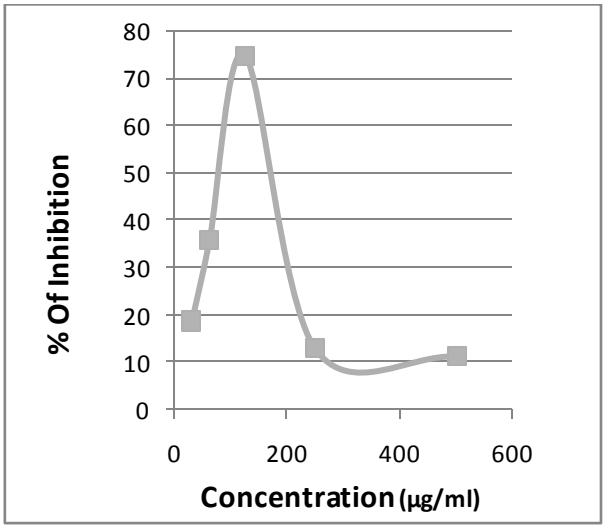



Figure : % Free radical scavenging activity of Ethyl Acetate fraction of n-hexan extract *Senna alata leaves*

Table:Antioxidant activity of oil extract of *Senna alata leaves*

| **Sample Name** | **Concentration µg/ml** | **Absorbance 1st Time** | **Absorbance 2nd time** | **% of Inhibition** |
|---|---|---|---|---|
| | 500 | 0.173 | 0.123 | ±110.56 |
| | 250 | 0.105 | 0.127 | ±23.22 |
| | 125 | 0.074 | 0.108 | ±21.24 |
| Oil | 62.5 | 0.061 | 0.085 | ±43.25 |
| | 31.25 | 0.033 | 0.096 | ±21.33 |

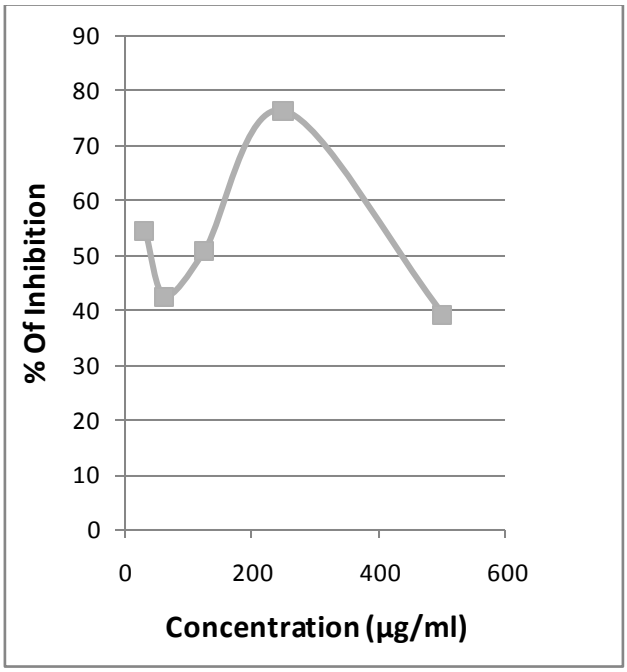



Figure : % Free radical scavenging activity of oil extract *Senna alata leaves*

**5.2 Antimicrobial Test :**

**Table 1: Zone of Inhibition for *Bacillus cereus* at 100 µg concentration of different solvent fraction**

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| 01 | Methanol →Methanol Fraction | 9 | 8.9 | 3.5 | |
| 02 | Methanol → DCM Fraction | 12 | 12 | 3.5 | |

| | | | | | |
|---|---|---|---|---|---|
| **03** | Methanol → Ethyl acetate Fraction | 12 | 12.1 | 3.5 | **11.1 ± 2.635** |
| **04** | DCM → Methanol Fraction | 9 | 8 | 3.5 | |
| **05** | DCM → n-hexane Fraction | 13 | 12.7 | 3.5 | |
| **06** | DCM → DCM Fraction | 12 | 10.8 | 3.5 | |
| **07** | DCM → Ethyl acetate Fraction | 10 | 11 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | 10 | 10.5 | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 9 | 8.7 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 9 | 8.9 | 3.5 | |
| **11** | Oil | 18 | 17.6 | 3.5 | |

**Table 2: Zone of Inhibition for *Bacillus subtilis* at 100 µg concentration of different solvent fraction**

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| **01** | Methanol →Methanol Fraction | 12 | 11 | 3.5 | |
| **02** | Methanol → DCM Fraction | 13 | 12 | 3.5 | |
| **03** | Methanol → Ethyl acetate Fraction | 12 | 12.2 | 3.5 | |
| **04** | DCM → Methanol Fraction | 7 | 6.9 | 3.5 | |

| | | | | | |
|---|---|---|---|---|---|
| **05** | DCM → n-hexane Fraction | 9 | 9 | 3.5 | **10.918 ± 3.943** |
| **06** | DCM → DCM Fraction | 8 | 8.1 | 3.5 | |
| **07** | DCM → Ethyl acetate Fraction | 9 | 9 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | 8 | 8 | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 9 | 9 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 12 | 13 | 3.5 | |
| **11** | Oil | 22 | 21 | 3.5 | |

**Table 3: Zone of Inhibition for *Salmonella paratyphi* at 100 µg concentration of different solvent fraction**

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| **01** | Methanol →Methanol Fraction | 3 | 3.1 | 3.5 | **10.768 ± 3.633** |
| **02** | Methanol → DCM Fraction | 13 | 12 | 3.5 | |
| **03** | Methanol → Ethyl acetate Fraction | 13 | 12.6 | 3.5 | |
| **04** | DCM → Methanol Fraction | 7 | 7.2 | 3.5 | |
| **05** | DCM → n-hexane Fraction | 13 | 12.3 | 3.5 | |

| | | | | | |
|---|---|---|---|---|---|
| **06** | DCM → DCM Fraction | 9 | 9.1 | 3.5 | |
| **07** | DCM → Ethyl acetate Fraction | 13 | 12.8 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | 10 | 9.8 | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 11 | 10 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 10 | 11 | 3.5 | |
| **11** | Oil | 18 | 17 | 3.5 | |

**Table 4: Zone of Inhibition for *Salmonella typhi* at 100 µg concentration of different solvent fraction**

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| **01** | Methanol →Methanol Fraction | 7 | 6.8 | 3.5 | **9.522 ± 2.658** |
| **02** | Methanol → DCM action | 9 | 9 | 3.5 | |
| **03** | Methanol → Ethyl acetate Fraction | 11 | 12 | 3.5 | |
| **04** | DCM → Methanol Fraction | 7.2 | 7 | 3.5 | |
| **05** | DCM → n-hexane Fraction | 11 | 10 | 3.5 | |
| **06** | DCM → DCM Fraction | 9 | 8.9 | 3.5 | |

| | | | | | |
|---|---|---|---|---|---|
| **07** | DCM → Ethyl acetate Fraction | 9 | 9 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | 7.2 | 7 | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 9 | 9.2 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 9.1 | 9.1 | 3.5 | |
| **11** | Oil | 17 | 16 | 3.5 | |

**Table 5: Zone of Inhibition for *Vibrio parahemolyticus* at 100 µg concentration of different solvent fraction**

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| **01** | Methanol →Methanol Fraction | 10 | 11.1 | 3.5 | **12.231 ± 3.223** |
| **02** | Methanol → DCM Fraction | 11 | 10 | 3.5 | |
| **03** | Methanol → Ethyl acetate Fraction | 14 | 13 | 3.5 | |
| **04** | DCM → Methanol Fraction | 9 | 9.2 | 3.5 | |
| **05** | DCM → n-hexane Fraction | 13 | 12 | 3.5 | |

| | | | | | |
|---|---|---|---|---|---|
| **06** | DCM → DCM Fraction | 11 | 11.2 | 3.5 | |
| **07** | DCM → Ethyl acetate Fraction | 12.1 | 12 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | 11 | 11.2 | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 12 | 11 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 11 | 11.3 | 3.5 | |
| **11** | Oil | 22 | 21 | 3.5 | |

**Table 6: Zone of Inhibition for *Vibrio mimicus* at 100 µg concentration of different solvent fraction**

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| **01** | Methanol →Methanol Fraction | 9 | 8.9 | 3.5 | |
| **02** | Methanol → DCM Fraction | 12.2 | 12 | 3.5 | |
| **03** | Methanol → Ethyl acetate Fraction | 14 | 13 | 3.5 | |
| **04** | DCM → Methanol Fraction | 9 | 9.1 | 3.5 | |
| **05** | DCM → n-hexane | 12 | 11 | 3.5 | **12.15 ± 2.799** |

| | | | | | |
|---|---|---|---|---|---|
| | Fraction | | | | |
| **06** | DCM → DCM Fraction | 11 | 12 | 3.5 | |
| **07** | DCM → Ethyl acetate Fraction | 14 | 13 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | 11 | 11 | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 11 | 11 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 12 | 12.1 | 3.5 | |
| **11** | Oil | 20 | 19 | 3.5 | |

**Table 7: Zone of Inhibition for *Escherichia coli* at 100 µg concentration of different solvent fraction**

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| **01** | Methanol →Methanol Fraction | 8 | 8.2 | 3.5 | **9.754 ± 1.382** |
| **02** | Methanol → DCM Fraction | 10 | 11 | 3.5 | |
| **03** | Methanol → Ethyl acetate Fraction | 10.2 | 10 | 3.5 | |
| **04** | DCM → Methanol Fraction | 8 | 8.1 | 3.5 | |
| **05** | DCM → n-hexane Fraction | 10 | 10.8 | 3.5 | |

| | | | | | |
|---|---|---|---|---|---|
| **06** | DCM → DCM Fraction | 8 | 7.8 | 3.5 | |
| **07** | DCM → Ethyl acetate Fraction | 11 | 11.3 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | 8 | 8.2 | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 11 | 11 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 11 | 11.3 | 3.5 | |
| **11** | Oil | 11 | 10.7 | 3.5 | |

**Table 8: Zone of Inhibition for *Shigella dysenteriae* at 100 µg concentration of different solvent fraction**

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| **01** | Methanol →Methanol Fraction | 8 | 8.1 | 3.5 | |
| **02** | Methanol → DCM Fraction | 13.3 | 12.9 | 3.5 | |
| **03** | Methanol → Ethyl acetate Fraction | 13 | 13.1 | 3.5 | |
| **04** | DCM → Methanol Fraction | 11 | 10.7 | 3.5 | |
| **05** | DCM → n-hexane | 12.3 | 12 | 3.5 | **11.895 ± 1.618** |

| | | | | | |
|---|---|---|---|---|---|
| | Fraction | | | | |
| **06** | DCM → DCM Fraction | 11.5 | 11 | 3.5 | |
| **07** | DCM → Ethyl acetate Fraction | 13 | 12 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | | | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 12 | 11.1 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 13 | 12.1 | 3.5 | |
| **11** | Oil | 14 | 13.8 | 3.5 | |

**Table 9: Zone of Inhibition for *Pseudomonas aureus* at 100 µg concentration of different solvent fraction**

.

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| **01** | Methanol →Methanol Fraction | 7.8 | 8 | 3.5 | **8.543 ± 2.633** |
| **02** | Methanol → DCM Fraction | 11 | 10.8 | 3.5 | |
| **03** | Methanol → Ethyl acetate Fraction | 11.1 | 11 | 3.5 | |
| **04** | DCM → Methanol Fraction | 7 | 7.6 | 3.5 | |
| **05** | DCM → n-hexane Fraction | 10 | 10.1 | 3.5 | |

| | | | | | |
|---|---|---|---|---|---|
| **06** | DCM → DCM Fraction | 7 | 7.2 | 3.5 | |
| **07** | DCM → Ethyl acetate Fraction | 9 | 8.7 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | 8 | 8.8 | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 7 | 7.1 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 7 | 7.3 | 3.5 | |
| **11** | Oil | 13 | 12.8 | 3.5 | |

**Table 10: Zone of Inhibition for *Shigella boydii* at 100 µg concentration of different solvent fraction**

| SL. No. | Sample Name | Zone of Inhibition (mm) (1st test) | Zone of Inhibition (mm) (2nd test) | Control Test (mm) | Average ± SD |
|---|---|---|---|---|---|
| **01** | Methanol →Methanol Fraction | 7 | 7.1 | 3.5 | **11.809 ± 3.054** |
| **02** | Methanol → DCM Fraction | 10 | 11 | 3.5 | |
| **03** | Methanol → Ethyl acetate Fraction | 13 | 12.8 | 3.5 | |
| **04** | DCM → Methanol Fraction | 7 | 7.1 | 3.5 | |
| **05** | DCM → n-hexane Fraction | 14 | 13.8 | 3.5 | |
| **06** | DCM → DCM Fraction | 11 | 12.2 | 3.5 | |

| | | | | | |
|---|---|---|---|---|---|
| **07** | DCM → Ethyl acetate Fraction | 14 | 13.9 | 3.5 | |
| **08** | Ethyl acetate → DCM Fraction | 10 | 11.1 | 3.5 | |
| **09** | Ethyl acetate → Ethyl acetate Fraction | 12 | 12.3 | 3.5 | |
| **10** | Ethyl acetate →n-hexane Fraction | 12 | 12.6 | 3.5 | |
| **11** | Oil | 18 | 17.9 | 3.5 | |

**5.3 Antidiabetic Result :**

Table : Anti-diabetic activity Metformin of *Senna alata* leave

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 0.830 | 0.424 | ±53.91 |
| | 250 | 0.753 | 0.387 | ±42.32 |
| Metformin | 125 | 1.048 | 0.399 | ±102.55 |
| | 62.5 | 1.197 | 0.36 | ±12.90 |
| | 31.25 | 1.341 | 0.333 | ±0.30 |

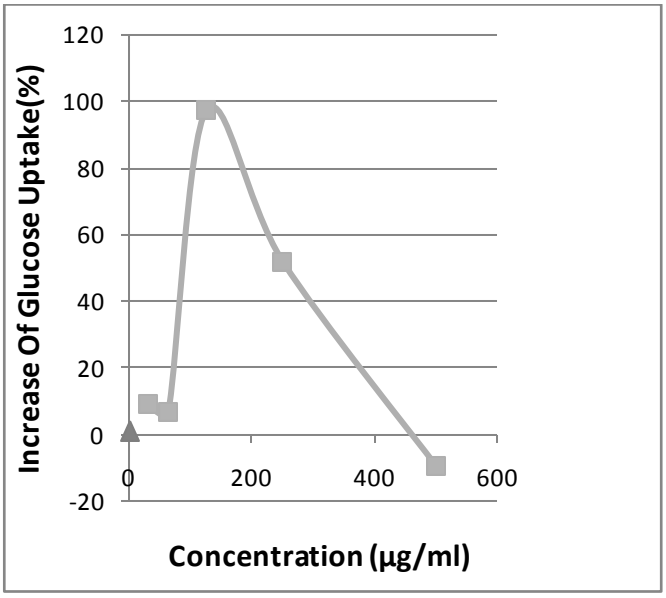



Figure : Anti-diabetic activity of Metformin

Table : Anti-diabetic activity of Methanol Fraction of Methanol Extarct of *Senna alata* leave

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 2.13 | 0.689 | ±9.60 |
| | 250 | 1.605 | 0.472 | ±8.62 |
| Methanol-Methanol | 125 | 1.416 | 0.443 | ±12.85 |
| | 62.5 | 1.343 | 0.362 | ±5.36 |
| | 31.25 | 1.245 | 0.346 | ±7.70 |

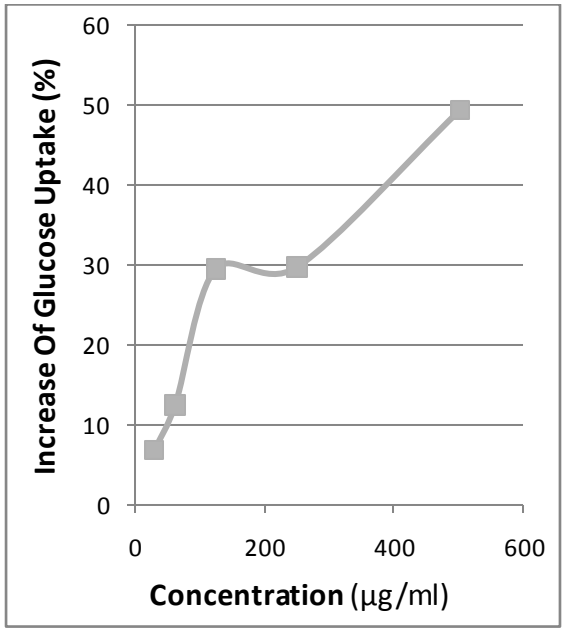



Figure: Anti-diabetic activity of Methanol-Methanol Fraction

Table : Anti-diabetic activity of DCM Fraction of Methanol Extarct of *Senna alata* leave

| Sample name | Concentration µg/ml | Absorbance 1st time | Absorbance 2nd time | % of inhibition |
|---|---|---|---|---|
| | 500 | 1.4 | 0.747 | ±33.24 |
| | 250 | 1.339 | 0.514 | ±23.05 |
| Methanol - DCM fraction | 125 | 1.351 | 0.43 | ±14.34 |
| | 62.5 | 1.349 | 0.384 | ±8.46 |
| | 31.25 | 1.267 | 0.325 | ±2.49 |

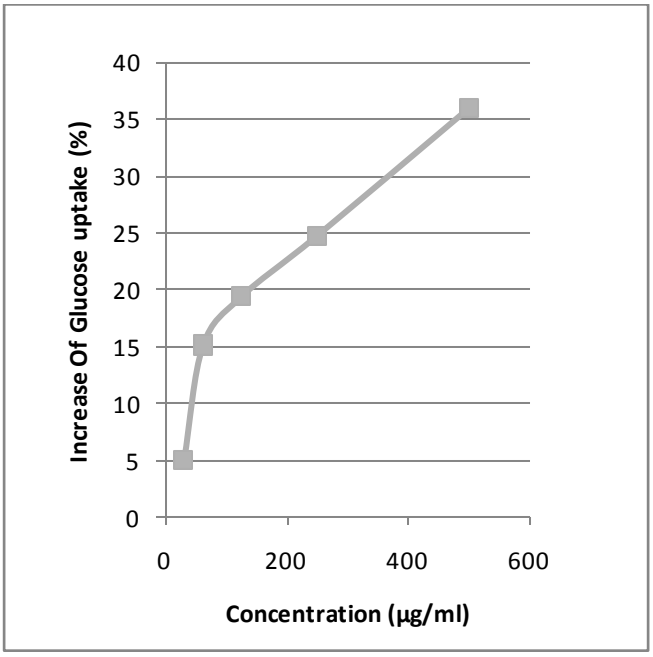



Figure : Anti-diabetic activity of Methanol-DCM FRaction

Table : Anti-diabetic activity of Ethyl acetate Fraction of Methanol Extarct of *Senna alata* leave

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 1.241 | 0.833 | ±44.13 |
| | 250 | 0.399 | 0.615 | ±182.43 |
| Methanol -Ethyl acetate | 125 | 0.246 | 0.462 | ±306.02 |
| | 62.5 | 0.291 | 0.388 | ±242.68 |
| | 31.25 | 0.289 | 0.348 | ±238.40 |

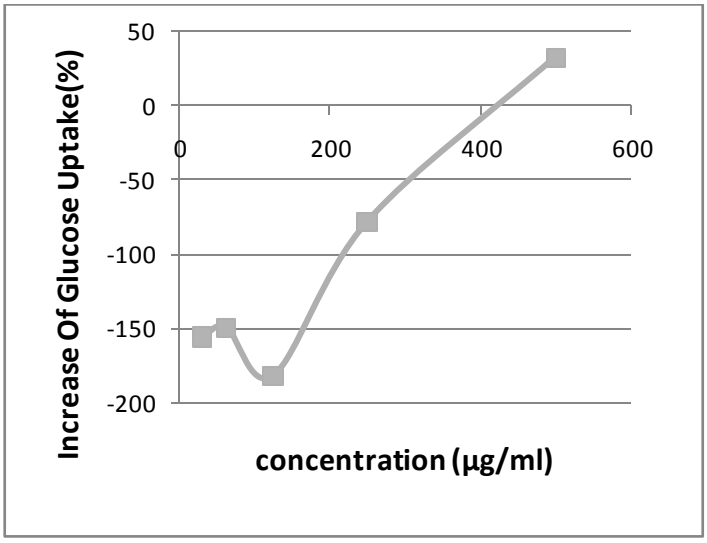



Figure : Anti-diabetic activity of Methanol-Ethyl acetate Fraction

Table : Anti-diabetic activity of Methanol Fraction of DCM Extarct of *Senna alata* leave

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 1.264 | 0.513 | ±26.8 |
| | 250 | 0.633 | 0.429 | ±87.009 |
| DCM-Methanol | 125 | 0.478 | 0.406 | ±128.59 |
| | 62.5 | 0.483 | 0.339 | ±116.28 |
| | 31.25 | 0.457 | 0.288 | ±115.30 |

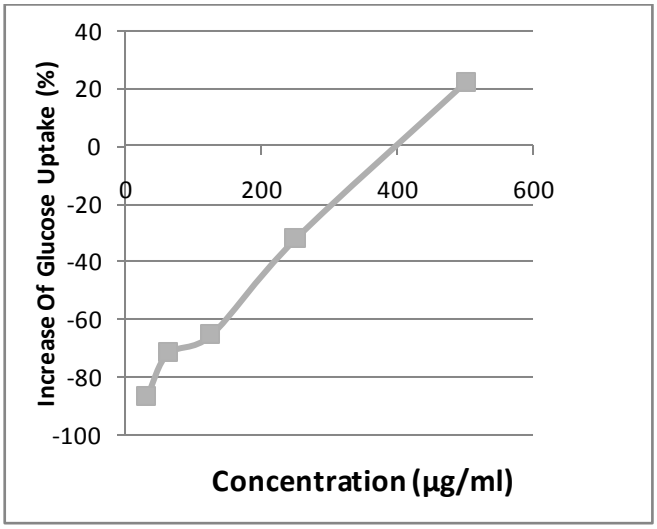



Figure : Anti-diabetic activity of DCM-Methanol Fraction

Table : Anti-diabetic activity of n-hexan Fraction of DCM Extarct of *Senna alata* leaves

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 0.67 | 1.15 | ±110.75 |
| | 250 | 0.589 | 0.445 | ±99.03 |
| DCM-n hexan | 125 | 0.514 | 0.395 | ±114.41 |
| | 62.5 | 0.486 | 0.328 | ±113.05 |
| | 31.25 | 0.469 | 0.335 | ±120.88 |

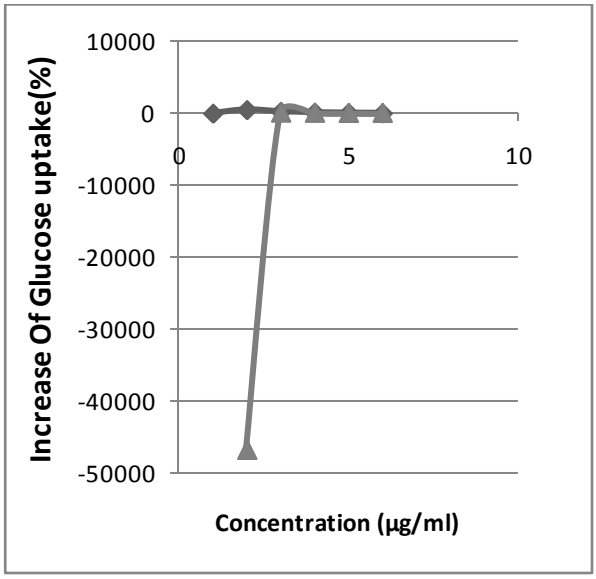



Figure: Anti-diabetic activity of DCM-n hexan Fraction

Table : Anti-diabetic activity of DCM Fraction of DCM Extarct of *Senna alata* leaves

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 1.283 | 1.044 | ±47.04 |
| | 250 | 1.216 | 0.606 | ±35.93 |
| DCM-DCM | 125 | 1.009 | 0.413 | ±34.04 |
| | 62.5 | 1.19 | 0.391 | ±18.053 |
| | 31.25 | 1.123 | 0.378 | ±20.514 |

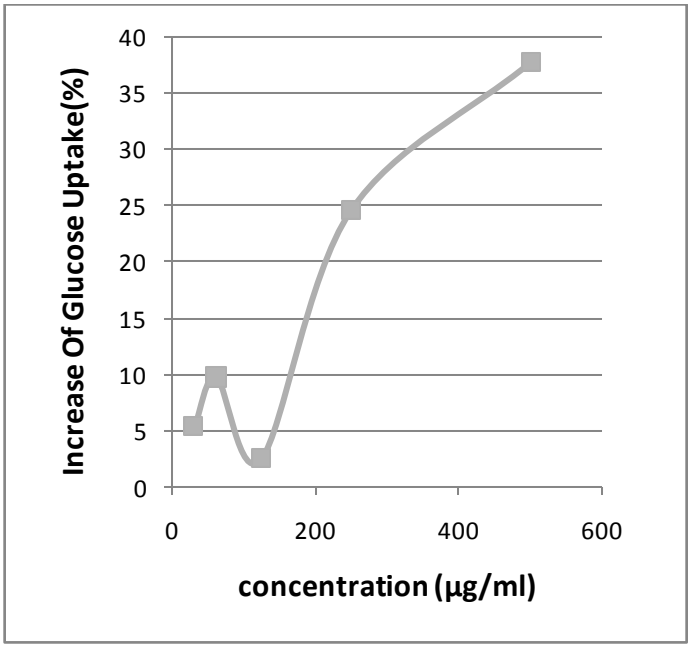



Figure : Anti-diabetic activity of DCM-DCM Fraction

Table : Anti-diabetic activity of oil Extract of *Senna alata* leaves

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 4 | 4 | ±7.67 |
| | 250 | 2.602 | 4 | ±27.96 |
| **Oil** | 125 | 1.041 | 2.473 | ±74.61 |
| | 62.5 | 0.632 | 1.16 | ±118.69 |
| | 31.25 | 0.55 | 0.75 | ±129.05 |

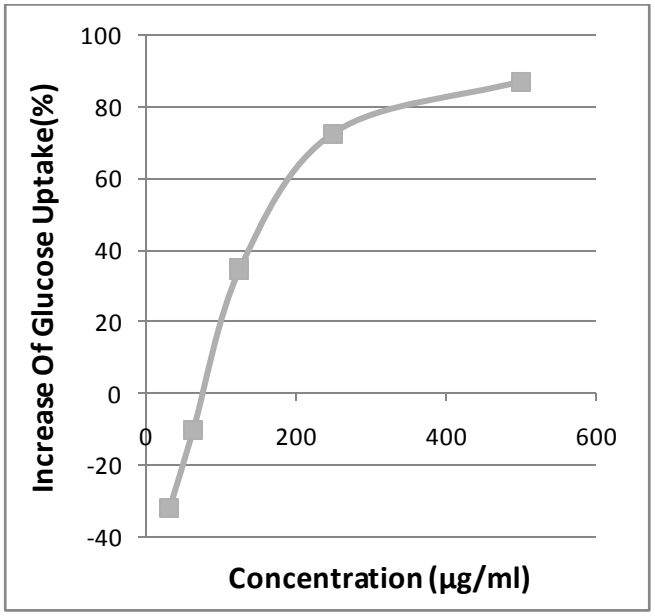



Figure : Anti-diabetic activity of oil Extract

Table : Anti-diabetic activity of Ethyl Acetate Fraction of DCM Extract of *Senna alata* leaves

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 0.588 | 0.343 | ±84.96 |
| | 250 | 0.475 | 0.242 | ±93.97 |
| DCM-Ethyl acetate | 125 | 0.421 | 0.312 | ±137.24 |
| | 62.5 | 0.462 | 0.322 | ±121.10 |
| | 31.25 | 0.418 | 0.266 | ±126.84 |

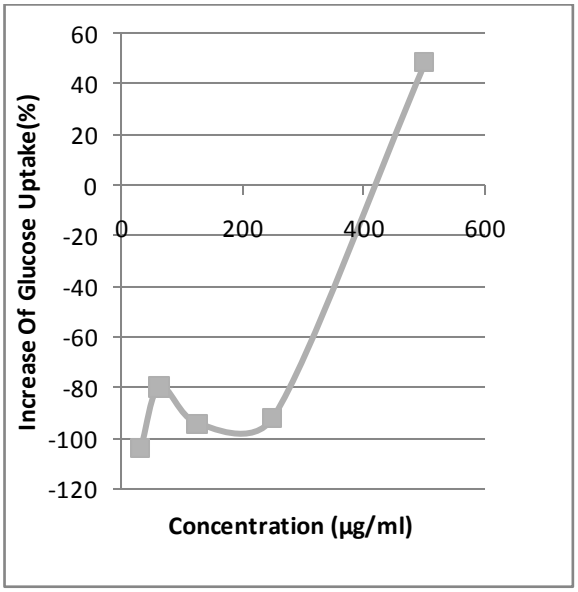



Figure : Anti-diabetic activity of DCM-Ethyl Acetate Fraction

Table : Anti-diabetic activity of Ethyl Acetate Fraction of Ethyl acetate Extarct of *Senna alata* leaves

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 1.671 | 0.392 | ±2.77 |
| | 250 | 1.791 | 0.362 | ±10.78 |
| Ethyl acetate - Ethyl acetate | 125 | 1.744 | 0.3 | ±21.70 |
| | 62.5 | 1.161 | 0.347 | ±12.92 |
| | 31.25 | 1.184 | 0.247 | ±13.52 |

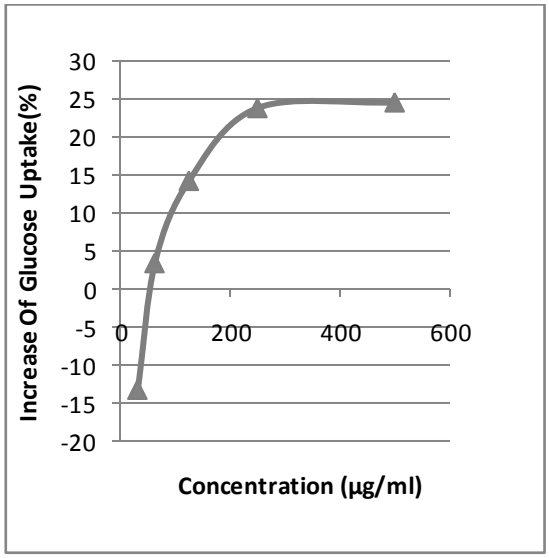



Fig : Anti-diabetic activity of Ethyl Acetate-Ethyl Acetate Fraction

Table : Anti-diabetic activity of DCM Fraction of Ethyl acetate Extract of *Senna alata* leaves

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 1.138 | 0.562 | ±38.05 |
| | 250 | 1.214 | 0.416 | ±19.90 |
| Ethyl acetate - DCM | 125 | 1.155 | 0.254 | ±9.29 |
| | 62.5 | 1.163 | 0.163 | ±56.902 |
| | 31.25 | 1.172 | 0.093 | ±156.41 |

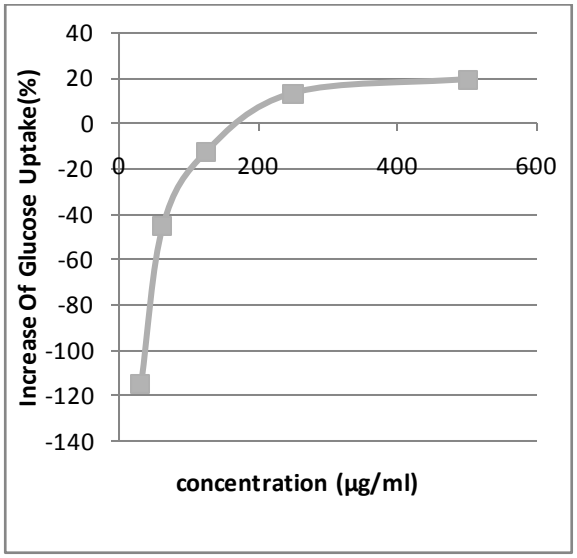



Figure: Anti-diabetic activity of Ethyl Acetate- DCM Fraction

Table : Anti-diabetic activity of n- Haxen Fraction of Ethyl acetate Extract of *Senna alata* leaves

| **Sample name** | **Concentration µg/ml** | **Absorbance 1st time** | **Absorbance 2nd time** | **% of inhibition** |
|---|---|---|---|---|
| | 500 | 1.25 | 1.264 | ±52.40 |
| | 250 | 1.161 | 526 | ±33.93 |
| Ethyl acetate -n Haxen | 125 | 1.21 | 0.391 | ±16.84 |
| | 62.5 | 1.203 | 0.207 | ±31.44 |
| | 31.25 | 1.17 | 0.156 | ±63.24 |

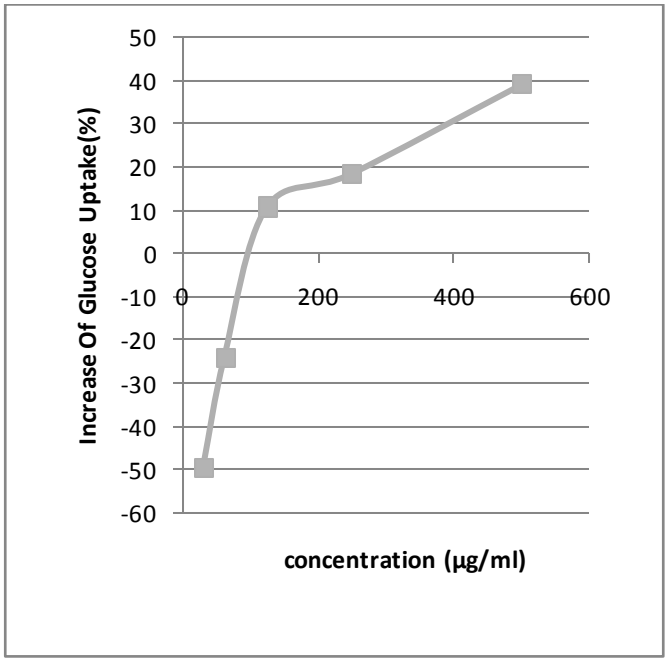



Fig : Anti-diabetic activity of Ethyl Acetate- n hexan Fraction

## DISCUSSIONS

## IN VITRO ANTI-DIABETIC TEST

**5.4 Dicussion** :

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. Although active phytochemicals may have been identified, in general, many pathways for the biosynthesis of specific medicinal compounds and the factors (biotic and abiotic) regulating their production remain unclear. At present, a major concern with the use of phytomedicines regards the maintenance of consistent medicinal quality in botanical medicines.

In the Glucose uptake in Yeast cells method the mechanism of glucose transport across the yeast cell membrane has been receiving attention as in vitro screening method for hypoglycemic effect of various compounds / medicinal plants. Recent studies on the transport of non metabolizable sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereo specific membrane carriers. It is reported that in yeast cells (Saccharomyces cerevisiae) glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast is by a facilitated diffusion process.In our result, It has shown that, ,oil extract and Ethyl acetate fraction of DCM of *Senna alata* give higher antidiabetic activity 83% and 58%. Methanol Fraction of methanol extract,ethyl acetate fraction of methanol extract and n-hexan fraction of ethyl acetate extract give moderate antidiabetic activity 50%,49% and 41%.Whereas,DCM fraction of methanol extract,n-hexan fraction of ethyl acetate DCM-DCM,DCM-n hexan and ethyl acetate fraction of ethyl acetae extract and DCM fraction of ethyl acetate extract of *Senna alata* give lower antidiabetic activity like 36%,23%,8%38%25% and 21% of inhibition.

## ANTIBACTERIAL TEST

### 5.5 Discussion :

Various strains of Gram positive, Gram negative bacteria and fungi were used in this test. The positive control used was ciprofloxacin (30µg/disc). methanol,DCM,ethyl acetate fraction of methanol extract,methanol,n-hexan,DCM,ethyl acetate fraction of DCM extract,ethyl acetate,DCM,n-hexan fraction of ethyl acetate extract and oil extract was used to evaluate the activity against different types of microrganism. The zones of inhibition for the microbes were measured in millimeters using a transparent ruler after 24hrs of incubation.extract was showed zone of inhibition up to 22 mm antibacterial activity at the concentrations used against, *B.Subtilis* than other strain. A study showed that, The leaves of *senna alata* possessing antimicrobial activity can be employed against human pathogens. The use of leaves of senna alata might promote human health by preventing bacterial pathogenesis.

## DPPH TEST

### 5.6 Discussion :

DPPH is a stable free radical that can accept an electron of hydrogen radical to become diamagnetic molecule. The reduction in DPPH radical was determined by the decrease of its absorbance at 517 nm (in methanol) induced by antioxidants. To evaluate the antioxidant activities of different fraction of metthanolic,ethyl acetate,DCM and oil extract of the *leaves of Senna alata* ,DPPH Free Radical Scavenging Assay was used. DPPH reaction has been widely used to test the ability of compounds to act as free-radical scavengers or hydrogen donors and to evaluate the antioxidant activity of foods and plant extracts (Ziying et.al, 2007).In our result,it has shown that DCM fraction of DCM extract,DCM fraction of ethyl acetate extract,ethyl acetate fraction of DCM extract,n-hexan fraction of ethyl acetate extract,Ethyl acetate fraction of Methanol extract of Senna alata leaves give 81%,83%,98%,78% and 79%antioxidant activity.

## Thin layer chromatography

### 5.7 Discussion :

TLC plates were developed with n-hexane,Dichloromeehane,ethyl acetate,Methanol, crude using solvent system-1 (Benzene,Ethanol,Ammonium hydroxide) and 2 (water, ethanol, ethyl acetate),and solvent system-3(Benzene,ethanol). The best result was found using solvent system-(Benzene,Ethanol,9:1) Then the plate was observed UV lamp, at 254 nm which is shown in the plate (1). It showed some spots which indicate the presence of different compounds in that sample. After charring of the TLC plate with sulfuric acid was showed (plate 2). In the crude extract layer three spots were observed. Spraying of DPPH solution on the TLC plate have

shown significant formation of plate yellow color (plate 3). This provides us a preliminary idea of the various types of compounds that may be present in the methanolic extract of the leaves of *Senna alata*.. Further extractions and purifications from these crude drugs may lead to the possible isolation of these compounds from the crude extracts.when used pola solvent then the result or Rf value is 0.87, Non Polar solvent given value of Rf is 0.329 and semi polar given the value of Rf is 0.96 .

Thin Layer Chromatography of Methanolic Extract ( Primary five fraction of VLC extract).TLC was done with primary five fraction of VLC. After TLC, it was found that the five fractions made some sports Under UV (Plate 2). After charring of the TLC plate with sulfuric acid was showed (plate 3) very visible when it was sprayed by 10% sulphuric acid solution.Every fraction(without n-hexane,which showed at least two spot),Dichloromeehane,n-Butanol, ethyl acetate,Methanol showed at least three spot.(plate-4)Some spots was found after TLC plates were dipped in DPPH solution

# Chapter 6: Conclusion

**CONCLUSION :**

In conclusion, medicinal plants play an important role in providing primary health care. The use of medicinal plants from requires adequate control measures to safeguard the future use of these resources. Herbal medicine is paving the way for novel and efficacious treatments, providing an integration of empirical and scientific data. The present study discusses the significance of *Senna alata* leaves as a valuable source for medicinally important compounds besides its leave which is a store house of minerals, oils, vitamins, antioxidants and other nutrients.

Thus, The present study on the different fraction of DCM,methanol,ethyl acetate and oil extract of the *Senna alata* leaves showed the potentiality of its as an antioxidant, in vitro anti-diabetic activities and antibacterial,activity. Besides, the leaves showed anti-inflammatory activity which may be induced due to its antioxidant activity.So, the isolated compounds in those fractions may be used as future therapeutic tools if further therapeutic investigations are carried out.

# Chapter 7: Reference

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