East West University

Phytochemical and Biological Investigation of *Tridax procumben* 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.

Submitted by:

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ID :2011-1-70-023

Dissertation Supervisor:
Tirtha Nandi
Lecturer
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Endorsement by the Chairperson

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Certificate by the Invigilator

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ABSTRACT

The crude methanolic extracts derived from the *Tridax procumben* 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 *Tridax procumben* 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 leaves was very good.

Tridax procumben 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 *Tridax procumben* evealed 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 *Tridax procumben* 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 *Tridax procumben* 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 *Tridax procumben* in the successful treatment of haemorroids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes.

Tridax procumben 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.

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INTRODUCTION

Plants are one of five big groups (kingdoms) of living things. They are autotrophic eukaryotes, which means they have complex cells. Trees, herbs, bushes, grasses, vines, ferns, mosses, and green algae are included in plant. The scientific study of plants, known as botany, has identified about 350,000 extant (living) species of plants. Plants help maintain gaseous balance in the air also prevent soil erosion. They help to reduce heat and prevent drying up of moisture. Thus they are environmental savvy. Plants like blue green algae and bacteria are also extensively used to fix nitrogen in the soil for agriculture (Ranga et al, 2015).

A large group of plants used in medicine or veterinary practice for therapeutic or prophylactic purposes. 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 as insects,fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total(Tap and sell et al, 2006). Although, there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties termed as medicinal plants (Samy et al, 2008).

Plants are the natural reservoir of many antimicrobial, anticancer agents, analgesics, anti-diarrheal as well as various therapeutic activities. Bangladeshi people have traditional medical practice as an integral part of their culture. A lot of medicinal plants are available for the treatment of various diseases. However, scientific studies have been conducted on only a relatively few medicinal plants, and then only to a superficial extent (Faysal, 2008). The use of plants as medicines predates written human history. Ethnobotany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant (Fabricant and Farnsworth, 2001)

There are hundreds of drugs and biologically active compounds developed from the traditional medicinal plants, a few of which are mentioned here; the antispasmodic agent vasicin isolated from Justicia a*dhatoda*, anticancer agents such as vincristine, vinblastine and D-tubocurarine isolated from *Catharanthus roseus* (Gurib-Fakim, 2006), antibacterial agents isolated from *Diospyros melanoxylon* (Mallavadhani et al, 1998), antimalarial agent isolated from *Sida acuta* (Karou et al., 2006), steroid and lancamarone with cardiotonic properties, lantamine with antipyretic and antispasmodic properties from *Lantana camara* (Ghisalberti, 2000), antimicrobial agents isolated from *Acorus calamus* (Chowdhury et al, 1993), antiviral, antibacterial and anti-inflammatory agents isolated from *Urtica dioica* (Harborne and Buxter, 1993), anticancer agents isolated from *Aloe vera*, *Allium sativum, Andrographis paniculata*, *Curcuma longa*, *Moringa oleifera*, *Phyllanthus amarus*, *Piper longum*, *Semecarpus anacardium*, *Tinospora cordifolia* and *Withanica somnifera* (Balachandran and Govindarajan 2005), promising and potent antimalarial drug artemisinin isolated from *Artemesia annua* (Dhingra et al, 2000).

1.1 PHYTOCHEMICALS

Phytochemistry is the study of phytochemicals produced in plants, describing the isolation, purification, identification, and structure of the large number of secondary metabolic compounds found in plants. Effect of extracted plant phytochemicals depends on:

- The nature of the plant material
- Its origin
- Degree of processing
- Moisture content
- Particle size (Tiwari and Kumer, 2011).

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 predationand others are pheromones used to attract insects for pollination (Meskin and Mark, 2002).Carbon dioxide gas deals with the photosynthesis process in plants in the presence of light energy. Photosynthesis and pentose pathway together pools the phosphate group present in the sugar molecules of plants which leads to glycolysis process and which is accounted for producing many of phytochemicals of plants, such as, shikimic acid, proteins, aliphatic and aromatic acids, mevalonic acids, fatty acids, flavanoids, terpinoids, steroids etc. There are lots of medicinal plants which contain a number of phytochemicals and those phytochemicals are used medicine purpose to treat various kinds of diseases. In the following table a list is shown of phytochemicals having medicinal values (Tiwari and Kumer, 2011).

1.2 NECESSITY OF STUDYING OF MEDICINAL PLANTS

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world.

- Medicinal plants have played an essential role in the development of human culture, for example religions and different ceremonies. (E.g. Dutura has long been associated with the worship of Shiva, the Indian god).
- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- Many food crops have medicinal effects, for example garlic.

Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species (Andrew, 2004)

- Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
- Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants.
- Plant resources (E.g. Angiosperm, Gymnosperm, Seedless vascular plants, Bryophytes) for new medicine.
- The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry (Andrew, 2004)
- With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today□s synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20% of the plant flora has been studied and 60%of synthetic medicines owe their origin to plants. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases.
- To identify alternative and complementary medicine.
- To reduce the toxicity of drug therapy especially toxicity reduction of synthetic and semi synthetic drugs.
- To find the lead compound diversification to treat various diseases (Andrew, 2004)

1.2.1 HISTORY OF TRADITIONAL HERBAL MEDICINE IN BANGLADESH

"Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating. Plant, animal and mineral based medicines, spiritual, therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being.By definition, 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines' .In many developing countries, a large (Allison et al, 2001).

The earliest mention of traditional medicine is found in *Rigveda,* the oldest repository of knowledge in this subcontinent. Later *Ayurveda,* developed from the *Vedic* concept of

life,became the important source of all systems of medical sciences. In course of time it became a part of culture and heritage of the people of the Indian subcontinent .

Traditional medicine involves the use of both material and non-material components. The material components invariably comprise parts or organs of plants and their products. They also consist of animal organs, minerals and other natural substances. The non-material components, which constitute important items of religious and spiritual medicines, include torture, charms, magic, incantations, religious verses, amulets and rituals like sacrifices, appeasement of evil spirits, etc.

Treatments in traditional medicine are carried out by internal and external application of medicaments, physical manipulation of various parts of the body, performing rituals, psychological treatment, and also by minor surgery. *Ayurvedic* medicinal preparations consist mainly of plant materials in the form of powders, semi-solid preparations, decoctions, elixirs and distillates. Many of them also contain inorganic chemical substances, minerals and animal products. Alcoholic extracts and alcoholic solutions of the ingredients, tinctures and elixirs are also frequently used in *Ayurvedi*c medicine **.**Whole plants or their powders or pastes or products and their extracts, infusions, decoctions and distillates constitute the major constituents of *Unani* medicine. Minerals, inorganic chemicals and animal products are also frequently used in preparing these medicines(Samy,Pushparaj & Gopalakrishnakone, 2008).

The desire to capture the wisdom of traditional healing systems has led to a resurgence of interest in herbal medicines (Tyler, 2000), particularly in Europe and North America, where herbal products have been incorporated into so-called ‘alternative’, ‘complementary’, ‘holistic’ or ‘integrative’ medical system.

The practice of Traditional medicine is deeply rooted in the cultural heritage of Bangladesh and constitutes an integral part of the culture of the people of this country. Different forms of Traditional medicines have been used in this country as an essential means of treatment of diseases and management of various health problems from time immemorial. The practice of traditional medicine in this country has flourished tremendously in the recent years along with that of modern medicine. As a result, even at this age of highly advanced allopathic medicine, a large majority (75-80%) of the population of this country, particularly in the rural and semi-urban areas, still prefer to use traditional medicine in the treatment of most of their diseases even though modern medical facilities may be available in the neighbourhood. However, the concept,

practice, type and method of application of traditional medicine vary widely among the different ethnic groups living in different parts of the country according to their culture, living standard, economic status, religious belief and level of education. Thus traditional medicine practice in Bangladesh includes both the most primitive forms of folk medicine (based on cultural habits, superstitions, religious customs and spiritualism) as well as the highly modernised Unani and Ayurvedic systems (based on scientific knowledge and modern pharmaceutical methods and technology). These various aspects of Traditional medicine practice in Bangladesh, their current official status (acceptability, recognition, etc.) in the country as a means of treatment, and their contribution to, and impact on, the overall health management programmes of the country are described and discussed in this paper supported by documentary evidences and scientific data (Ghani and Abdul, 1998).

Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. As only a certain percentage of plants are used in traditional medicines, it is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field.

Table1.2.1:Some Crude drugs used as medicine in bangladesh (Samy,Pushparaj,& Gopalakrishnakone, 2008:P.24)

Common name	Botanical name	Uses
Amla	*Emblica officinalis*	Vitamin - C, Cough, Diabetes, cold, Laxative, hyper acidity.
Ashok	*Saraca asoca*	Menstrual Pain, uterine, disorder, Deiabetes.
Bael / Bilva	*Aegle marmelous*	Diarrrhoea, Dysentry, Constipation.

Chiraita	*Swertia chiraita*	Skin Desease, Burning, censation, fever.
Kalmegh/ Bhui neem	*Andrographis paniculata*	Fever, weekness, release of gas.
Long peeper / Pippali	*Peeper longum*	Appetizer, enlarged spleen,
Sandal Wood	*Santalum album*	Skin disorder, Burning, sensation, Jaundice, Cough.
Satavari	*Asparagus racemosus*	Enhance lactation, general weekness, fatigue, cough.
Senna	*Casia augustifolia*	General debility tonic, aphrodisiac.
Tulsi	*Ocimum sanclum*	Cough, Cold, bronchitis,expectorand
Pippermint	*Mentha pipertia*	Digestive, Pain killer
Henna/Mehd	*Lawsennia iermis*	Burning, Steam, Anti Imflamatary
Gritkumari	*Aloe verra*	Laxative, Wound healing, Skin burns & care,Ulcer
Sada Bahar	*Vincea rosea*	Leaukamia, Hypotensiv, Antispasmodic , Atidot

Vringraj	*Eclipta alba*	Anti-inflamatory, Digestive, hairtonic
Neem	*Azardirchata indica*	Sdedative, analgesic, epilepsy, hypertensive
Anantamool/sariva	*Hemibi smus indicus*	Appetiser, Carminative, aphrodisiac, Astringent
Kantakari	*Solanum xanthocarpum*	Diuretic, Antiinflamatory, Appetiser, Stomachic
Shankhamul	*Geodorum denciflorum*	Antidiabetic

1.3 Plant metabolite

Metabolites are compounds synthesized by plants for both essential functions, such as growth and development (primary metabolites), and specific functions, such as pollinator attraction or defense against herbivory (secondary metabolites). Metabolites are organic compounds synthesized by organisms using enzyme-mediated chemical reactions called metabolic pathways. Primary metabolites have functions that are essential to growth and development and are therefore present in all plants.In contrast, secondary metabolites are variously distributed in the plant kingdom, and their functions are specific to the plants in which they are found. Secondary metabolites are often colored, fragrant, or flavorful compounds and they typically mediate the interaction of plants with other organisms. Such interactions include those of plant-pollinator, plant-pathogen, and plant-herbivore).

1.3.1 Primary metabolite

A plant produces primary metabolites that are involved in growth and metabolism. Primary metabolites comprise many different types of organic compounds, including, but not limited to, carbohydrates, lipids, proteins, and nucleic acids. They are found universally in the plant kingdom because they are the components or products of fundamental metabolic pathways or cycles such as glycolysis, the Krebs cycle, and the Calvin cycle. Because of the importance of these and other primary pathways in enabling a plant to synthesize, assimilate, and degrade organic compounds, primary metabolites are essential. Examples of primary metabolites include energy rich fuel molecules, such as sucrose and starch, structural components such as cellulose, informational molecules such as DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), and pigments, such as chlorophyll. In addition to having fundamental roles in plant growth and development, some primary metabolites are precursors (starting materials) for the synthesis of secondary metabolites.

1.3.2 Secondary metabolite

Secondary metabolites are those metabolites which are often produced in a phase of subsequent to growth, have no function in growth (although they may have survival function), are produced by certain restricted taxonomic groups of microorganisms, have unusual chemicals structures, and are often formed as mixtures of closely related members of a chemical family. The simplest definition of secondary products is that they are not generally included in standard metabolic charts. A metabolic intermediate or product, found as a differentiation product in restricted taxonomic groups, not essential to growth and the life of the producing organism, and biosynthesis from one or more general metabolites by a wider variety of pathways than is available in general metabolism. Secondary metabolites are not essential for growth and tend to be strain specific. They have a wide range of chemical structures and biological activities. They are derived by unique biosynthetic pathways from primary metabolites and intermediates (David and Wang, 2014)

Of the estimated 400,000 – 500,000 plant species around the globe, only a small percentage has been investigated phytochemically and the fraction subjected to biological or pharmacological screening is even lower. The ability to synthesize secondary metabolites has been selected

through the course of evolution in different plant lineage when such compounds address specific needs

- Floral scent volatiles and pigments have evolved to attract insect pollinators and thus enhance fertilization.
- To synthesize toxic chemical has evolved to ward off pathogens and herbivores or to suppress the growth of neighboring plants.
- Chemicals found in fruits prevent spoilage and act as signals (in the form of color, aroma, and flavor) of the presence of potential rewards (sugars, vitamins and flavor) for animals that eat the fruit and thereby help to disperse the seeds.
- Other chemicals serve cellular functions that are unique to the particular plant in which they occur (e.g. resistance to salt or drought)((David and Wang, 2014)

1.4 Overview of family

The Asteraceae or Compositae (commonly referred to as the aster, daisy, composite, or sunflower family) are an exceedingly large and widespread family of flowering plants (Angiospermae**).**

The family has more than 23,600 currently accepted species, spread across 1,620 genera (list) and 13 subfamilies.[citation needed] In terms of numbers of species, the Asteraceae are rivaled only by the Orchidaceae. (Which of the two families is actually larger is unclear, owing to uncertainty about exactly how many species exist in each family.) Many members have composite flowers in the form of flower heads (capitula or pseudanthia) surrounded by involucral bracts. When viewed from a distance, each capitulum may have the appearance of being a single flower. The name "Asteraceae" comes from Aster, the most prominent genus in the family, that derives from the Greek □στήρ, meaning star, and is connected with its inflorescence star form. "Compositae" is an older but still valid name which refers to the fact that the family is one of the few angiosperm ones to have composite flowers**.**

Most members of Asteraceae are herbaceous, but a significant number are also shrubs, vines, or trees. The family has a worldwide distribution, from the polar regions to the tropics, colonizing a wide variety of habitats. It is most common in the arid and semiarid regions of subtropical and lower temperate latitudes. The Asteraceae may represent as much as 10% of autochthonous flora in many regions of the world.

The Asteraceae are an economically important family, providing products such as cooking oils, lettuce, sunflower seeds, artichokes, sweetening agents, coffee substitutes and herbal teas. Several genera are of horticultural importance, including pot marigold, Calendula officinalis, Echinacea (cone flowers), various daisies, fleabane, chrysanthemums, dahlias, zinnias, and heleniums. Asteraceae are important in herbal medicine, including Grindelia, yarrow, and many others. A number of species are considered invasive, including, most notably in North America, dandelion, which was originally introduced by European settlers who used the young leaves as a salad green.

1.5.1 Some common names

Its common names include coat buttons and tridax daisy in English, jayanthi in Kannada, cadillo chisaca in Spanish, herbe caille in French, jayanti veda in Sanskrit, ghamra in Hindi, bishalya karani in Oriya, kambarmodi in Marathi, gaddi chemanthi in Telugu,vettukaaya poondu in Tamil, and kotobukigiku in Japanese,

1.5.2BOARD OF TAXONOMICAL CLASSIFICATION

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Asteridae

Order: Asterales

Family: Asteraceae

Genus: Tridax L.

Species: Tridax procumbens L.

1.6 ORIGIN AND DISTRIBUTION

Tridax procumbens 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, Tridax procumbens 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)

1.7 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. Tridax procumbens 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)

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)

CONSIDRATION

All parts of the Tridax procumbens 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 Tridax procumbens to garden and keep any eye on where it goes to prevent its invasion into natural habitats (Bonnie Singleton, 2015).

1.8 PLANT PARTS

1.8.1 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).

Leaves

Figure 1.1:Leaves of *Tridax Procumben* (Navie, 2004).

1.8.2 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)

1.8.2 Fruits

Fruit an achene, narrowly obconic to cylindrical, tapering to a blunt base, 1.5-2.5 mm long, 0.5-1.4 mm in diameter (not including pappus). Blackish-brown, pilose, with pale ascending hairs, giving achene grayish-brown appearance. Pappus persistent, one row of ca. 20 straw-colored scalelike bristles, copiously long-plumose. Ray achene pappus 0.5-2.5 mm long, disc achene pappus alternately long and short, 3.5-6 mm long. Scar basal, a raised +/- elliptic pad, semi-transparent, striate. Apex horizontal, round, blackish, rough, with central style base; style base reddish-brown, cylindrical and hollow, or inconspicuous. Embryo linear; endosperm absent.

1.9 CHEMICAL CONSTITUENTS

A new flavonoid (procumbenetin), isolated from the aerial parts of Tridax procumbens, has been characterised as 3,6-dimethoxy-5,7,2',3',4'-pentahydroxyflavone 7-O-β-D-gluco- pyranoside (1) on the basis of spectroscopic techniques and by chemical means. Tridax procumbens; Flavonoids Plant. Uses in traditional medicine. Commonly used in Indian traditional medicine as anticoagulant, hair tonic, antifungal and insect repellent, in bronchial catarrh, diarrhoea, dysentery, and wound healing. Previously isolated constituents. Alkyl esters, sterols,[2] pentacyclic triterpenes,[2][3] fatty acids[4] and polysaccharides.[5] New isolated constituent. 3,6-Dimethoxy-5,7,2',3',4'-pentahydroxyflavone 7-O-β- D-glucopyranoside (1), named procumbetin Žyield: 0.016% on dried basis.

1.10 Uses

1.10.1 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 *Tridax procumbens* 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 *Tridax procumbens* 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 *Tridax procumbens* 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

Other eilments treated in tropical Africa with *Tridax Procumbens* 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. *Tridax procumben* can become a weed in pastures; it is not eaten by livestock and is reported to be poisonous, especially for goats. The bark is used as fish poison and for tanning leather. The roots and the bark are reported to be used for tattooing. *Tridax procumben* is widely appreciated as a garden ornamental and bee forage (Protabase , 2015)

1.11 NUTRITIONAL FACTS

Table 1.11.1: Mineral composition of *Tridax procumben* leaf (Abdulwaliyu et al, 2013:p.4)

Elements	**Leaf (mg/100g)**
k	23.46
Zn	0.17
Cu	0.47
Na	1106.56
Mg	49.02
Fe	3.6
Ca	333.32

Table 1.11.2: Vitamin composition of *Tridax procumben* leaf (Abdulwaliyu et al, 2013:p.4)

Vitamins	**Leaf**
β-Carotene (IU)	50.37
Vitamin C (mg/L)	9.09
Vitamin E (IU)	31.50

Table 1.11.3: Antinutrient composition of *Tridax procumben* 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 1.11.4: Proximate analysis of *Tridax procumben* leaf (Abdulwaliyu et al, 2013:p.3)

Parameters	**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)

Table 1.11.5: Essential oil constituents of *Tridax procumben* (Isiaka A et al, 2010:p.214)

Compounds a	LRI b	%	Compounds a	LRI b	%
(E)-2-hexenal	854	3.3	germacrene D	1483	5.5
tricyclene	926	t	(E)-β-ionone	1487	t
benzaldehyde	961	t	bicyclogermacrene	1497	t
α-phellandrene	1008	3.7	α-selinene	1498	5.4
α-terpinene	1021	t	n-pentadecane	1500	t

p-cymene	1029	t	α-bulnesene	1506	1.0
limonene	1034	5.2	δ-cadinene	1525	t
1,8-cineole	1037	39.8	caryophyllene oxide	1583	12.7
β-elemene	1394	t	n-hexadecane	1600	t
β-caryophyllene	1421	19.1	humulene epoxide II	1609	t
(E)-geranyl	1457	t	tetradecanal	1614	t
acetone			α-cadinol	1656	4.2
α-humulene	1458	t			
E)-β-farnesene	1461	t			Total= 99.9 %

2.1 PHYTOCHEMICAL REVIEW

2.1.1 NUTRITIVE COMPOSITION

The leaves of *Tridax procumben* is a good source of many mineral content.In a survay,The analyses performed by Energy Dispersive X-Ray Fluorescence (EDXRF) revealed the following mineral elements: k, Zn, Cd, Na ,Mg ,Fe ,Ca. And the vitamin elements are β-Carotene (IU), Vitamin C (mg/L), Vitamin E (IU). The results obtained from mineral analysis on the leaf of *Tridax procumben* revealed low content of sodium and high contents of calcium, potassium, iron and magnesium. The result showed that the magnesium, potassium and iron contents of the

leaf and flower of *Tridax procumben* were high compared to magnesium (19.16 mg/kg), iron (3.80 mg/kg) and potassium (0.6 mg/kg) contents of shear butter leaf (Abidemi et al, 2009)

Tridax procumben leaves have also been found to contain 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 (Fuzellier et al,1982; Abo et al,1999). Phytochemical screening of the leaves and roots of *Tridax procumben* revealed the presence of alkaloids, carbohydrates, tannins, saponins, phenols, flavonoids, anthraquinones and cardiac glycosidess (Elmahmood and Amey, 2007). 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.The quantitatively significant constituents of the leaf oil of *Tridax procumben* Roxb.,(Fabaceae) were 1, 8-cineole (39.8%),-caryophyllene (19.1%) and caryophyllene oxide (12.7%). Limonene (5.2%),germacrene D (5.5%) and _-selinene (5.4%) constituted the other significant compounds present in the oil.(Isiaka et al, 2010).

The plant is a source of chrysoeriol,quercetin, 5,7,4'-trihydroflavanone, kaempferol-3-O-D-glucopyranoside,kaempferol-3-O-D-glucopyranosyl-(1->6)-_-D-glucopyranoside,17-hydrotetratriacontane, n-dotriacontanol, n-triacontanol, palmitic acid ceryl ester,stearic acid, palmitic acid. There is only a report on the constituents of its volatile oil.(Isiaka et al, 2010).

2.2 PHARMACOLOGICAL REVIEW

Tridax procumben leaves. has been ethnobotanically used extensively in traditional medicines for the treatment of a variety of diseases such as skin problems, arthritis, HBP (high blood pressure), and laxative or purgative.It is also used in boils, wound, eye, urinary and gastrointestinal tract infections, diarrhoea and scarlet fever (Benjamin and Lamikanra, 1981).Recent reports have credited the use of *Tridax procumben* in the successful treatment of haemorroids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes (Makinde et al, 2007).

2.2.1ANTIMICROBIAL ACTIVITY

The antimicrobial activities of ethanolic leaf extract of *Tridax procumben* against five bacteria (Staphylococcus aureus, Staphylococcus albus, Klebsiella pneumonia, Pseudomonas aeruginosa and Proteus mirabilis) and six fungi (Rhizopus spp, Penicillum oxalicum, Aspergillus tamari, Aspergillus niger, Fusarium oxysporum and Fusarium vacitilus) were examined using agar diffusion method. The result revealed that the ethanolic leaf extract had high inhibitory activity against S. albus, P. mirabilis and all the fungi tested. The eight antibacterial drugs produced varied reactions on the microbes with streptomycin having the highest inhibitory activity against all the bacteria(Odunbaku and Ilusanya, 2011).

Crude methanol extracts from leaves of *Tridax procumben* , fistula and tora were investigated for their antifungal activities on three pathogenic fungi (Microsporum gypseum, Trichophyton rubrum and Penicillium marneffei). Among 3 species, was the most effective leaf extract against T. rubrum and M. gypseum with the 50% inhibition concentration (IC50) of hyphal growth at 0.5 and 0.8 mg/ml, respectively, whereas the extract of C. fistula was the most potent inhibitor of P. marneffei with the IC50 of 0.9 mg/ml. In addition, it was found that all three Cassia leaf extracts also affected M. gypseum conidial germination. Microscopic observation revealed that the treated hyphae and macroconidia with leaf extracts were shrunken and collapsed, which might be due to cell fluid leakage.(Souwalak Phongpaichit et al, 2004)

2.2.2 ANTIALLERGIC ACTIVITY

Leaves of *Tridax procumben* are ethnomedically claimed as anti-asthmatic. In the current study it is aimed to investigate the anti-allergic activities of hydro-methanolic extract of *Tridax procumben* and its constituents rhein and kaempferol on triple antigen/sheep serum-induced mast-cell degranulation in rats. Antiallergic activity of hydroalcoholic extract of *Tridax procumben* with its two components rhein and kaempferol was evaluated using *in vivo* mast cell stabilization assay. The hydroalcoholic extract of *Tridax procumben* significantly inhibited mast

cell degranulation at 200 mg/kg dose. Both chemical constituents rhein and kaempferol also showed potent (>76%) inhibition of mast-cell degranulation at 5 mg/kg. Extract and rhein inhibited LOX enzyme with IC_{50} values of 90.2 and 3.9 µg/mL, respectively, whereas kaempferol was inactive.(Baljinder Singh et al, 2012,The hydroalcoholic extract of *Tridax procumben* leaves and its major compound rhein exhibits antiallergic activity *via* mast cell stabilization and lipoxygenase inhibition).

2.2.3 ANTIOXIDENT ACTIVITY

Aqueous extract of *Tridax procumben* showed strong antioxidant activity and high total phenolic content. The antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'– azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods. The strongest antioxidant activities of aqueous extract of *Tridax procumben* were 22.11 ± 0.324 mg gallic/g extract and 214.99 ± 17.279 mg trolox/g extract when determined by DPPH and ABTS assay, respectively. Moreover, the highest total phenolic content of 70.90 ± 1.048 mg gallic/g extract was measured from the aqueous extract of *Tridax procumben* . Therefore, the biological activities of these plants observed in this study will be useful to develop the plant extracts for primary treatment of diseases as new therapeutic agents.(Wipawan et al, 2012, Total phenolic contents, antibacterial and antioxidant activities of some Thai medicinal plant extracts).

Methanolic extract of the leaves of *Tridax procumben* was assayed for determining the antioxidant compounds present in this plant. Estimation of total phenols, Vitamin-C, Vitamin-A, flavonoids, carotenoidsand anthra quinones was done. DPPH radical scavenging activity of the methanolic extract of leaves was also tested against a synthetic antioxidant, Butylated hydroxytoluene (BHT). It is evident from the results that the plant *Tridax procumben* possesses strong antioxidant activity, as it contains good quantity of antioxidant compounds like phenols, Vitamin-C,Vitamin- A, flavonoids, carotenoids and anthraquinone. In addition, it has very high DPPH radical scavenging activity in contrast to the synthetic antioxidant compound, BHT. (Saheli Chatterjee et al .2013. Study of Antioxidant Activity and Immune Stimulating Potency of the Ethnomedicinal Plant, *Tridax procumbens*

2.2.4 ANTIDIABETIC ACTIVITY

The methanol extract of leaves of *Tridax procumben*, which showed potent α-glucosidase inhibitory activity (IC_{50}, 63.75±12.81 µg/ml), was fractionated. The α-glucosidase inhibitory effect of the crude extract was far better than the standard clinically used drug, acarbose (IC_{50}, 107.31±12.31 µg/ml). A subsequent fractionation of the crude extract was made using solvents of ascending polarity (petroleum ether, chloroform, ethyl acetate, butanol and water). The ethyl acetate (IC_{50}, 2.95±0.47 µg/ml) and butanol (IC_{50}, 25.80±2.01 µg/ml) fractions which contained predominantly kaempferol (56.7±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 o *Tridax procumben* f 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 *Tridax procumben* leaves: Identification through α-glucosidase inhibition studies).

2.2.5 ANTICANCER ACTIVITY

The present study reports the effects of *Tridax procumben* extract on the metabolism of polyamines resulting from the proliferation of leukaemia cells (L1210). The results established that the inhibition of cell proliferation was significantly increased with the concentration of extract from 28 to 32.80 % after 72 h. The percentage of cells viability changed significantly from 9.72 to 80 % when cells are treated with extract alone, in combination with DFMO or putrescine. The levels of the intracellular yield of putrescine, spermidine and spermine were also reduced by the extract compared to the control. The DFMO-extract complex enhanced the inhibition of the production polyamines up to 95 %. In opposite, the *Tridax procumben*-putrescine complex stimulated significantly its biosynthesis of polyamines. A significant reduction of the level of protein after 72 h of treatment was observed. This result corroborated with the reduction of polyamines resulting from inhibition cellproliferation.(Pieme et al, 2009, In vitro effects of extract of *Tridax procumben* on the polyamines produced by Leukaemia cells).

2.2.6 ABORTIFACIENT PROPERTIES

This study has provided evidence to the age-long claim of *Tridax procumben* leaves in "washing the uterus". The abortifacient properties were most pronounced at 500 and 1000 mg/kg body weight of the extract and were similar to the animals treated with 2.85 mg/kg body weight of mifepristone. Hormonal influence, changes in implantation site, estrogenicity and uterogenicity are suggested as possible mechanism of abortifacient activity of aqueous extract of *Tridax procumben* leaves. Overall, the extract may be used as an abortifacient especially at 500 and 1000 mg/kg body weight and therefore not safe for consumption as oral remedy during pregnancy) (Yakubu et al, 2010, Abortifacient Potential of Aqueous Extract of *Tridax procumben* Leaves in Rats**).**

2.2.7 BIOFILM FORMATION

Five bioactive fractions were detected and chemically characterized, using high-resolution mass spectrometry (qTOF-MS/MS). Six compounds from four fractions could be characterized as kaempferol, kaempferol-O-diglucoside, kaempferol-O-glucoside, quercetin-O-glucoside, rhein, and danthron. In the Salmonella/microsome assay CaRP showed weak mutagenicity (M I < 3) only in strain TA98, pointing to a frameshift mutation activity. These results indicate that *Tridax procumben* leaf extract contains a minimum of 7 compounds with antimicrobial activity and that these together or as single substance are active in preventing formation of bacterial biofilm, indicating potential for therapeutic applications.(Samuel et al, 2012, Bioguided Fractionation Shows *Tridax procumbens* Extract to Inhibit Staphylococcus epidermidis and Pseudomonas aeruginosa Growth and Biofilm Formation).

2.2.8 LARVICIDAL EFFECT

The larvicidal activities of aqueous and ethanolic leaf and stem extracts of *Tridax procumben* were evaluated in static bioassays, on fourth instar larvae of Anopheles gambiae, Culex quinquefasciatus and Aedes aegypti, at extract concentrations of 0.15, 0.30, 0.45, 0.60 and 0.75% w/v, for 72 hours. Mortality of larvae exposed to the different extracts increased with increase in extract concentration and time of exposure. This study revealed a differential potency of the

extracts used and a difference in susceptibility of larvae to the extracts as evident by the 72hLC50 values obtained. The leaf extract proved to be more lethal to the larvae than the stem extract as judged by the 72hLC50 values obtained both for the aqueous as well as the ethanolic extracts assayed. Phytochemical screening of the plant parts investigated revealed the presence of some plant metabolites, which have been reported in separate studies to be lethal to mosquito larvae. Results obtained from this study suggest that the leaf and stem extracts of *Tridax procumbens* possess a promising larvicidal potential which can be exploited in mosquito vector control.(Ubulom et al, 2013, Larvicidal effect of aqueous and ethanolic extracts of *Tridax procumbens* on Anopheles gambiae, Culex quinquefasciatus and Aedes aegypti).

2.2.9 ACARICIDAL ACTIVITY

The leaves of *Tridax procumben* has a Acaricidal activity against Rhipicephalus annulatus (Reghu rabindran et al,2010).

2.2.10 ANTHELMINTIC ACTIVITY

Tridax procumben leaf was extracted with 70% acetone and fractions were obtained by solvent: solvent group separation procedures. The acetone extract and the fractions were tested by egg hatch assay (EHA) and larval development and viability assay to assess relative bioactivity against *H. contortus* eggs and larvae The extracts inhibited egg hatchability and killed infective larvae of *H. contortus* in a concentration-dependent manner. The best-fit LC_{50} values were 0.562, 0.243, 0.490, 0.314, and 0.119mg/mL for the acetone extract, chloroform, hexane, butanol and 35% water in methanol fractions, respectively, when tested against nematode eggs. The best-fit LC_{50} values were 0.191, 0.505, 1.444, 0.306, and 0.040mg/mL for acetone extract, chloroform, hexane, butanol and 35% water in methanol fractions, respectively, when tested against larvae. The 35% water in methanol fraction was the most active against the larvae and eggs of *H. contortus* demonstrating the lowest LC_{50} values. This study demonstrates that the leaf extracts of *Tridax procumben* have anthelmintic activity; therefore it could find application in the

control of helminths in livestock.(Ademola, and Eloff, 2011, Ovicidal and larvicidal activity of *Tridax procumben* leaf acetone extract and fractions on *Haemonchus contortus*: *In vitro* studies).

2.2.11 ANTI HIV ACTIVITY

The Petroleum Ether and ethanol extracts of *Tridax procumben* are reported to have a potent inhibitory activity against opportunistic HIV patients' infections(Crockett et al., 1992).The Petroleum Ether and ethanol extractsof *Tridax procumben* are reported to have a potent inhibitory activity against *Chrysomya megacephale* (Kumarasinghe et al, 2002).

2.2.12 IMMUNO STIMULATING AGENT

The plant *Tridax procumben* has strong immune-modulating orimmune-stimulating potency, as evidenced by a steep rise in the total count of leucocytes with concomitant increasing in granulocyte: a granulocyte ratio as well as remarkable increase in the total number of peritoneal macrophages in the rabbits treated with the aqueous extract of leaves of *Tridax procumben*. Thus, the plant *Tridax procumben* may extensively be used in therapeutic medicines as a resource of natural and immune stimulating agent. (Saheli Chatterjee et al .2013**.** Study of Antioxidant Activity and Immune Stimulating Potency of the Ethnomedicinal Plant, *Tridax procumben*(L.) Roxb.)

2.2.13 SYNERGISTIC EFFECT

The synergism between the extract and synthetic drugs produced higher inhibitory activity against the organisms. The broth of cultured bacteria and fungi were spread on nutrient and potato dextrose agar using flooding method. A well sterilized cork borer (5mm) was used to make 'wells' in the media. The mixture of different antibiotics (0.4mg/ml) /antifungal drugs (0.4mg/ml) and plant extract were poured into the punched wells. The plates were incubated for 24-36 hours at 370 C and the zones of inhibition were measured and recorded. (Odunbaku, 2011. Synergistic Effect of Ethanol Leaf Extract of *Tridax procumbens* and Antimicrobial Drugs on Some Pathogenic Microbes)

The synergism effect of plant extracts and antibiotics drugs from this study supports the use of drug combinations in treating diseases because some organism are now known to be resistance to

antibiotics(Ajaiyeoba, Onocha and Olarenwaju, 2000. Invitro Anthelmintic properties of Buchholzia coriacea and Gynandropsis gynandra. J. Pharmaceut. Biol).

2.2.14 MIMIC PLANT GROWTH HORMONE

The efficacy of crude extracts of *Tridax procumben* in the improvement of vegetative and reproductive growth in *Tridax rocumben* was investigated. Fresh leaves of *Tridax procumben* were blended with a homogenizer in 1 litre of distilled water. The resultant green paste was filtered under suction. Different concentrations (75%, 50%, 40%, 30%, 25%, 12%, 10%, and 5%) were prepared from the 100% crude extract. Seeds of were presoaked in these different concentrations including a control (0%) and planted out after 24 hours. Results obtained showed that seedling height, leaf area, dry weight and leaf area ratio were promoted and enhanced by presoaking seeds in the extract. At the end of the experimental period (six weeks), seedling height in 75% and 100% treatments were 109 ± 16.12 cm and 117 ± 19.32 cm, leaf area 128 ± 17.91 cm^2 and 125 ± 18.12 cm^2, dry weight 7.48 kg and 7.0 kg respectively. Seedlings raised from seeds presoaked in water (control) however, flowered earlier (8 weeks) than the treatments (10 weeks in 75% and 100%). Presoaking seeds in crude extracts of *Tridax procumben* before planting is recommended for optimum production of the leafy vegetable. The procedure is cheap and easily implementable by resource-poor farmers who are the main growers. *(*Ikechukwu, 2014. Crude Extracts of *Tridax procumben* (L.) Roxb. Mimics Plant Growth Hormones in Promotion of Vegetative and Reproductive Growth.)

2.2.15 REDUCING PHOTO-INDUCED DAMAGE ON DNA AT THE MITOCHONDRIAL LEVEL

Chronic repetitive exposure to UVA radiation induces mtDNA deletions (missing fragments in circular mtDNA) in human dermal fibroblasts. The main consequences of mtDNA deletions in dermal fibroblasts are improper mitochondria functioning and reduction of cell metabolism, which results in accelerated skin aging. Cassia Alata acts on the deep effects of UV radiation on the skin, by reducing the photo induced damages on DNA at the nuclear and mitochondrial levels, but also by acting on the visible signs of photo-aging such as the prevention of micro relief alteration due to sun exposure. This auto regulating protective activity against the chronic harmful effects of the sun helps the cells to keep their “youth”. Specifically selected for its high

amount of K30S (Kaempferol-3-0-Sophoroside). K03S is over expressed in the sun-exposed leaves, as a natural protective system of the plant against UV radiations. It is fixed in and around the cell nucleus of the plants (Rhonda and Allison.2009).

2.2.16 ANTI-CORROSIVE EFFECT

The inhibition effect of *Tridax procumben* leaves extract on corrosion of mild steel in 1N HCl was investigated through mass loss measurements with various time and temperature. The observed result indicated that the corrosion inhibition efficiency and degree of surface coverage were increased with increase of inhibitor concentration and temperature. The thermodynamic parameters (E_a, ΔH_{ads}, ΔG_{ads}, ΔS_{ads}) were evaluated for corrosion inhibition process which suggests that the adsorption is endothermic, spontaneous and chemisorptions and also the inhibitor follows Langmuir adsorption isotherm. The protective film formed on metal surface was analyzed using spectroscopic studies viz, UV, FT-IR and EDX techniques. (*Petchiammal A.p et al.2013.*Anti-corrosive effect of *Tridax procumben* leaves extract on Mild steel in 1.0N Hydrochloric acid).

2.2.17 WOUND HEALING ACTIVITY

The ethanol extracts of leaves of *Tridax procumben* were investigated on excision wound model in Rats by, the leaf extract accelerated the wound healing potential by reducing the epithelial isationperiod,prevent high risk of sepsis and prolongation of inflammatory phase.(Midawa, 2010. Cutaneous wound healing activity of the ethanolic extracts of the leaf of *Tridax procumben).*

2.2.18 ANTI INFLAMMATORY ACTIVITY

Kaempferol-3-O-gentiobioside(K3G) flavoniod glycoside isolated from *Tridax procumben* leaves have anti-inflammatory activity (Moriyama et al, 2001,Antiinflammatory activity of Heat-treated *Tridax procumben* Leaf extract and its flavonoid glycoside).

2.2.19 HEPATOPROTECTIVE ACTIVITY

Aqueous extract of the leaves of *Tridax procumben* has hepatoprotective activity (.Effraim KD et al.1999, Antihepatotoxic activity of aqueous extract of *] Tridax procumben* (Linn) leaves against carbon tetrachloride induced liver damage in rats.) *Tridax procumbent* petals havehepatoprotective effect by decreasing the levels of Serum aspartate aminotransferase and alanine aminotransferase in carbon tetrachloride (CCl4) –induced hepatotoxicity in rats.(Wegwu et al, 2005, Anti-Oxidant Protective Effects of *Tridax procumben* in Rats Exposed to Carbon Tetrachloride. J Appl Sci Environ.)

2.2.20 ANTI-CRYPTOCOCCUS ACTIVITY

Combination of ethanolic extracts of leaves of *Tridax procumben* and *Ocimum sanctum* showed anti-Cryptococcus activity (Ranganathan and Balajeen, 2000. Anti-cryptococcus activity of combination of extracts of *Tridax procumben* and *Ocimum sanctum*)

2.2.21 INSECTICIDAL ACTIVITY

Hexane extract of *Tridax procumben* fruits cause high lethality and toxic to control insect pests. Cut down the glycogen, protein DNA, RNA amino acids and lipid content cause physiological imbalance in *C.chinensis* leads to death(Ravi Kant upadhyay et al,2011,Toxic effects of solvent and aqueous extracts of *Tridax procumben* against bio-molecules and enzymaticparameters of *Callosobruchuschinensis* L).

2.2.22 BRONCHORELAXANT EFFECT

Aqueous-ethanolic extract of *Tridax procumben* produce relaxation of tracheal smooth muscles exhibits broncho relaxant effect(Ouédraogo et al, 2013, Evaluation of the Bronchorelaxant, Genotoxic and Antigenotoxic Effects of *Tridax procumben* L. Evidence-Based Complementary and Alternative Medicine).

2.2.23 ANTIGENOTOXIC EFFECT

Genotoxic studies are useful to identify the level of DNA damage induced by xenobiotics. The antigenotoxic potential of was evaluated by aqueous-ethanolic extract of *Tridax procumben* did not induce DNA migration(Ouédraogo et al, 2013, Evaluation of the Broncho relaxant,

Genotoxic and Antigenotoxic Effects. Evidence-Based Complementary and Alternative Medicine).

2.2.24 ANALGESIC ACTIVITY

Kaempferol 3-O-sophoroside was isolated from the leaves of *Tridax procumben* exhibited analgesic activity (Owoyale J A et al, 2005 ,Antifungal and Antibacterial Activities of an Alcoholic Extract of *Tridax procumben* Leaves).The hexane, chloroform and ethyl acetate extract of the leaves of *Tridax procumben* exhibites analgesic activity(Irene et al, 2002, Bioactivity studies on leaf extracts).

2.2.25 CHOLERETIC ACTIVITY

The Choleretic activity of *Tridax procumben* extract proved to be better than that of hydroxycyclohexenyl-butyrate (Hebucol) asyntheyic, choleretic in rats (Assane et al, 1993,Choleretic effects of Linn in Rats)

3.1 PLANT SELECTION

Throughout medical history, plant products have been shown to be valuable sources of novel compound for discovery of drugs. Topical forest are on of the most diverse and endangered habitats on earth. They have also been portrayed as a source of future pharmaceuticals, yet finding useful compounds can be both scientifically and politically challenging. Over the past decade the potential value for medicinal compound derives from plants ,microorganism , animal has been proposed as tangible benefit of biodiversity and therefore a basis for promoting its prevention. Ecological theories of plant defense can increase the probability of discovering with activity in bioassy against human disease target.

There are thousands of medicinal plants in Bangladesh and in this Indian subcontinent. Among these plants it was not easy to select a few plants for the research purpose. The selection of plant greatly affects the research work if there is carelessness takes place. Plant secondary metabolites

often accumulate in specific plant parts. Thus, unless it is already known which parts contain the highest level of the compounds of interest, it is important to collect multiple plant parts, or the whole plant to ensure the extracts prepared representative of the range of secondary metabolites. For drug discovery from plants, sample may be selected using a number following criteria by which the research work will run smoothly.

From the literature review it is seen that there is a lot of work on the plant *Tridax procumben* about the pharmacological activity of the plant. But there is a least of work has been found about chemical investigation of this plant, especially about the leaves of this plant. So I got a chance to select the leaves of *Tridax procumben* for my research work to see whether the leaves have antioxidant and anti-diabetic and antimicrobial activity or not.

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3.2 PLANT COLLECTION

After selection of plant it is must to collect the plant parts for the research purpose. But the plant *Tridax procumben* is not available throughout the bangladesh . The plant sample was collected from Jhalokathi District, under Barisal division on 9th October, 2015.

3.2.1 DRYING OF PLANT SAMPLE

After the collection of sample it needs to be dried to make the sample extract. In general the plant material should be dried at temperature below 30 degree C to avoid the decomposition of thermo labile compounds. So sun drying can be very effective but drawback is sometimes water molecules are absorbed by the sample and hence fungus growth can affect the phytochemical study. The seeds along with the testa were dried in the sun light thus chemical decomposition can not take place.

3.2.2 GRINDING OF DRIED SAMPLE

Small amount of plant material can be milled using grinder or blender. But if the sample is in high amount then it is easier to get powdered sample by grinding from a spice mill. Grinding improves the efficiency of extraction by increasing surface area. It also decreases the amount of solvent required for the extraction. The dried samples were ground to coarse powder with a mechanical grinder (Blender) and powdered samples were kept in clean closed containers

pending extraction. During grinding of samples, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign matter deposited on the grinder.

3.3 MACERATION OF DRIED POWDERED SAMPLE

3.3.1 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

3.3.2 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.

3.4 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

3.5 SAMPLE CONCENTRATION BY ROTARY EVAPORATION TECHNIQUE

3.5.1 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).

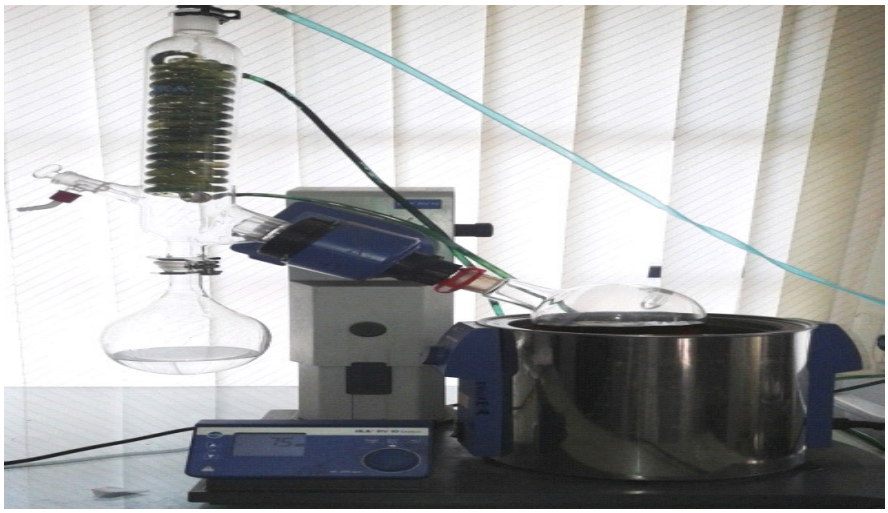


Figure 3.5.1: Rotary Evaporato

Figure 3.5.1: Rotary machine of east west university

3.5.2 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

3.5.3 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 130 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.

3.6 SAMPLE CONCENTRATION BY VACUUM LIQUID CHROMATOGRAPHY(VLC) TECHNIQUE

3.6.1 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.

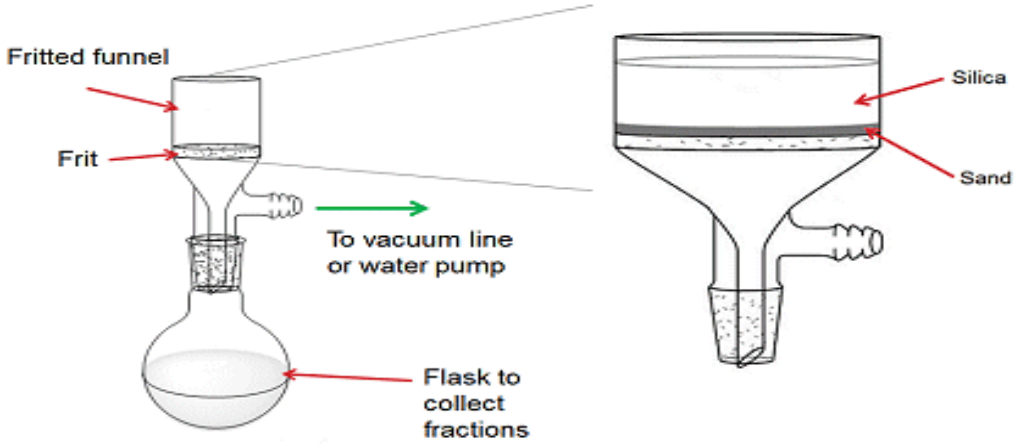


Figure 3.6.1: Vacuum Liquid Chromatography

3.6.2 Apparatus

- VLC chamber.
- Filter paper

3.6.3 Reagents

- Silica gel

  Methanol
- Chloroform
- Dichloromethane.

  Butanol
- Ethanol

3.6.4 Procedure

The 500gm Methanol extract of *Tridax procumben* 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. Dichloromethane, 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.

3.7 Equipments and other necessary tools

During the extraction procedure and for various phytochemical tests many equipments and materials were used. Some of them are TLC plate, TLC tank, scale, pencil, TLC plate cutter, capillary tube, mortar and pestle, laminar air flow cabinet, loop, burner, micropipette tip, petri dishes, glass rod, cotton, filter paper, funnel, hot plate, centrifugal machine, autoclave, glassware washers, stirrer, UV spectroscopy, knife, ephedrine tube, Whatman□s filter paper, paper disc, incubator, vortex machine, PH meter. analytical balance, beaker (in various size), pipette, micro-pipette, rotary evaporator, hot air oven, dryer, storage cabinet, spatula, test tube, volumetric flask, conical flask, test tube holder, test tube rack, aluminum foil paper, scotch tape, refrigerator, water bath, electronic shaker, ultra violate lamp, mask, gloves, lab coat, sprayer, reagent bottle.

3.8 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)

3.9 Solvents for experiments

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

4.1 THIN LAYER CHROMATOGRAPHY(TLC)

4.1.1 Principle

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase. The solvent or solvent system that runs on the stationery phase by capillary action and conducts the separation, this is known as the mobile phase.Once the sample has been spotted on the plate and the mobile phase run through it, the different components of the mixture separate differently owing to their relative affinities for the stationery and mobile phases. Heavier components or the ones more attracted to the stationery phase remain at the bottom while components that are light and more soluble in the mobile phase travel up with it. The relative separation of the components can be studied by calculating the Retardation Factor (Rf), which is the ratio of the distance of migration of a particular substance to the distance of migration of the solvent front.

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. The goal of TLC is to obtain well defined, well separated spots.

4.1.2 Materials Required

- Silica coated TLC plate
- TLC tank
- Spotting capillary tubes
- Tweezers
- Pipette
- Pipette filter
- Test tubes
- Solvents
- UV lamp

4.1.3 Reagents

- Benzene
- Ethanol
- Ammonium Hydroxide
- Chloroform
- Ethyl Acetate
- Formic Acid
- Water
- Di-chloromethane
- Butanol
- Methanol

The experiment was conducted on three solvent systems, first one was non-polar, the second one was intermediate polar and the third one was polar. The compositions of the three solvent systems are as follows:

Table 4.1.4: The composition of various solvent systems for TLC

		Intermediate polar Basic Solvent		Polar Basic Solvent
Benzene 9ml		Chloroform 5ml		Ethyl acetate 8ml
Ethanol 1ml		Ethyl acetate 4ml		Ethanol 1.2ml
AlOH 0.1ml		Formic acid 1ml		Water 0.8ml

4.1.5 Procedure

- Using a pencil the baseline and the solvent front line was drawn on the TLC plate and the plate was labeled for the individual spots.
- The fraction of methanolic extract after VLC and Column Chromatography was spotted on TLC plate and the plate was dried completely in the air.
- In a TLC tank the solvent system was added. A strip of filter paper was inserted into the tank so that its bottom touched the solvent. The lid of the tank was closed and left to rest for a few minutes so that the solvent system could travel up the filter paper and saturate the chamber.
- Using a pair of tweezers the TLC plates were placed in the chamber carefully so that the baseline did not touch the solvent
- The plate was left in the tank so that the solvent system could run up the plate by capillary action and develop the spots.
- The plate was removed from the tank using a pair of tweezers once the solvent had reached reached the solvent line. The plate was then allowed to dry completely.

- Three types of solvent system were used based on difference in polarity for the detection of different compounds.
- The developed plate was then viewed under UV light for the detection of bands and spots.

4.1.6 Acid Charing of TLC plates

4.1.6.1 Materials

- Tweezers
- Conc. Sulfuric acid
- Distilled water
- Hot plate
- Petri dish

4.1.6.2 Procedure

- 9 ml of distilled water was added to 1 ml of concentrated sulfuric acid to produce a 10% solution of sulfuric acid which was taken in a petri dish.
- The TLC plate was dipped in this solution using tweezers with the silica face down.
- The plate was left in the open for 10 minutes to allow for drying.
- A hot plate was heated to about 90 degree C and the plates were heated until the spots developed.

4.2 CHARRING PROCESS OF TLC PLATE

4.2.1 Concentrated H2SO4 (98%)

1 ml concentrated H2SO4 (98%) is added to 9 ml distilled water. And TLC plate is sprayed with this reagent for 1 minute, dried and heated for spots visualization (Brand-Willams, W., Cuvelier, M. E,& Berset, C1995).

2. 2,2'-Diphenylpicrylhydrazyl

Reagent: 1 ml 0.4% DPPH is added to 9 ml methanol to produce 0.04% DPPH solution. TLC plate is sprayed with this reagent in dark room for 1 minute; then spots are visualized in daylight and immediate picture of TLC plate is captured (Duke JA, W.K,(1981).

4.3 APPLICATION OF TLC TECHNIQUE

- Purity of any sample : Purity of sample can be carried out with TLC. Direct comparison is done between the sample and the standard or authentic sample; if any impurity is detected, then it shows extra spots and this can be detected easily.
- Identification of compounds*:* Thin layer chromatography can be employed in purification, isolation and identification of natural products like volatile oil or essential oil, fixed oil, waxes, terpenes, alkaloids, glycosides, steriods etc.
- Examination of reactions*:* Reaction mixture can be examined by Thin layer chromatography to access whether the reaction is complete or not. This method is also used in checking other separational processes and purification processes like distillation, molecular distillation etc.
- Biochemical analysis*:* TLC is extremely useful in isolation or separation of biochemical metabolites or constituent from its body fluids, blood plasma, serum, urine etc.
- In chemistry*:* TLC methodology is increasingly used in chemistry for the separation and identification of compounds which are closely related to each other. It is also used for identification of cations and anions in inorganic chemistry.

- In pharmaceutical industry*:* Various pharmacopoeias have adopted TLC technique for detection of impurity in a pharmacopoeial chemical.
- Various medicines like hypnotics, sedatives, anticonvulsant tranquillisers, antihistaminics, analgesics, local anaesthetics, steroida have been tested qualitatively by TLC method.
- One of the most important application of TLC is in separation of multicomponent pharmaceutical formulations.
- In food and cosmetic industry , TLC method is used for separation and identification of colours, preservatives, sweetening agent, and various cosmetic products.
- This are some of the applications of Thin layer Chromatography (TLC)

4.4 Advantages of TLC technique

- TLC is very simple to use and inexpensive.
- Undergraduates can be taught this technique and apply its similar principles to other chromatographic techniques.
- There are little materials needed for TLC (chamber,watch glass, capillary, plate, solvent, pencil, and UV-light). Therefore, once the best solvent is found, it can be applied to other techniques such as High performance liquid chromatography.
- More than 1 compound can be separated on a TLC plate as long as the mobile phase is preferred for each compound.
- The solvents for the TLC plate can be changed easily and it is possible to use several different solvents depending on desired results.
- As stated earlier, TLC can be used to ensure purity of a compound. It is very easy to check the purity using a UV-light.
- Identification of most compounds can be done simply by checking Rf literature values.And can modify the chromatography conditions easily to increase the optimization for resolution of a specific component (ChemWiki,2015, http://chemwiki.ucdavis.edu)

4.5 Disadvantages of TLC technique

- TLC plates do not have long stationary phases. Therefore, the length of separation is limited compared to other chromatographic techniques.
- Also, the detection limit is a lot higher. If one would need a lower detection limit, one would have to use other chromatographic techniques.
- TLC operates as an open system, so factors such as humidity and temperature can be consequences to the results of your chromatogram (ChemWiki,2015, http://chemwiki.ucdavis.edu)

4.6 Common Problems in TLC

There are common problems in TLC that should be avoided. Normally, these problems can be solved or avoided if taught proper techniques.

- **Over-large Spot**s: Spotting sizes of sample should be not be larger than 1-2 mm in diameter. The component spots will never be larger than or smaller than sample origin spot. If the spot is large, this could cause overlapping of other component spots with similar Rf values on TLC plate. If overlapping occurs, it would prove difficult to resolve the different components.
- **Uneven Advance of Solvent Front**: Uneven advance of the mobile phase is a common problem encountered in TLC. Consequences would be inaccurate R_f values due to the uneven advance of sample origin spots. This uneven advance can be caused by a few factors listed below.
- *No flat bottom*. When placing the TLC plate into the chamber, place the bottom of the plate on the edge of the chamber (normally glass container (e.g. beaker)) and lean the top of the plate along the other side of the chamber. Also, make sure that the TLC plate is placed in the chamber evenly. Do not tilt the plate or sit it at an angle.
- *Not enough solvent.* There should be enough solvent (depends on size of chamber) to travel up the length of the TLC plate.

- *Plate is not cut evenly*. It is recommended that a ruler is used so that the plate is cut evenly.
- Rarely, water is used as a solvent because it produces an uneven curve front which is mainly accounted for by its surface tension.
- **Streaking**: If the sample spot is too concentrated, the substance will travel up the stationary phase as a streak rather than a single separated spot. In other words, the solvent can not handle the concentrated sample and in result, moves as much of the substance as it can up the stationary phase. The substance that it can not move is left behind. This can be eliminated by diluting the sample solution. To ensure that one has enough solution,one should use a short-wave UV light to see if the spot is visible (normally purple in color), as stated earlier.
- **Spotting**: The sample should be above the solvent level. If the solvent level covers the sample, the sample spot will be washed off into the solvent before it travels up the TLC plate (ChemWiki,2015, http://chemwiki.ucdavis.edu)

4.7 DPPH CHARRING PROCESS OF TLC PLATE

4.7.1 Materials Required

4% DPPH stock solution (1%), Methanol (9 ml), Test Tube, Pipette, Pipette filter, Petridish and Tweezers.

Procedure:

1. 0.4% solution of DPPH was prepared by adding 9 ml of methanol to 1 ml of 4% DPPH stock solution. The procedure was carried out in a dark room as DPPH is light sensitive.

2. By using tweezers the developed TLC plates would be dipped into this solution on the silica face down.

3. The plates were left in the dark room for 30 minutes for the color to develop after which they were observed for the formation of yellow, golden / brown color on the background of purple. This coloration indicates the presence of compounds that have antioxidant properties (Neeraj et al, 2013).

4.8 ANTI-OXIDANT TESTS

DPPH Test (1,1 -diphenyl-2-picrylhydrazyl radical)

4.8.1 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).

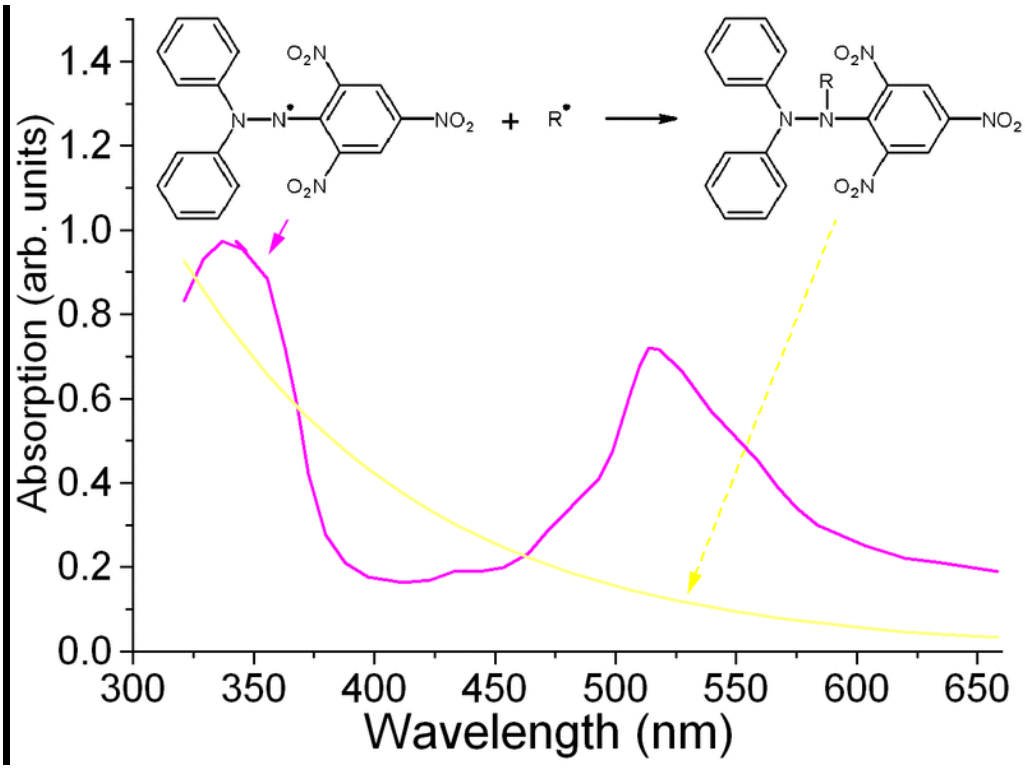


Figure4.8.1: Change in absorption spectrum (from magenta to yellow) upon reaction of DPPH with a radical (ABUIN, 2002:p.145-149).

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)

4.8.2 Apparatus

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

4.8.3 Reagents

- DPPH
- L-ascorbic acid
- Methanol
- Water

4.8.4 Procedure

4.8.4.1 Sample Preparation

- The methanolic extract of the *Tridax procumben 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.

4.8.4.2 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.

4.8.4.3 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.

4.9 IN VITRO ANTI-DIABETIC TEST

4.9.1 Introduction

Diabetes 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 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, *tridax procumben* 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).

4.9.2 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.

4.9.3 Procedure:

Antidiabetic activity of leaves of *Tridax procumben* 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).

4.10 Antimicrobial Screening of Methanolic Extract Using Agar Diffusion Method

The discovery of disease-causing pathogens is an important activity in the field of medical science, as many viruses, bacteria, protozoa, fungi, helminthes and prions are identified as a confirmed or potential pathogen. A Centers for Disease Control program begun in 1995 identified over a hundred patients, with life-threatening illnesses which were considered to be of an infectious cause, but could not be linked to a known pathogen. The association of pathogens with disease can be a complex and controversial process, in some cases requiring decades or even centuries to achieve (Day, 1997).There are many factors impairing identification of pathogens including Lack of animal models, Pre-existing theories of disease, Variable pathogenicity, Organisms that look alike but behave differently, Lack of research effort.

Control of many infectious diseases became possible with the pioneering work of Robert Koch and Louis Pasteur and the introduction of the germ theory of disease. With bacteriologic cultivation techniques came the first isolation and identification of etiologic agents; virus cultivation and identification became available some decades later. Reservoirs of microorganisms and their life cycles were identified; the epidemiology and natural history of many infectious diseases were described, and successful control measures were initiated. Water treatment, vector control, and rodent reduction programs followed. By the beginning of the 20th century, the principles of vaccination, established empirically by Edward Jenner more than 100 years earlier, began to be realized in earnest. Antibiotics were discovered, and disinfectants were developed. Collectively, these control measures dramatically decreased the incidence and prevalence of many infectious diseases and their fatality rates. The early part of this century is appropriately regarded as a golden age in public health (David Satcher, 2015; Emerging Infections: Getting Ahead of the Curve)

Death from infectious disease rankd 5th in 1981, has become the 3rd leading leading cause of death in 1992; an increasing of 58%. It is estimated that infectious disease is the underlying cause 58% of the death occurring in the US (Ahmed et al, 2011). The respiratory tract infection are increased and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosicomial and community acquired infections. Furthermore, the most increasr are occurring in the 25-44years old age group (Khosa, et al, 2011).

The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability estimated by disc diffusion (Kowti, et al, 2010).

Some investigator use the diameter of Zone of inhibition and the minimum weight of extract inhibit the growth of microorganisms/ However, a great number of factors, the extraction methods, inoculums volume, culture medium composition, pH and incubation temperature can presence the results (Britto ,et al, 2011).

Among the above mentioned techniques the disc diffusion is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity. It is essentially a quantitative and qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method.

4.10.1 Principle of disc diffusion Method

The agar diffusion assay is one method for quantifying the ability of antibiotics to inhibit bacterial growth. The agar diffusion test, or the Kirby-Bauer disk-diffusion method, is a means of measuring the effect of an antimicrobial agent against bacteria grown in culture. The bacteria in question is swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is

greater than or equal to the effective concentration. This is the zone of inhibition. This along with the rate of antibiotic diffusion are used to estimate the bacteria's sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that bacteria. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection (Mohanty A et al, 2010).

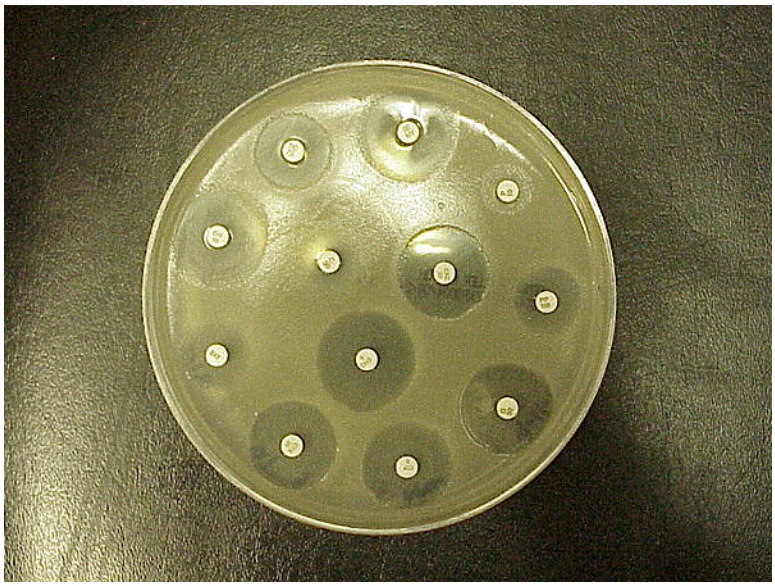


Figure 4.10.1:Discs containing antibiotics are placed on agar where bacteria are growing and inhibited.

4.10.2 Materials Required

- Filter paper discs
- Autoclave
- Nutrient Agar Medium
- Laminar air flow hood

- Petri dishes
- Spirit burner
- Sterile cotton swabs
- Refrigerator
- Micropipette
- Incubator
- Inoculating loop
- Ethanol
- Sterile forceps
- Nose mask and Hand gloves
- Screw cap test tube

4.10.3 Test Organisms

4.10.3.1 Gram Negative Bacteria

- *Salmonella typhi*
- *E.coli*
- *Pseudomonas*
- *Shigella dysentery*
- *Salmonella paratyphi*
- *Vibriomimicus*

4.10.3.2 Gram Positive Bacteria

- *Staphylococcus aureus*
- *Bacillus cereus*
- *Bacillus subtilis*

4.10.3.3 Fungi

- *Candia albicans*
- *Bacillus megaterium*
- *Aspergillus niger*

4.11 The Culture Medium and Its Composition

Nutrient agar was used to conduct the antimicrobial screening using the disc diffusion method. The nutrient agar was bought from the market. Nutrient agar contains the following substances:

4.11.1 Ingredients

- Bacto peptone - 0.5gm
- Sodium chloride - 0.5gm
- Bacto yeast extract - 1.0gm
- Bacto agar - 2.0gm
- Distilled water (Qs) - 100ml

4.11.2 Preparation of the Medium

First of all, The amount of nutrient agar needed was calculated and then added to distilled water in an agar bottle and mixed thoroughly. It was then autoclaved to dissolve the agar and sterilize it.

Figure 4.11.2: The autoclaved machine of East West University

4.11.3 Sterilization Procedure

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present in a specified region, such as a surface, a volume of fluid, medication, or in a compound such as biological culture media. 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. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121ºC and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Figure 4.11.3: The laminar hood of East West University

4.11.4 Preparation of the Test Plates

1. The test organisms were transferred from the subculture to petridish containing the required amount of melted and sterilized agar medium as required by the size of the dish.

2. The bacterial and fungal suspension was taken by a loop and mixed with normal saline with the help of vortex machine.

3. Then a sterilized cotton bud was taken and dipped into the bacterial/ fungal suspension. Then the bacterial/fungal sample is applied to the petridish with the help of this cotton bud.

4. The swabbing was done carefully so that the microorganisms would be spread out evenly on the dish.

Figure 4.11.4: The vortex machine of East West University

4.11.5 Preparation of Discs

4.11.5.1 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) standard disc was used as the reference.

4.11.5.2 Blank Discs

These were used as negative controls, which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves. Here the negative control used was methanol.

4.11.6 Preparation of sample discs with test samples

1. In a specific volume of solvent, Measured amount of each test sample was dissolved to obtain the desired concentrations in an aseptic condition.
2. For the each extract of husk, a stock solution of 10mg/ml was prepared and was used directly.

3. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of 10µl of test samples and dried.

4.11.7 Diffusion and Incubation

Here, incubation is done for maintaining controlled environmental conditions for the purpose of favoring growth or development of microbial or tissue cultures or to maintain optimal conditions for a chemical or immunologic reaction.

1.The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi.

2.The plates were then inverted and kept in an incubator at 370C for 24 hours.

Figure 4.11.7: Incubator for microorganisms at East West University

4.12 Determination of Antimicrobial Activity by using the Zones of Inhibition

The agar diffusion test, the size of the zone of inhibition indicates the degree of sensitivity of bacteria to a drug or testing agent. In general, a bigger area of bacteria-free media surrounding an antibiotic disk means the bacteria are more sensitive to the drug or the testing agent the disk contains .KB tests are performed under standard conditions, so the minimum inhibitory

concentration for a given antibiotic or testing agent can be calculated by comparing the observed zone of inhibition's size to known values.

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

RESULT

5.1 THIN LAYER CHROMATOGRAPHY(TLC)

TLCs were conducted on methanolic extract of the leaves of *Tridax procumben* by using all the three types of solvent systems, and the best results were obtained by using the non polar solvent system.

TLC was done in non polar solvent system which consist of Benzene 9ml, Ethanol 1ml.The naked eye view of the TLC was mentioned in the plate 1 which did not show any clear spot (1). Then the plate was observed under UV which is shown in the plate (2). 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 -3). In the crude extract layer three spot was observed.

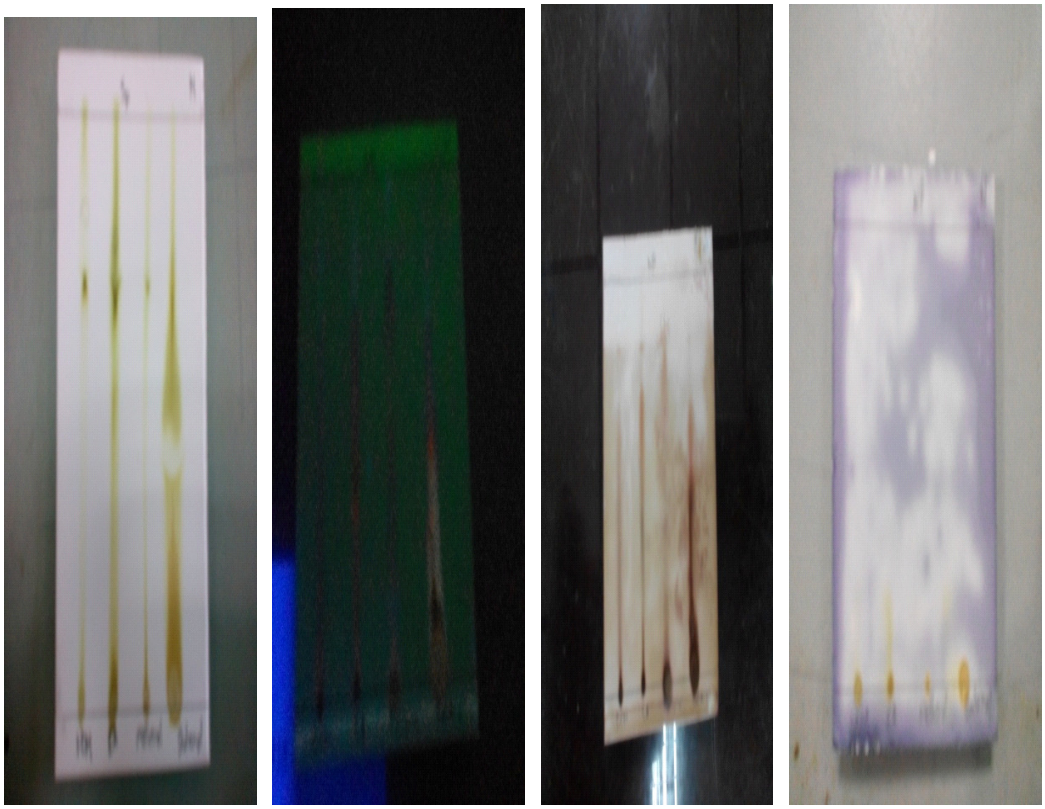
R.F (Retardation Factor) Value Calculation:

R_f = Distance spot travels/ Distance solvent travels

Table 5.1*:* R.F (Retardation Factor) Value Calculation of leave extract of for methanolic extract

Name of sample(Methanolic extract)	R_f value
1st spot	0.31
2^{nd} spot	0.50
3^{rd} spot	0.75

5.2 Thin layer Chromatography of Methanolic Extract of *Tridax procumben* leaves (Primary five fraction of VLC extract).



(1) (2) (3) (4)

Figure 5.2: (1)TLC plate in naked eye view, (2)TLC plate Under UV light,(3)TLC plate after charring with H_2SO_4, (4) TLC plate after application of DPPH.

TLC was done with primary five fraction of VLC. After TLC, it was found that the five fractions made some spots 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. There were some spot was found after TLC plates were dipped in DPPH solution (plate 4).

R.F (Retardation Factor) Value Calculation of primary five fraction of VLC

R_f = Distance spot travels/ Distance solvent travels

Table 5.2: R.F (Retardation Factor) Value Calculation of primary five fraction of VLC.

Name of sample	1st spot R_f value	2nd spot R_f value	3rd spot R_f value
DCM	0.53	0.77	0.89
Butanol	0.55	0.75	0.96
Ethyl Acetate	0.60	0.87	0.93
Methanol	0.2	0.5	

5.3 HYPOGLYCEMIC TEST

The result of hypoglycemic test of different fraction of methanolic extract of the *Tridax procumben* leaves are given below-

Table 5.3.2: Antidiabetic activity of DCM fraction of methanolic extract of *Tridax procumben* leaves

Sample Name	**Concentrations µg/ml**	**Absorbance (340nm)**	**% of inhibition**
	31.25	**0.280**	**7.68**
	62.5	0.305	15.25
DCM	125	0.352	26.56
	250	0.390	33.72
	500	0.479	46.03

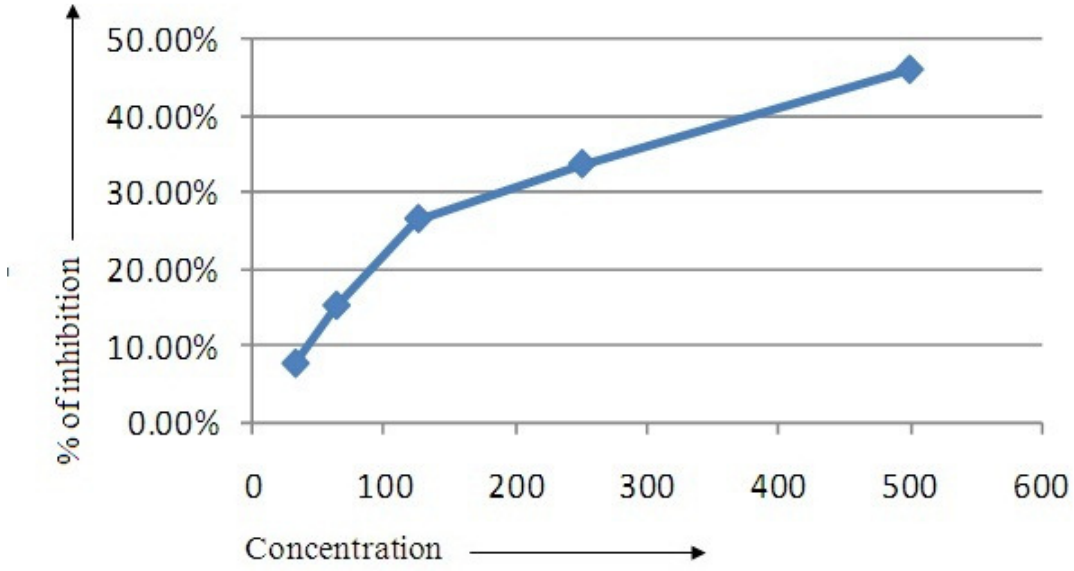


Fig 5.3.2 : Anti-diabetic activity of DCM fraction of *Tridax procumben* leaves.

Table 5.3.3: Antidiabetic activity of n-butanol fraction of methanolic extract of *Tridax procumben* leaves

Sample Name	**Concentrations µg/ml**	**Absorbance (340nm)**	**% of inhibition**
	31.25	0.294	12.07
	62.5	0.356	27.39
Butanol	125	0.540	52.13
	250	0.653	60.41
	500	0.694	62.75

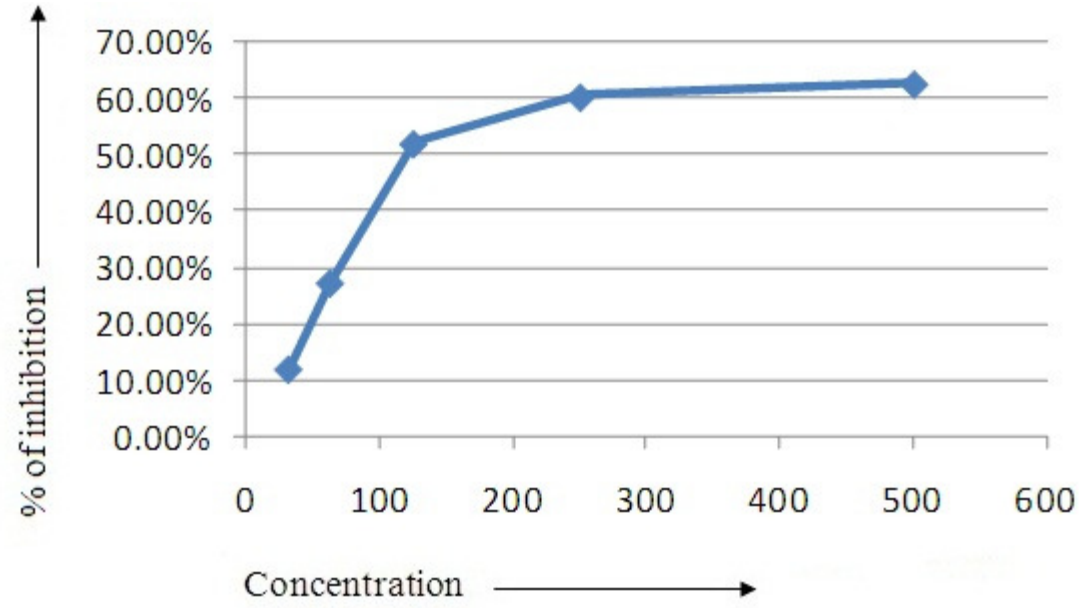


Fig 5.3.3: Anti-diabetic activity of butanol fraction of *Tridax procumben* leaves

Table 5.3.4: Antidiabetic activity of Ethyl acetate fraction of methanolic extract of *Tridax rocumben* leaves

Sample Name	Concentrations µg/ml	Absorbance (340nm)	% of inhibition
	31.25	0.300	13.83
	62.5	0.325	20.46
Ethyl acetate	125	0.423	39.01
	250	0.471	45.12
	500	0.622	58.44

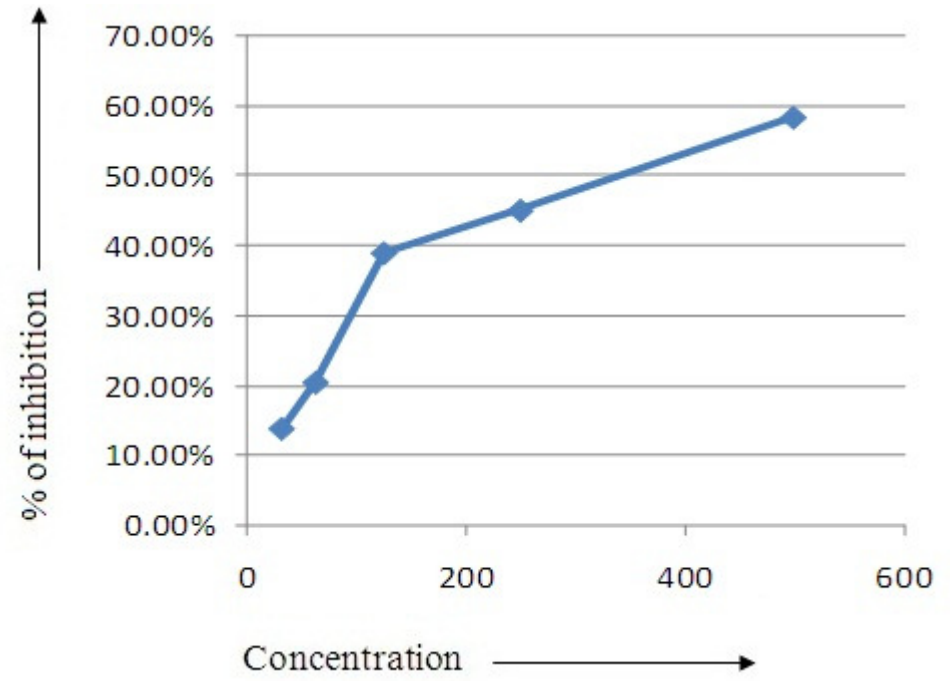


Fig 5.3.4: Anti-diabetic activity of Ethyl acetate fraction of *Tridax rocumben* leaves

Sample Name	**Concentrations µg/ml**	**Absorbance (340nm)**	**%of inhibition**
	31.25	0.294	12.07
	62.5	0.331	21.90
Methanol	125	0.343	22.89
	250	0.369	29.95
	500	0.530	51.22

Table 5.3.5: Antidiabetic activity of Methanol fraction of methanolic extract of *Tridax procumben leaves*

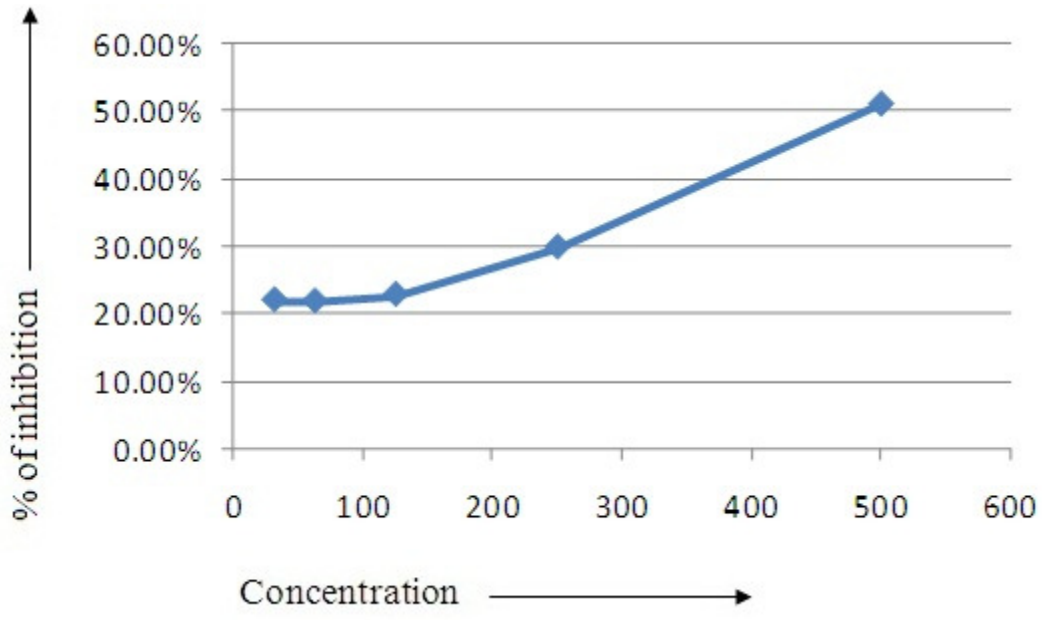


Fig 5.3.5: Anti-diabetic activity of Methanol fraction of *Tridax procumben* leaves.

Table 15.3.6: Antidiabetic activity of standard Metformin

Sample Name	**Concentrations µg/ml**	**Absorbance (340nm)**	**%of inhibition**
	31.25	0.333	22.37
	62.5	0.360	28.19
Metformin	125	0.387	33.20
	250	0.399	35.74
	500	0.429	39.74

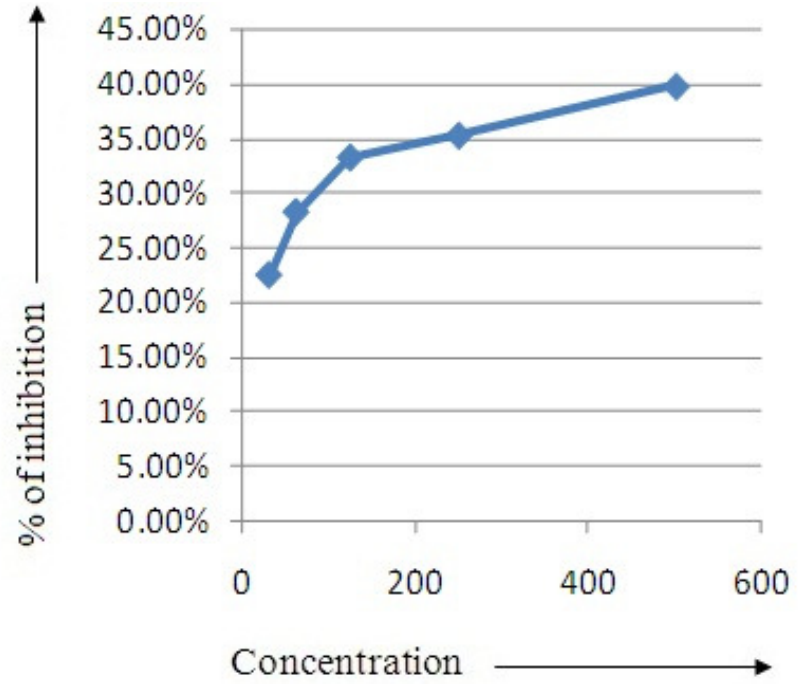


Fig 5.3.5: Anti-diabetic activity of Metformin

Anti oxidant activity

Table 5.3.8:Antioxidant activity of DCM fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**% of inhibition**
	31.25	0.523	1 2 . 6 1
	62.5	0.519	13.28
DCM	125	0.448	89.72
	250	0.355	40.68
	500	0.178	70.25

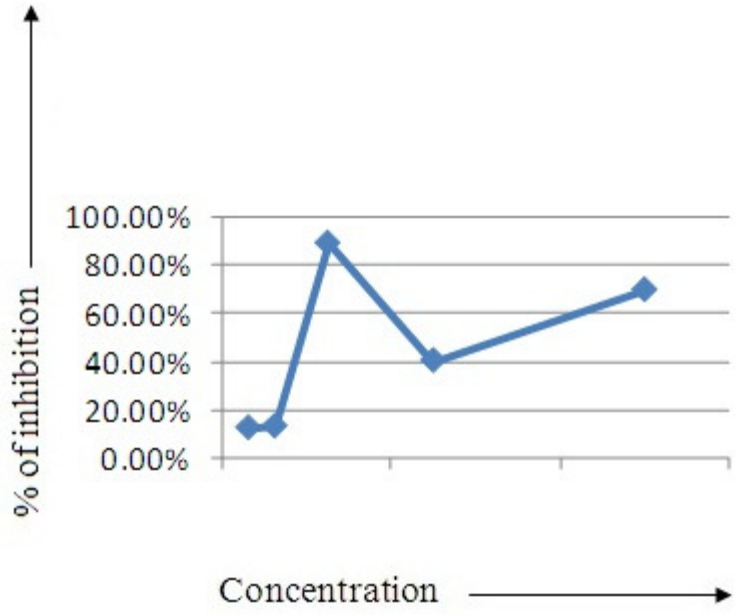


Fig 5.4.3 : % Free radical scavenging activity of DCM fraction of *Tridax procumben leaves*

Table 4.3.5: .Antioxidant activity of standard fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**% of inhibition**
	31.25	.246	5 8 . 8 9
	62.5	0.246	60.73
Standard	125	0.219	63.40
	250	0.199	66.75
	500	0.168	71.90

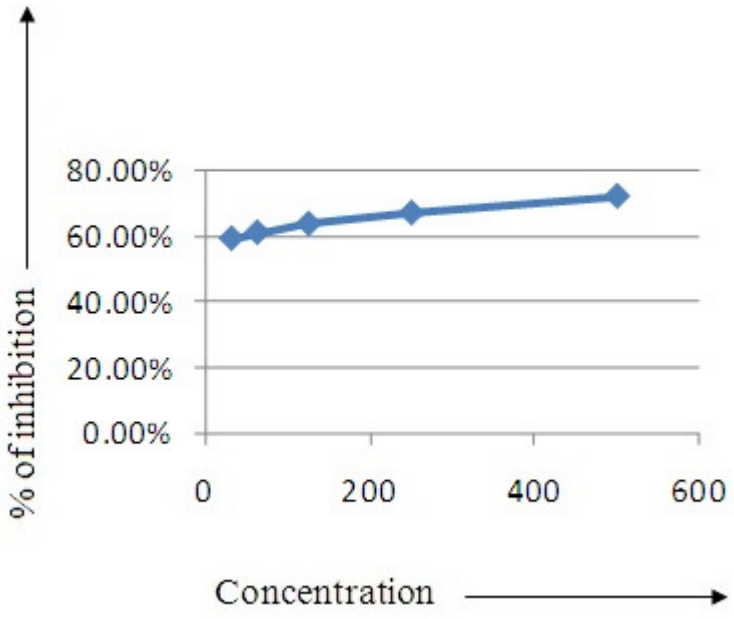


Fig 4.3.4 : % Free radical scavenging activity of Ascorbic acid

Table 4.4.5:Antioxidant activity of Ethyl acetate fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**% of inhibition**
	31.25	.226	6 2 . 2 3
	62.5	0.165	72.43
Ethyl acetate	125	0.115	80.78
	250	0.098	83.62
	500	0.092	84.62

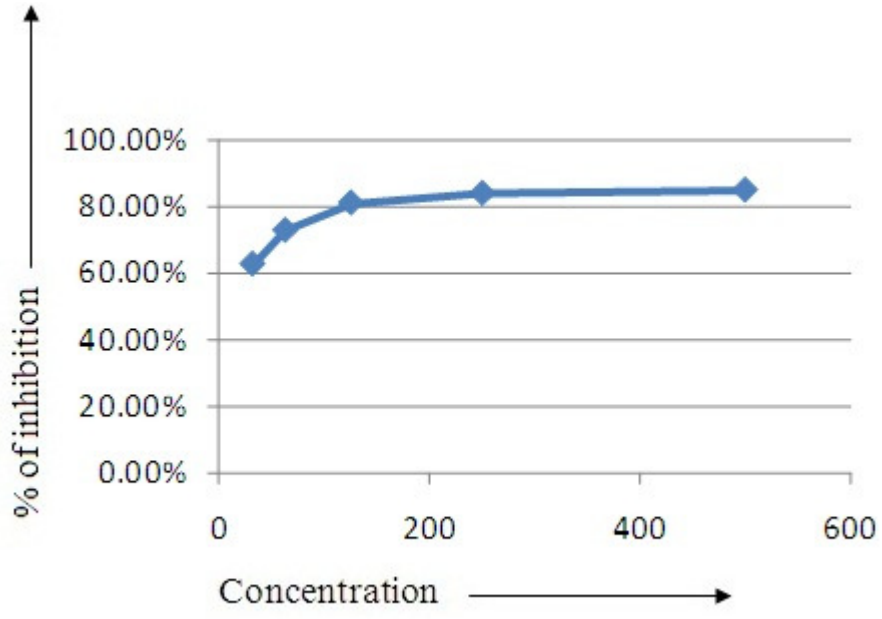


Fig 4.5.7 : % Free radical scavenging activity of Ethyl Acetate fraction of *Tridax procumben leaves ac*tivity

Table 4.4.6:Antioxidant of Methanol fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**%of inhibition**
	31.25	0.254	57.56
Methanol	62.5	0.178	70.25
	125	0.124	79.28
	250	0.108	81.95
	500	0.100	83.29

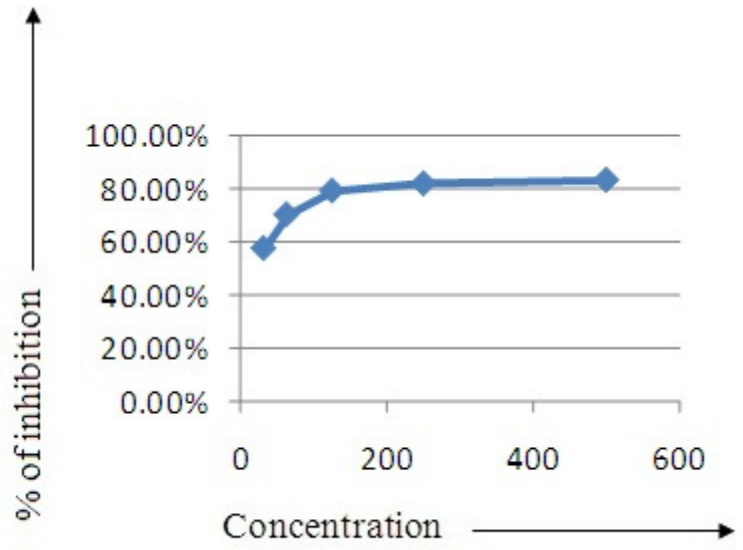


Fig 4.5.3 : % Free radical scavenging activity of Methanol fraction of *Tridax procumben leaves*

Table 4.4.7:Antioxidant of Butanol fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**% of inhibition**
	31.25	0.480	19.80
Butanol	62.5	0.344	42.52
	125	0.312	47.86
	250	0.288	51.89
	500	0.264	55.89

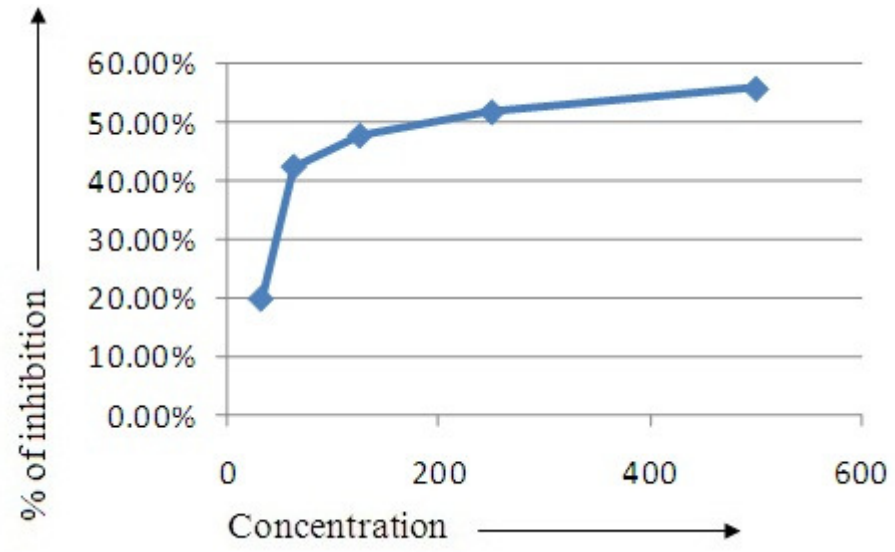


Fig 4.5.3 : % Free radical scavenging activity of Butanol fraction of *Tridax procumben leaves*

Antibacterial activity

Table 6.2: Antibacterial activity of the methanolic extract of Ethyl acetate ,Dichloromethane,Butanol, Methanol,standard and control test

Tested bacteria	**Zone of inhibition (mm)**					
	Methanol	**Ethyl acetate**	**DCM**	**Butanol**	**Standard**	**Control**
S. paratyphi	9	9	8	14	24	0
Bacillus sereus	8	9	7	7	23	0
Bacillus subtilis	13	11	8	9	26	0

Staphylocuccos aureas	13	12	9	21	30	0
Salmonela typhi	9	8	8	11	31	0
Shigella dysentriae	10	8	10	7	27	0
Vibrio mimicus	9	9	8	14	28	0
Candida albicans	9	10	9	11	27	**0**
Aspergillus niger	9	10	9	11	27	0
E.Coli	10	11	9	11	25	0
Vibrio parahemoliticus	11	9	8	13	30	0
Bacillus megaterium	13	11	8	9	27	0
Pseudomonas aurea	11	12	9	12	22	0

DISCUSSION

6.1 Thin layer chromatography

6.1.1 Discussion

TLC plates were developed with Dichloromethane, Butanol,ethyl acetate,Methanol crude using solvent system-1 (Benzene,Ethanol,Ammonium hydroxide) and 3 (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 (2). 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 3). 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 4). This provides us a preliminary idea of the various types of compounds that may be present in the methanolic extract of the leaves of *TridaxpProcumben* Further extractions and purifications from these crude drugs may lead to the possible isolation of these compounds from the crude extracts.

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,Dichloromeehane,Butanol, ethyl acetate,Methanol showed at least three spot.(plate-4)Some spots was found after TLC plates were dipped in DPPH solution

6.2 IN VITRO ANTI-DIABETIC TEST

6.2.1 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,Butanol and Ethyl acetate fraction of methanolic extract of give higher antidiabetic activity 62% and 58%.Whereas, Methanol and Dichloromethane fraction of methanolic extract of give 51%,46% lower antidiabetic activity .

6.3 DPPH TEST

6.3.1 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 methanolic extract of the *leaves of Tridax procumben* 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 Dichloromethane(DCM), Butanol, Ethyl acetate and Methanol fraction of *Tridax procumben* leaves give 89%,55% and 84%,83% antioxidant activity.

6.4 ANTIBACTERIAL TEST

6.4.1Discussion

Various strains of Gram positive, Gram negative bacteria and fungi were used in this test. The positive control used was ciprofloxacin (30µg/disc). For methanolic extract of,Ethyl acetate ,Dichloromethane,Butanol**,**was used to evaluate the activity against different types of microrganism. The zones of inhibition for the microbes were measured in centimeters using a transparent ruler after 24hrs of incubation.butanol extract was showed zone of inhibition up to 21cm antibacterial activity at the concentrations used against*, Staphylocuccos aureas* than other strain. A study showed that, The leaves of *Tridax procumben* possessing antimicrobial activity can be employed against human pathogens. The use of leaves of *Tridax procumben* might promote human health by preventing bacterial pathogenesis.

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 *Tridax procumben* 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 methanolic extract of the *Tridax procumben* 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.

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INTRODUCTION

Plants are one of five big groups (kingdoms) of living things. They are autotrophic eukaryotes, which means they have complex cells. Trees, herbs, bushes, grasses, vines, ferns, mosses, and green algae are included in plant. The scientific study of plants, known as botany, has identified about 350,000 extant (living) species of plants. Plants help maintain gaseous balance in the air also prevent soil erosion. They help to reduce heat and prevent drying up of moisture. Thus they are environmental savvy. Plants like blue green algae and bacteria are also extensively used to fix nitrogen in the soil for agriculture (Ranga et al, 2015).

A large group of plants used in medicine or veterinary practice for therapeutic or prophylactic purposes. 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 as insects,fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total(Tap and sell et al, 2006). Although, there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties termed as medicinal plants (Samy et al, 2008).

Plants are the natural reservoir of many antimicrobial, anticancer agents, analgesics, anti-diarrheal as well as various therapeutic activities. Bangladeshi people have traditional medical practice as an integral part of their culture. A lot of medicinal plants are available for the treatment of various diseases. However, scientific studies have been conducted on only a relatively few medicinal plants, and then only to a superficial extent (Faysal, 2008). The use of plants as medicines predates written human history. Ethnobotany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant (Fabricant and Farnsworth, 2001)

There are hundreds of drugs and biologically active compounds developed from the traditional medicinal plants, a few of which are mentioned here; the antispasmodic agent vasicin isolated from Justicia a*dhatoda*, anticancer agents such as vincristine, vinblastine and D-tubocurarine isolated from *Catharanthus roseus* (Gurib-Fakim, 2006), antibacterial agents isolated from *Diospyros melanoxylon* (Mallavadhani et al, 1998), antimalarial agent isolated from *Sida acuta* (Karou et al., 2006), steroid and lancamarone with cardiotonic properties, lantamine with antipyretic and antispasmodic properties from *Lantana camara* (Ghisalberti, 2000), antimicrobial agents isolated from *Acorus calamus* (Chowdhury et al, 1993), antiviral, antibacterial and anti-inflammatory agents isolated from *Urtica dioica* (Harborne and Buxter, 1993), anticancer agents isolated from *Aloe vera*, *Allium sativum, Andrographis paniculata*, *Curcuma longa*, *Moringa oleifera*, *Phyllanthus amarus*, *Piper longum*, *Semecarpus anacardium*, *Tinospora cordifolia* and *Withanica somnifera* (Balachandran and Govindarajan 2005), promising and potent antimalarial drug artemisinin isolated from *Artemesia annua* (Dhingra et al, 2000).

1.1 PHYTOCHEMICALS

Phytochemistry is the study of phytochemicals produced in plants, describing the isolation, purification, identification, and structure of the large number of secondary metabolic compounds found in plants. Effect of extracted plant phytochemicals depends on:

- The nature of the plant material
- Its origin
- Degree of processing
- Moisture content
- Particle size (Tiwari and Kumer, 2011).

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 predationand others are pheromones used to attract insects for pollination (Meskin and Mark, 2002).Carbon dioxide gas deals with the photosynthesis process in plants in the presence of light energy. Photosynthesis and pentose pathway together pools the phosphate group present in the sugar molecules of plants which leads to glycolysis process and which is accounted for producing many of phytochemicals of plants, such as, shikimic acid, proteins, aliphatic and aromatic acids, mevalonic acids, fatty acids, flavanoids, terpinoids, steroids etc. There are lots of medicinal plants which contain a number of phytochemicals and those phytochemicals are used medicine purpose to treat various kinds of diseases. In the following table a list is shown of phytochemicals having medicinal values (Tiwari and Kumer, 2011).

1.2 NECESSITY OF STUDYING OF MEDICINAL PLANTS

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world.

- Medicinal plants have played an essential role in the development of human culture, for example religions and different ceremonies. (E.g. Dutura has long been associated with the worship of Shiva, the Indian god).
- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- Many food crops have medicinal effects, for example garlic.

Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species (Andrew, 2004)

- Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
- Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants.
- Plant resources (E.g. Angiosperm, Gymnosperm, Seedless vascular plants, Bryophytes) for new medicine.
- The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry (Andrew, 2004)
- With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today□s synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20% of the plant flora has been studied and 60%of synthetic medicines owe their origin to plants. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases.
- To identify alternative and complementary medicine.
- To reduce the toxicity of drug therapy especially toxicity reduction of synthetic and semi synthetic drugs.
- To find the lead compound diversification to treat various diseases (Andrew, 2004)

1.2.1 HISTORY OF TRADITIONAL HERBAL MEDICINE IN BANGLADESH

"Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating. Plant, animal and mineral based medicines, spiritual, therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being.By definition, 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines' .In many developing countries, a large (Allison et al, 2001).

The earliest mention of traditional medicine is found in *Rigveda,* the oldest repository of knowledge in this subcontinent. Later *Ayurveda,* developed from the *Vedic* concept of

life,became the important source of all systems of medical sciences. In course of time it became a part of culture and heritage of the people of the Indian subcontinent .

Traditional medicine involves the use of both material and non-material components. The material components invariably comprise parts or organs of plants and their products. They also consist of animal organs, minerals and other natural substances. The non-material components, which constitute important items of religious and spiritual medicines, include torture, charms, magic, incantations, religious verses, amulets and rituals like sacrifices, appeasement of evil spirits, etc.

Treatments in traditional medicine are carried out by internal and external application of medicaments, physical manipulation of various parts of the body, performing rituals, psychological treatment, and also by minor surgery. *Ayurvedic* medicinal preparations consist mainly of plant materials in the form of powders, semi-solid preparations, decoctions, elixirs and distillates. Many of them also contain inorganic chemical substances, minerals and animal products. Alcoholic extracts and alcoholic solutions of the ingredients, tinctures and elixirs are also frequently used in *Ayurvedi*c medicine **.**Whole plants or their powders or pastes or products and their extracts, infusions, decoctions and distillates constitute the major constituents of *Unani* medicine. Minerals, inorganic chemicals and animal products are also frequently used in preparing these medicines(Samy,Pushparaj & Gopalakrishnakone, 2008).

The desire to capture the wisdom of traditional healing systems has led to a resurgence of interest in herbal medicines (Tyler, 2000), particularly in Europe and North America, where herbal products have been incorporated into so-called ‘alternative’, ‘complementary’, ‘holistic’ or ‘integrative’ medical system.

The practice of Traditional medicine is deeply rooted in the cultural heritage of Bangladesh and constitutes an integral part of the culture of the people of this country. Different forms of Traditional medicines have been used in this country as an essential means of treatment of diseases and management of various health problems from time immemorial. The practice of traditional medicine in this country has flourished tremendously in the recent years along with that of modern medicine. As a result, even at this age of highly advanced allopathic medicine, a large majority (75-80%) of the population of this country, particularly in the rural and semi-urban areas, still prefer to use traditional medicine in the treatment of most of their diseases even though modern medical facilities may be available in the neighbourhood. However, the concept,

practice, type and method of application of traditional medicine vary widely among the different ethnic groups living in different parts of the country according to their culture, living standard, economic status, religious belief and level of education. Thus traditional medicine practice in Bangladesh includes both the most primitive forms of folk medicine (based on cultural habits, superstitions, religious customs and spiritualism) as well as the highly modernised Unani and Ayurvedic systems (based on scientific knowledge and modern pharmaceutical methods and technology). These various aspects of Traditional medicine practice in Bangladesh, their current official status (acceptability, recognition, etc.) in the country as a means of treatment, and their contribution to, and impact on, the overall health management programmes of the country are described and discussed in this paper supported by documentary evidences and scientific data (Ghani and Abdul, 1998).

Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. As only a certain percentage of plants are used in traditional medicines, it is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field.

Table1.2.1:Some Crude drugs used as medicine in bangladesh (Samy,Pushparaj,& Gopalakrishnakone, 2008:P.24)

Common name	Botanical name	Uses
Amla	*Emblica officinalis*	Vitamin - C, Cough, Diabetes, cold, Laxative, hyper acidity.
Ashok	*Saraca asoca*	Menstrual Pain, uterine, disorder, Deiabetes.
Bael / Bilva	*Aegle marmelous*	Diarrrhoea, Dysentry, Constipation.

Chiraita	*Swertia chiraita*	Skin Desease, Burning, censation, fever.
Kalmegh/ Bhui neem	*Andrographis paniculata*	Fever, weekness, release of gas.
Long peeper / Pippali	*Peeper longum*	Appetizer, enlarged spleen,
Sandal Wood	*Santalum album*	Skin disorder, Burning, sensation, Jaundice, Cough.
Satavari	*Asparagus racemosus*	Enhance lactation, general weekness, fatigue, cough.
Senna	*Casia augustifolia*	General debility tonic, aphrodisiac.
Tulsi	*Ocimum sanclum*	Cough, Cold, bronchitis,expectorand
Pippermint	*Mentha pipertia*	Digestive, Pain killer
Henna/Mehd	*Lawsennia iermis*	Burning, Steam, Anti Imflamatary
Gritkumari	*Aloe verra*	Laxative, Wound healing, Skin burns & care,Ulcer
Sada Bahar	*Vincea rosea*	Leaukamia, Hypotensiv, Antispasmodic , Atidot

Vringraj	*Eclipta alba*	Anti-inflamatory, Digestive, hairtonic
Neem	*Azardirchata indica*	Sdedative, analgesic, epilepsy, hypertensive
Anantamool/sariva	*Hemibi smus indicus*	Appetiser, Carminative, aphrodisiac, Astringent
Kantakari	*Solanum xanthocarpum*	Diuretic, Antiinflamatory, Appetiser, Stomachic
Shankhamul	*Geodorum denciflorum*	Antidiabetic

1.3 Plant metabolite

Metabolites are compounds synthesized by plants for both essential functions, such as growth and development (primary metabolites), and specific functions, such as pollinator attraction or defense against herbivory (secondary metabolites). Metabolites are organic compounds synthesized by organisms using enzyme-mediated chemical reactions called metabolic pathways. Primary metabolites have functions that are essential to growth and development and are therefore present in all plants.In contrast, secondary metabolites are variously distributed in the plant kingdom, and their functions are specific to the plants in which they are found. Secondary metabolites are often colored, fragrant, or flavorful compounds and they typically mediate the interaction of plants with other organisms. Such interactions include those of plant-pollinator, plant-pathogen, and plant-herbivore).

1.3.1 Primary metabolite

A plant produces primary metabolites that are involved in growth and metabolism. Primary metabolites comprise many different types of organic compounds, including, but not limited to, carbohydrates, lipids, proteins, and nucleic acids. They are found universally in the plant kingdom because they are the components or products of fundamental metabolic pathways or cycles such as glycolysis, the Krebs cycle, and the Calvin cycle. Because of the importance of these and other primary pathways in enabling a plant to synthesize, assimilate, and degrade organic compounds, primary metabolites are essential. Examples of primary metabolites include energy rich fuel molecules, such as sucrose and starch, structural components such as cellulose, informational molecules such as DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), and pigments, such as chlorophyll. In addition to having fundamental roles in plant growth and development, some primary metabolites are precursors (starting materials) for the synthesis of secondary metabolites.

1.3.2 Secondary metabolite

Secondary metabolites are those metabolites which are often produced in a phase of subsequent to growth, have no function in growth (although they may have survival function), are produced by certain restricted taxonomic groups of microorganisms, have unusual chemicals structures, and are often formed as mixtures of closely related members of a chemical family. The simplest definition of secondary products is that they are not generally included in standard metabolic charts. A metabolic intermediate or product, found as a differentiation product in restricted taxonomic groups, not essential to growth and the life of the producing organism, and biosynthesis from one or more general metabolites by a wider variety of pathways than is available in general metabolism. Secondary metabolites are not essential for growth and tend to be strain specific. They have a wide range of chemical structures and biological activities. They are derived by unique biosynthetic pathways from primary metabolites and intermediates (David and Wang, 2014)

Of the estimated 400,000 – 500,000 plant species around the globe, only a small percentage has been investigated phytochemically and the fraction subjected to biological or pharmacological screening is even lower. The ability to synthesize secondary metabolites has been selected

through the course of evolution in different plant lineage when such compounds address specific needs

- Floral scent volatiles and pigments have evolved to attract insect pollinators and thus enhance fertilization.
- To synthesize toxic chemical has evolved to ward off pathogens and herbivores or to suppress the growth of neighboring plants.
- Chemicals found in fruits prevent spoilage and act as signals (in the form of color, aroma, and flavor) of the presence of potential rewards (sugars, vitamins and flavor) for animals that eat the fruit and thereby help to disperse the seeds.
- Other chemicals serve cellular functions that are unique to the particular plant in which they occur (e.g. resistance to salt or drought)((David and Wang, 2014)

1.4 Overview of family

The Asteraceae or Compositae (commonly referred to as the aster, daisy, composite, or sunflower family) are an exceedingly large and widespread family of flowering plants (Angiospermae**).**

The family has more than 23,600 currently accepted species, spread across 1,620 genera (list) and 13 subfamilies.[citation needed] In terms of numbers of species, the Asteraceae are rivaled only by the Orchidaceae. (Which of the two families is actually larger is unclear, owing to uncertainty about exactly how many species exist in each family.) Many members have composite flowers in the form of flower heads (capitula or pseudanthia) surrounded by involucral bracts. When viewed from a distance, each capitulum may have the appearance of being a single flower. The name "Asteraceae" comes from Aster, the most prominent genus in the family, that derives from the Greek □στήρ, meaning star, and is connected with its inflorescence star form. "Compositae" is an older but still valid name which refers to the fact that the family is one of the few angiosperm ones to have composite flowers**.**

Most members of Asteraceae are herbaceous, but a significant number are also shrubs, vines, or trees. The family has a worldwide distribution, from the polar regions to the tropics, colonizing a wide variety of habitats. It is most common in the arid and semiarid regions of subtropical and lower temperate latitudes. The Asteraceae may represent as much as 10% of autochthonous flora in many regions of the world.

The Asteraceae are an economically important family, providing products such as cooking oils, lettuce, sunflower seeds, artichokes, sweetening agents, coffee substitutes and herbal teas. Several genera are of horticultural importance, including pot marigold, Calendula officinalis, Echinacea (cone flowers), various daisies, fleabane, chrysanthemums, dahlias, zinnias, and heleniums. Asteraceae are important in herbal medicine, including Grindelia, yarrow, and many others. A number of species are considered invasive, including, most notably in North America, dandelion, which was originally introduced by European settlers who used the young leaves as a salad green.

1.5.1 Some common names

Its common names include coat buttons and tridax daisy in English, jayanthi in Kannada, cadillo chisaca in Spanish, herbe caille in French, jayanti veda in Sanskrit, ghamra in Hindi, bishalya karani in Oriya, kambarmodi in Marathi, gaddi chemanthi in Telugu,vettukaaya poondu in Tamil, and kotobukigiku in Japanese,

1.5.2BOARD OF TAXONOMICAL CLASSIFICATION

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Asteridae

Order: Asterales

Family: Asteraceae

Genus: Tridax L.

Species: Tridax procumbens L.

1.6 ORIGIN AND DISTRIBUTION

Tridax procumbens 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, Tridax procumbens 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)

1.7 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. Tridax procumbens 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)

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)

CONSIDRATION

All parts of the Tridax procumbens 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 Tridax procumbens to garden and keep any eye on where it goes to prevent its invasion into natural habitats (Bonnie Singleton, 2015).

1.8 PLANT PARTS

1.8.1 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).

Leaves

Figure 1.1:Leaves of *Tridax Procumben* (Navie, 2004).

1.8.2 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)

1.8.2 Fruits

Fruit an achene, narrowly obconic to cylindrical, tapering to a blunt base, 1.5-2.5 mm long, 0.5-1.4 mm in diameter (not including pappus). Blackish-brown, pilose, with pale ascending hairs, giving achene grayish-brown appearance. Pappus persistent, one row of ca. 20 straw-colored scalelike bristles, copiously long-plumose. Ray achene pappus 0.5-2.5 mm long, disc achene pappus alternately long and short, 3.5-6 mm long. Scar basal, a raised +/- elliptic pad, semi-transparent, striate. Apex horizontal, round, blackish, rough, with central style base; style base reddish-brown, cylindrical and hollow, or inconspicuous. Embryo linear; endosperm absent.

1.9 CHEMICAL CONSTITUENTS

A new flavonoid (procumbenetin), isolated from the aerial parts of Tridax procumbens, has been characterised as 3,6-dimethoxy-5,7,2',3',4'-pentahydroxyflavone 7-O-β-D-gluco- pyranoside (1) on the basis of spectroscopic techniques and by chemical means. Tridax procumbens; Flavonoids Plant. Uses in traditional medicine. Commonly used in Indian traditional medicine as anticoagulant, hair tonic, antifungal and insect repellent, in bronchial catarrh, diarrhoea, dysentery, and wound healing. Previously isolated constituents. Alkyl esters, sterols,[2] pentacyclic triterpenes,[2][3] fatty acids[4] and polysaccharides.[5] New isolated constituent. 3,6-Dimethoxy-5,7,2',3',4'-pentahydroxyflavone 7-O-β- D-glucopyranoside (1), named procumbetin Žyield: 0.016% on dried basis.

1.10 Uses

1.10.1 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 *Tridax procumbens* 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 *Tridax procumbens* 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 *Tridax procumbens* 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

Other eilments treated in tropical Africa with *Tridax Procumbens* 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. *Tridax procumben* can become a weed in pastures; it is not eaten by livestock and is reported to be poisonous, especially for goats. The bark is used as fish poison and for tanning leather. The roots and the bark are reported to be used for tattooing. *Tridax procumben* is widely appreciated as a garden ornamental and bee forage (Protabase , 2015)

1.11 NUTRITIONAL FACTS

Table 1.11.1: Mineral composition of *Tridax procumben* leaf (Abdulwaliyu et al, 2013:p.4)

Elements	**Leaf (mg/100g)**
k	23.46
Zn	0.17
Cu	0.47
Na	1106.56
Mg	49.02
Fe	3.6
Ca	333.32

Table 1.11.2: Vitamin composition of *Tridax procumben* leaf (Abdulwaliyu et al, 2013:p.4)

Vitamins	**Leaf**
β-Carotene (IU)	50.37
Vitamin C (mg/L)	9.09
Vitamin E (IU)	31.50

Table 1.11.3: Antinutrient composition of *Tridax procumben* 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 1.11.4: Proximate analysis of *Tridax procumben* leaf (Abdulwaliyu et al, 2013:p.3)

Parameters	**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)

Table 1.11.5: Essential oil constituents of *Tridax procumben* (Isiaka A et al, 2010:p.214)

Compounds a	LRI b	%	Compounds a	LRI b	%
(E)-2-hexenal	854	3.3	germacrene D	1483	5.5
tricyclene	926	t	(E)-β-ionone	1487	t
benzaldehyde	961	t	bicyclogermacrene	1497	t
α-phellandrene	1008	3.7	α-selinene	1498	5.4
α-terpinene	1021	t	n-pentadecane	1500	t

p-cymene	1029	t	α-bulnesene	1506	1.0
limonene	1034	5.2	δ-cadinene	1525	t
1,8-cineole	1037	39.8	caryophyllene oxide	1583	12.7
β-elemene	1394	t	n-hexadecane	1600	t
β-caryophyllene	1421	19.1	humulene epoxide II	1609	t
(E)-geranyl	1457	t	tetradecanal	1614	t
acetone			α-cadinol	1656	4.2
α-humulene	1458	t			
E)-β-farnesene	1461	t			Total= 99.9 %

2.1 PHYTOCHEMICAL REVIEW

2.1.1 NUTRITIVE COMPOSITION

The leaves of *Tridax procumben* is a good source of many mineral content.In a survay,The analyses performed by Energy Dispersive X-Ray Fluorescence (EDXRF) revealed the following mineral elements: k, Zn, Cd, Na ,Mg ,Fe ,Ca. And the vitamin elements are β-Carotene (IU), Vitamin C (mg/L), Vitamin E (IU). The results obtained from mineral analysis on the leaf of *Tridax procumben* revealed low content of sodium and high contents of calcium, potassium, iron and magnesium. The result showed that the magnesium, potassium and iron contents of the

leaf and flower of *Tridax procumben* were high compared to magnesium (19.16 mg/kg), iron (3.80 mg/kg) and potassium (0.6 mg/kg) contents of shear butter leaf (Abidemi et al, 2009)

Tridax procumben leaves have also been found to contain 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 (Fuzellier et al,1982; Abo et al,1999). Phytochemical screening of the leaves and roots of *Tridax procumben* revealed the presence of alkaloids, carbohydrates, tannins, saponins, phenols, flavonoids, anthraquinones and cardiac glycosidess (Elmahmood and Amey, 2007). 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.The quantitatively significant constituents of the leaf oil of *Tridax procumben* Roxb.,(Fabaceae) were 1, 8-cineole (39.8%),-caryophyllene (19.1%) and caryophyllene oxide (12.7%). Limonene (5.2%),germacrene D (5.5%) and _-selinene (5.4%) constituted the other significant compounds present in the oil.(Isiaka et al, 2010).

The plant is a source of chrysoeriol,quercetin, 5,7,4'-trihydroflavanone, kaempferol-3-O-D-glucopyranoside,kaempferol-3-O-D-glucopyranosyl-(1->6)-_-D-glucopyranoside,17-hydrotetratriacontane, n-dotriacontanol, n-triacontanol, palmitic acid ceryl ester,stearic acid, palmitic acid. There is only a report on the constituents of its volatile oil.(Isiaka et al, 2010).

2.2 PHARMACOLOGICAL REVIEW

Tridax procumben leaves. has been ethnobotanically used extensively in traditional medicines for the treatment of a variety of diseases such as skin problems, arthritis, HBP (high blood pressure), and laxative or purgative.It is also used in boils, wound, eye, urinary and gastrointestinal tract infections, diarrhoea and scarlet fever (Benjamin and Lamikanra, 1981).Recent reports have credited the use of *Tridax procumben* in the successful treatment of haemorroids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes (Makinde et al, 2007).

2.2.1ANTIMICROBIAL ACTIVITY

The antimicrobial activities of ethanolic leaf extract of *Tridax procumben* against five bacteria (Staphylococcus aureus, Staphylococcus albus, Klebsiella pneumonia, Pseudomonas aeruginosa and Proteus mirabilis) and six fungi (Rhizopus spp, Penicillum oxalicum, Aspergillus tamari, Aspergillus niger, Fusarium oxysporum and Fusarium vacitilus) were examined using agar diffusion method. The result revealed that the ethanolic leaf extract had high inhibitory activity against S. albus, P. mirabilis and all the fungi tested. The eight antibacterial drugs produced varied reactions on the microbes with streptomycin having the highest inhibitory activity against all the bacteria(Odunbaku and Ilusanya, 2011).

Crude methanol extracts from leaves of *Tridax procumben* , fistula and tora were investigated for their antifungal activities on three pathogenic fungi (Microsporum gypseum, Trichophyton rubrum and Penicillium marneffei). Among 3 species, was the most effective leaf extract against T. rubrum and M. gypseum with the 50% inhibition concentration (IC50) of hyphal growth at 0.5 and 0.8 mg/ml, respectively, whereas the extract of C. fistula was the most potent inhibitor of P. marneffei with the IC50 of 0.9 mg/ml. In addition, it was found that all three Cassia leaf extracts also affected M. gypseum conidial germination. Microscopic observation revealed that the treated hyphae and macroconidia with leaf extracts were shrunken and collapsed, which might be due to cell fluid leakage.(Souwalak Phongpaichit et al, 2004)

2.2.2 ANTIALLERGIC ACTIVITY

Leaves of *Tridax procumben* are ethnomedically claimed as anti-asthmatic. In the current study it is aimed to investigate the anti-allergic activities of hydro-methanolic extract of *Tridax procumben* and its constituents rhein and kaempferol on triple antigen/sheep serum-induced mast-cell degranulation in rats. Antiallergic activity of hydroalcoholic extract of *Tridax procumben* with its two components rhein and kaempferol was evaluated using *in vivo* mast cell stabilization assay. The hydroalcoholic extract of *Tridax procumben* significantly inhibited mast

cell degranulation at 200 mg/kg dose. Both chemical constituents rhein and kaempferol also showed potent (>76%) inhibition of mast-cell degranulation at 5 mg/kg. Extract and rhein inhibited LOX enzyme with IC_{50} values of 90.2 and 3.9 µg/mL, respectively, whereas kaempferol was inactive.(Baljinder Singh et al, 2012,The hydroalcoholic extract of *Tridax procumben* leaves and its major compound rhein exhibits antiallergic activity *via* mast cell stabilization and lipoxygenase inhibition).

2.2.3 ANTIOXIDENT ACTIVITY

Aqueous extract of *Tridax procumben* showed strong antioxidant activity and high total phenolic content. The antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'– azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods. The strongest antioxidant activities of aqueous extract of *Tridax procumben* were 22.11 ± 0.324 mg gallic/g extract and 214.99 ± 17.279 mg trolox/g extract when determined by DPPH and ABTS assay, respectively. Moreover, the highest total phenolic content of 70.90 ± 1.048 mg gallic/g extract was measured from the aqueous extract of *Tridax procumben* . Therefore, the biological activities of these plants observed in this study will be useful to develop the plant extracts for primary treatment of diseases as new therapeutic agents.(Wipawan et al, 2012, Total phenolic contents, antibacterial and antioxidant activities of some Thai medicinal plant extracts).

Methanolic extract of the leaves of *Tridax procumben* was assayed for determining the antioxidant compounds present in this plant. Estimation of total phenols, Vitamin-C, Vitamin-A, flavonoids, carotenoidsand anthra quinones was done. DPPH radical scavenging activity of the methanolic extract of leaves was also tested against a synthetic antioxidant, Butylated hydroxytoluene (BHT). It is evident from the results that the plant *Tridax procumben* possesses strong antioxidant activity, as it contains good quantity of antioxidant compounds like phenols, Vitamin-C,Vitamin- A, flavonoids, carotenoids and anthraquinone. In addition, it has very high DPPH radical scavenging activity in contrast to the synthetic antioxidant compound, BHT. (Saheli Chatterjee et al .2013. Study of Antioxidant Activity and Immune Stimulating Potency of the Ethnomedicinal Plant, *Tridax procumbens*

2.2.4 ANTIDIABETIC ACTIVITY

The methanol extract of leaves of *Tridax procumben*, which showed potent α-glucosidase inhibitory activity (IC_{50}, 63.75±12.81 µg/ml), was fractionated. The α-glucosidase inhibitory effect of the crude extract was far better than the standard clinically used drug, acarbose (IC_{50}, 107.31±12.31 µg/ml). A subsequent fractionation of the crude extract was made using solvents of ascending polarity (petroleum ether, chloroform, ethyl acetate, butanol and water). The ethyl acetate (IC_{50}, 2.95±0.47 µg/ml) and butanol (IC_{50}, 25.80±2.01 µg/ml) fractions which contained predominantly kaempferol (56.7±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 o *Tridax procumben* f 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 *Tridax procumben* leaves: Identification through α-glucosidase inhibition studies).

2.2.5 ANTICANCER ACTIVITY

The present study reports the effects of *Tridax procumben* extract on the metabolism of polyamines resulting from the proliferation of leukaemia cells (L1210). The results established that the inhibition of cell proliferation was significantly increased with the concentration of extract from 28 to 32.80 % after 72 h. The percentage of cells viability changed significantly from 9.72 to 80 % when cells are treated with extract alone, in combination with DFMO or putrescine. The levels of the intracellular yield of putrescine, spermidine and spermine were also reduced by the extract compared to the control. The DFMO-extract complex enhanced the inhibition of the production polyamines up to 95 %. In opposite, the *Tridax procumben*-putrescine complex stimulated significantly its biosynthesis of polyamines. A significant reduction of the level of protein after 72 h of treatment was observed. This result corroborated with the reduction of polyamines resulting from inhibition cellproliferation.(Pieme et al, 2009, In vitro effects of extract of *Tridax procumben* on the polyamines produced by Leukaemia cells).

2.2.6 ABORTIFACIENT PROPERTIES

This study has provided evidence to the age-long claim of *Tridax procumben* leaves in "washing the uterus". The abortifacient properties were most pronounced at 500 and 1000 mg/kg body weight of the extract and were similar to the animals treated with 2.85 mg/kg body weight of mifepristone. Hormonal influence, changes in implantation site, estrogenicity and uterogenicity are suggested as possible mechanism of abortifacient activity of aqueous extract of *Tridax procumben* leaves. Overall, the extract may be used as an abortifacient especially at 500 and 1000 mg/kg body weight and therefore not safe for consumption as oral remedy during pregnancy) (Yakubu et al, 2010, Abortifacient Potential of Aqueous Extract of *Tridax procumben* Leaves in Rats**).**

2.2.7 BIOFILM FORMATION

Five bioactive fractions were detected and chemically characterized, using high-resolution mass spectrometry (qTOF-MS/MS). Six compounds from four fractions could be characterized as kaempferol, kaempferol-O-diglucoside, kaempferol-O-glucoside, quercetin-O-glucoside, rhein, and danthron. In the Salmonella/microsome assay CaRP showed weak mutagenicity (M I < 3) only in strain TA98, pointing to a frameshift mutation activity. These results indicate that *Tridax procumben* leaf extract contains a minimum of 7 compounds with antimicrobial activity and that these together or as single substance are active in preventing formation of bacterial biofilm, indicating potential for therapeutic applications.(Samuel et al, 2012, Bioguided Fractionation Shows *Tridax procumbens* Extract to Inhibit Staphylococcus epidermidis and Pseudomonas aeruginosa Growth and Biofilm Formation).

2.2.8 LARVICIDAL EFFECT

The larvicidal activities of aqueous and ethanolic leaf and stem extracts of *Tridax procumben* were evaluated in static bioassays, on fourth instar larvae of Anopheles gambiae, Culex quinquefasciatus and Aedes aegypti, at extract concentrations of 0.15, 0.30, 0.45, 0.60 and 0.75% w/v, for 72 hours. Mortality of larvae exposed to the different extracts increased with increase in extract concentration and time of exposure. This study revealed a differential potency of the

extracts used and a difference in susceptibility of larvae to the extracts as evident by the 72hLC50 values obtained. The leaf extract proved to be more lethal to the larvae than the stem extract as judged by the 72hLC50 values obtained both for the aqueous as well as the ethanolic extracts assayed. Phytochemical screening of the plant parts investigated revealed the presence of some plant metabolites, which have been reported in separate studies to be lethal to mosquito larvae. Results obtained from this study suggest that the leaf and stem extracts of *Tridax procumbens* possess a promising larvicidal potential which can be exploited in mosquito vector control.(Ubulom et al, 2013, Larvicidal effect of aqueous and ethanolic extracts of *Tridax procumbens* on Anopheles gambiae, Culex quinquefasciatus and Aedes aegypti).

2.2.9 ACARICIDAL ACTIVITY

The leaves of *Tridax procumben* has a Acaricidal activity against Rhipicephalus annulatus (Reghu rabindran et al,2010).

2.2.10 ANTHELMINTIC ACTIVITY

Tridax procumben leaf was extracted with 70% acetone and fractions were obtained by solvent: solvent group separation procedures. The acetone extract and the fractions were tested by egg hatch assay (EHA) and larval development and viability assay to assess relative bioactivity against *H. contortus* eggs and larvae The extracts inhibited egg hatchability and killed infective larvae of *H. contortus* in a concentration-dependent manner. The best-fit LC_{50} values were 0.562, 0.243, 0.490, 0.314, and 0.119mg/mL for the acetone extract, chloroform, hexane, butanol and 35% water in methanol fractions, respectively, when tested against nematode eggs. The best-fit LC_{50} values were 0.191, 0.505, 1.444, 0.306, and 0.040mg/mL for acetone extract, chloroform, hexane, butanol and 35% water in methanol fractions, respectively, when tested against larvae. The 35% water in methanol fraction was the most active against the larvae and eggs of *H. contortus* demonstrating the lowest LC_{50} values. This study demonstrates that the leaf extracts of *Tridax procumben* have anthelmintic activity; therefore it could find application in the

control of helminths in livestock.(Ademola, and Eloff, 2011, Ovicidal and larvicidal activity of *Tridax procumben* leaf acetone extract and fractions on *Haemonchus contortus*: *In vitro* studies).

2.2.11 ANTI HIV ACTIVITY

The Petroleum Ether and ethanol extracts of *Tridax procumben* are reported to have a potent inhibitory activity against opportunistic HIV patients' infections(Crockett et al., 1992).The Petroleum Ether and ethanol extractsof *Tridax procumben* are reported to have a potent inhibitory activity against *Chrysomya megacephale* (Kumarasinghe et al, 2002).

2.2.12 IMMUNO STIMULATING AGENT

The plant *Tridax procumben* has strong immune-modulating orimmune-stimulating potency, as evidenced by a steep rise in the total count of leucocytes with concomitant increasing in granulocyte: a granulocyte ratio as well as remarkable increase in the total number of peritoneal macrophages in the rabbits treated with the aqueous extract of leaves of *Tridax procumben*. Thus, the plant *Tridax procumben* may extensively be used in therapeutic medicines as a resource of natural and immune stimulating agent. (Saheli Chatterjee et al .2013**.** Study of Antioxidant Activity and Immune Stimulating Potency of the Ethnomedicinal Plant, *Tridax procumben*(L.) Roxb.)

2.2.13 SYNERGISTIC EFFECT

The synergism between the extract and synthetic drugs produced higher inhibitory activity against the organisms. The broth of cultured bacteria and fungi were spread on nutrient and potato dextrose agar using flooding method. A well sterilized cork borer (5mm) was used to make 'wells' in the media. The mixture of different antibiotics (0.4mg/ml) /antifungal drugs (0.4mg/ml) and plant extract were poured into the punched wells. The plates were incubated for 24-36 hours at 370 C and the zones of inhibition were measured and recorded. (Odunbaku, 2011. Synergistic Effect of Ethanol Leaf Extract of *Tridax procumbens* and Antimicrobial Drugs on Some Pathogenic Microbes)

The synergism effect of plant extracts and antibiotics drugs from this study supports the use of drug combinations in treating diseases because some organism are now known to be resistance to

antibiotics(Ajaiyeoba, Onocha and Olarenwaju, 2000. Invitro Anthelmintic properties of Buchholzia coriacea and Gynandropsis gynandra. J. Pharmaceut. Biol).

2.2.14 MIMIC PLANT GROWTH HORMONE

The efficacy of crude extracts of *Tridax procumben* in the improvement of vegetative and reproductive growth in *Tridax rocumben* was investigated. Fresh leaves of *Tridax procumben* were blended with a homogenizer in 1 litre of distilled water. The resultant green paste was filtered under suction. Different concentrations (75%, 50%, 40%, 30%, 25%, 12%, 10%, and 5%) were prepared from the 100% crude extract. Seeds of were presoaked in these different concentrations including a control (0%) and planted out after 24 hours. Results obtained showed that seedling height, leaf area, dry weight and leaf area ratio were promoted and enhanced by presoaking seeds in the extract. At the end of the experimental period (six weeks), seedling height in 75% and 100% treatments were 109 ± 16.12 cm and 117 ± 19.32 cm, leaf area 128 ± 17.91 cm^2 and 125 ± 18.12 cm^2, dry weight 7.48 kg and 7.0 kg respectively. Seedlings raised from seeds presoaked in water (control) however, flowered earlier (8 weeks) than the treatments (10 weeks in 75% and 100%). Presoaking seeds in crude extracts of *Tridax procumben* before planting is recommended for optimum production of the leafy vegetable. The procedure is cheap and easily implementable by resource-poor farmers who are the main growers. *(*Ikechukwu, 2014. Crude Extracts of *Tridax procumben* (L.) Roxb. Mimics Plant Growth Hormones in Promotion of Vegetative and Reproductive Growth.)

2.2.15 REDUCING PHOTO-INDUCED DAMAGE ON DNA AT THE MITOCHONDRIAL LEVEL

Chronic repetitive exposure to UVA radiation induces mtDNA deletions (missing fragments in circular mtDNA) in human dermal fibroblasts. The main consequences of mtDNA deletions in dermal fibroblasts are improper mitochondria functioning and reduction of cell metabolism, which results in accelerated skin aging. Cassia Alata acts on the deep effects of UV radiation on the skin, by reducing the photo induced damages on DNA at the nuclear and mitochondrial levels, but also by acting on the visible signs of photo-aging such as the prevention of micro relief alteration due to sun exposure. This auto regulating protective activity against the chronic harmful effects of the sun helps the cells to keep their “youth”. Specifically selected for its high

amount of K30S (Kaempferol-3-0-Sophoroside). K03S is over expressed in the sun-exposed leaves, as a natural protective system of the plant against UV radiations. It is fixed in and around the cell nucleus of the plants (Rhonda and Allison.2009).

2.2.16 ANTI-CORROSIVE EFFECT

The inhibition effect of *Tridax procumben* leaves extract on corrosion of mild steel in 1N HCl was investigated through mass loss measurements with various time and temperature. The observed result indicated that the corrosion inhibition efficiency and degree of surface coverage were increased with increase of inhibitor concentration and temperature. The thermodynamic parameters (E_a, ΔH_{ads}, ΔG_{ads}, ΔS_{ads}) were evaluated for corrosion inhibition process which suggests that the adsorption is endothermic, spontaneous and chemisorptions and also the inhibitor follows Langmuir adsorption isotherm. The protective film formed on metal surface was analyzed using spectroscopic studies viz, UV, FT-IR and EDX techniques. (*Petchiammal A.p et al.2013.*Anti-corrosive effect of *Tridax procumben* leaves extract on Mild steel in 1.0N Hydrochloric acid).

2.2.17 WOUND HEALING ACTIVITY

The ethanol extracts of leaves of *Tridax procumben* were investigated on excision wound model in Rats by, the leaf extract accelerated the wound healing potential by reducing the epithelial isationperiod,prevent high risk of sepsis and prolongation of inflammatory phase.(Midawa, 2010. Cutaneous wound healing activity of the ethanolic extracts of the leaf of *Tridax procumben).*

2.2.18 ANTI INFLAMMATORY ACTIVITY

Kaempferol-3-O-gentiobioside(K3G) flavoniod glycoside isolated from *Tridax procumben* leaves have anti-inflammatory activity (Moriyama et al, 2001,Antiinflammatory activity of Heat-treated *Tridax procumben* Leaf extract and its flavonoid glycoside).

2.2.19 HEPATOPROTECTIVE ACTIVITY

Aqueous extract of the leaves of *Tridax procumben* has hepatoprotective activity (.Effraim KD et al.1999, Antihepatotoxic activity of aqueous extract of *] Tridax procumben* (Linn) leaves against carbon tetrachloride induced liver damage in rats.) *Tridax procumbent* petals havehepatoprotective effect by decreasing the levels of Serum aspartate aminotransferase and alanine aminotransferase in carbon tetrachloride (CCl4) –induced hepatotoxicity in rats.(Wegwu et al, 2005, Anti-Oxidant Protective Effects of *Tridax procumben* in Rats Exposed to Carbon Tetrachloride. J Appl Sci Environ.)

2.2.20 ANTI-CRYPTOCOCCUS ACTIVITY

Combination of ethanolic extracts of leaves of *Tridax procumben* and *Ocimum sanctum* showed anti-Cryptococcus activity (Ranganathan and Balajeen, 2000. Anti-cryptococcus activity of combination of extracts of *Tridax procumben* and *Ocimum sanctum*)

2.2.21 INSECTICIDAL ACTIVITY

Hexane extract of *Tridax procumben* fruits cause high lethality and toxic to control insect pests. Cut down the glycogen, protein DNA, RNA amino acids and lipid content cause physiological imbalance in *C.chinensis* leads to death(Ravi Kant upadhyay et al,2011,Toxic effects of solvent and aqueous extracts of *Tridax procumben* against bio-molecules and enzymaticparameters of *Callosobruchuschinensis* L).

2.2.22 BRONCHORELAXANT EFFECT

Aqueous-ethanolic extract of *Tridax procumben* produce relaxation of tracheal smooth muscles exhibits broncho relaxant effect(Ouédraogo et al, 2013, Evaluation of the Bronchorelaxant, Genotoxic and Antigenotoxic Effects of *Tridax procumben* L. Evidence-Based Complementary and Alternative Medicine).

2.2.23 ANTIGENOTOXIC EFFECT

Genotoxic studies are useful to identify the level of DNA damage induced by xenobiotics. The antigenotoxic potential of was evaluated by aqueous-ethanolic extract of *Tridax procumben* did not induce DNA migration(Ouédraogo et al, 2013, Evaluation of the Broncho relaxant,

Genotoxic and Antigenotoxic Effects. Evidence-Based Complementary and Alternative Medicine).

2.2.24 ANALGESIC ACTIVITY

Kaempferol 3-O-sophoroside was isolated from the leaves of *Tridax procumben* exhibited analgesic activity (Owoyale J A et al, 2005 ,Antifungal and Antibacterial Activities of an Alcoholic Extract of *Tridax procumben* Leaves).The hexane, chloroform and ethyl acetate extract of the leaves of *Tridax procumben* exhibites analgesic activity(Irene et al, 2002, Bioactivity studies on leaf extracts).

2.2.25 CHOLERETIC ACTIVITY

The Choleretic activity of *Tridax procumben* extract proved to be better than that of hydroxycyclohexenyl-butyrate (Hebucol) asyntheyic, choleretic in rats (Assane et al, 1993,Choleretic effects of Linn in Rats)

3.1 PLANT SELECTION

Throughout medical history, plant products have been shown to be valuable sources of novel compound for discovery of drugs. Topical forest are on of the most diverse and endangered habitats on earth. They have also been portrayed as a source of future pharmaceuticals, yet finding useful compounds can be both scientifically and politically challenging. Over the past decade the potential value for medicinal compound derives from plants ,microorganism , animal has been proposed as tangible benefit of biodiversity and therefore a basis for promoting its prevention. Ecological theories of plant defense can increase the probability of discovering with activity in bioassy against human disease target.

There are thousands of medicinal plants in Bangladesh and in this Indian subcontinent. Among these plants it was not easy to select a few plants for the research purpose. The selection of plant greatly affects the research work if there is carelessness takes place. Plant secondary metabolites

often accumulate in specific plant parts. Thus, unless it is already known which parts contain the highest level of the compounds of interest, it is important to collect multiple plant parts, or the whole plant to ensure the extracts prepared representative of the range of secondary metabolites. For drug discovery from plants, sample may be selected using a number following criteria by which the research work will run smoothly.

From the literature review it is seen that there is a lot of work on the plant *Tridax procumben* about the pharmacological activity of the plant. But there is a least of work has been found about chemical investigation of this plant, especially about the leaves of this plant. So I got a chance to select the leaves of *Tridax procumben* for my research work to see whether the leaves have antioxidant and anti-diabetic and antimicrobial activity or not.

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3.2 PLANT COLLECTION

After selection of plant it is must to collect the plant parts for the research purpose. But the plant *Tridax procumben* is not available throughout the bangladesh . The plant sample was collected from Jhalokathi District, under Barisal division on 9th October, 2015.

3.2.1 DRYING OF PLANT SAMPLE

After the collection of sample it needs to be dried to make the sample extract. In general the plant material should be dried at temperature below 30 degree C to avoid the decomposition of thermo labile compounds. So sun drying can be very effective but drawback is sometimes water molecules are absorbed by the sample and hence fungus growth can affect the phytochemical study. The seeds along with the testa were dried in the sun light thus chemical decomposition can not take place.

3.2.2 GRINDING OF DRIED SAMPLE

Small amount of plant material can be milled using grinder or blender. But if the sample is in high amount then it is easier to get powdered sample by grinding from a spice mill. Grinding improves the efficiency of extraction by increasing surface area. It also decreases the amount of solvent required for the extraction. The dried samples were ground to coarse powder with a mechanical grinder (Blender) and powdered samples were kept in clean closed containers

pending extraction. During grinding of samples, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign matter deposited on the grinder.

3.3 MACERATION OF DRIED POWDERED SAMPLE

3.3.1 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

3.3.2 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.

3.4 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

3.5 SAMPLE CONCENTRATION BY ROTARY EVAPORATION TECHNIQUE

3.5.1 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).

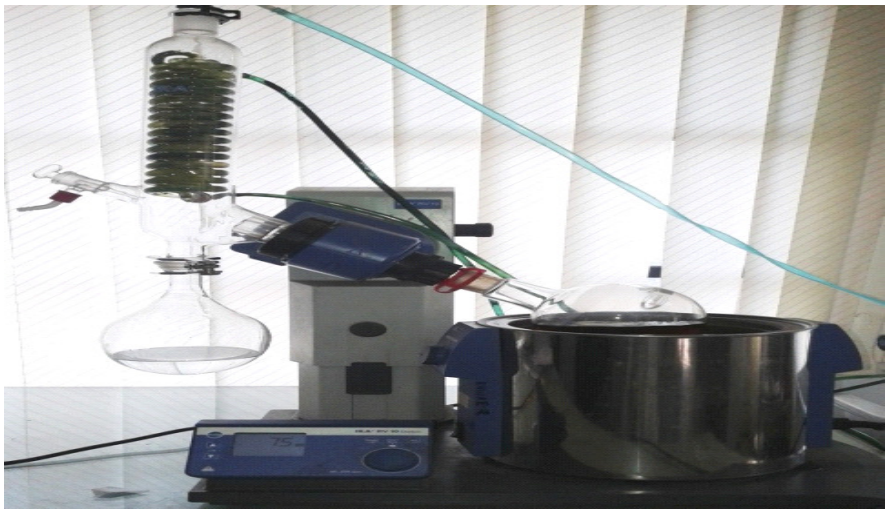


Figure 3.5.1: Rotary Evaporato

Figure 3.5.1: Rotary machine of east west university

3.5.2 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

3.5.3 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 130 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.

3.6 SAMPLE CONCENTRATION BY VACUUM LIQUID CHROMATOGRAPHY(VLC) TECHNIQUE

3.6.1 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.

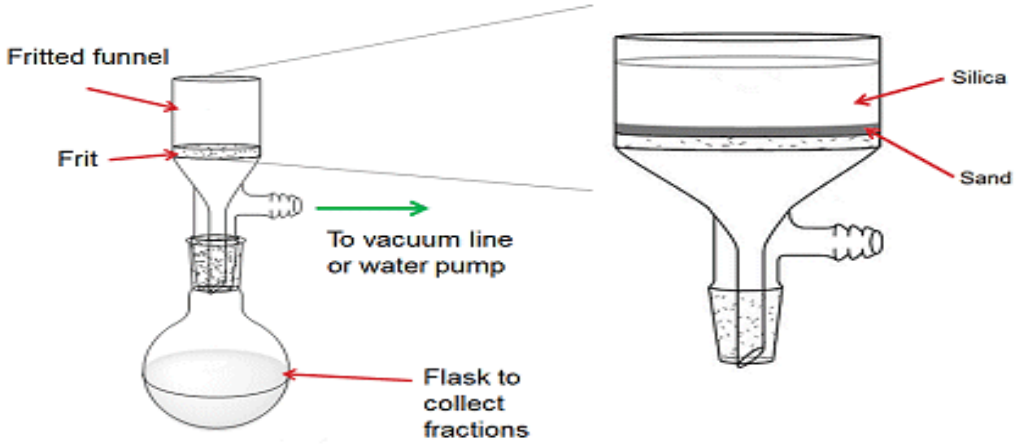


Figure 3.6.1: Vacuum Liquid Chromatography

3.6.2 Apparatus

- VLC chamber.
- Filter paper

3.6.3 Reagents

- Silica gel

  Methanol
- Chloroform
- Dichloromethane.

  Butanol
- Ethanol

3.6.4 Procedure

The 500gm Methanol extract of *Tridax procumben* 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. Dichloromethane, 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.

3.7 Equipments and other necessary tools

During the extraction procedure and for various phytochemical tests many equipments and materials were used. Some of them are TLC plate, TLC tank, scale, pencil, TLC plate cutter, capillary tube, mortar and pestle, laminar air flow cabinet, loop, burner, micropipette tip, petri dishes, glass rod, cotton, filter paper, funnel, hot plate, centrifugal machine, autoclave, glassware washers, stirrer, UV spectroscopy, knife, ephedrine tube, Whatman□s filter paper, paper disc, incubator, vortex machine, PH meter. analytical balance, beaker (in various size), pipette, micro-pipette, rotary evaporator, hot air oven, dryer, storage cabinet, spatula, test tube, volumetric flask, conical flask, test tube holder, test tube rack, aluminum foil paper, scotch tape, refrigerator, water bath, electronic shaker, ultra violate lamp, mask, gloves, lab coat, sprayer, reagent bottle.

3.8 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)

3.9 Solvents for experiments

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

4.1 THIN LAYER CHROMATOGRAPHY(TLC)

4.1.1 Principle

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase. The solvent or solvent system that runs on the stationery phase by capillary action and conducts the separation, this is known as the mobile phase.Once the sample has been spotted on the plate and the mobile phase run through it, the different components of the mixture separate differently owing to their relative affinities for the stationery and mobile phases. Heavier components or the ones more attracted to the stationery phase remain at the bottom while components that are light and more soluble in the mobile phase travel up with it. The relative separation of the components can be studied by calculating the Retardation Factor (Rf), which is the ratio of the distance of migration of a particular substance to the distance of migration of the solvent front.

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. The goal of TLC is to obtain well defined, well separated spots.

4.1.2 Materials Required

- Silica coated TLC plate
- TLC tank
- Spotting capillary tubes
- Tweezers
- Pipette
- Pipette filter
- Test tubes
- Solvents
- UV lamp

4.1.3 Reagents

- Benzene
- Ethanol
- Ammonium Hydroxide
- Chloroform
- Ethyl Acetate
- Formic Acid
- Water
- Di-chloromethane
- Butanol
- Methanol

The experiment was conducted on three solvent systems, first one was non-polar, the second one was intermediate polar and the third one was polar. The compositions of the three solvent systems are as follows:

Table 4.1.4: The composition of various solvent systems for TLC

		Intermediate polar Basic Solvent		Polar Basic Solvent
Benzene 9ml		Chloroform 5ml		Ethyl acetate 8ml
Ethanol 1ml		Ethyl acetate 4ml		Ethanol 1.2ml
AlOH 0.1ml		Formic acid 1ml		Water 0.8ml

4.1.5 Procedure

- Using a pencil the baseline and the solvent front line was drawn on the TLC plate and the plate was labeled for the individual spots.
- The fraction of methanolic extract after VLC and Column Chromatography was spotted on TLC plate and the plate was dried completely in the air.
- In a TLC tank the solvent system was added. A strip of filter paper was inserted into the tank so that its bottom touched the solvent. The lid of the tank was closed and left to rest for a few minutes so that the solvent system could travel up the filter paper and saturate the chamber.
- Using a pair of tweezers the TLC plates were placed in the chamber carefully so that the baseline did not touch the solvent
- The plate was left in the tank so that the solvent system could run up the plate by capillary action and develop the spots.
- The plate was removed from the tank using a pair of tweezers once the solvent had reached reached the solvent line. The plate was then allowed to dry completely.

- Three types of solvent system were used based on difference in polarity for the detection of different compounds.
- The developed plate was then viewed under UV light for the detection of bands and spots.

4.1.6 Acid Charing of TLC plates

4.1.6.1 Materials

- Tweezers
- Conc. Sulfuric acid
- Distilled water
- Hot plate
- Petri dish

4.1.6.2 Procedure

- 9 ml of distilled water was added to 1 ml of concentrated sulfuric acid to produce a 10% solution of sulfuric acid which was taken in a petri dish.
- The TLC plate was dipped in this solution using tweezers with the silica face down.
- The plate was left in the open for 10 minutes to allow for drying.
- A hot plate was heated to about 90 degree C and the plates were heated until the spots developed.

4.2 CHARRING PROCESS OF TLC PLATE

4.2.1 Concentrated H2SO4 (98%)

1 ml concentrated H2SO4 (98%) is added to 9 ml distilled water. And TLC plate is sprayed with this reagent for 1 minute, dried and heated for spots visualization (Brand-Willams, W., Cuvelier, M. E,& Berset, C1995).

2. 2,2'-Diphenylpicrylhydrazyl

Reagent: 1 ml 0.4% DPPH is added to 9 ml methanol to produce 0.04% DPPH solution. TLC plate is sprayed with this reagent in dark room for 1 minute; then spots are visualized in daylight and immediate picture of TLC plate is captured (Duke JA, W.K,(1981).

4.3 APPLICATION OF TLC TECHNIQUE

- Purity of any sample : Purity of sample can be carried out with TLC. Direct comparison is done between the sample and the standard or authentic sample; if any impurity is detected, then it shows extra spots and this can be detected easily.
- Identification of compounds*:* Thin layer chromatography can be employed in purification, isolation and identification of natural products like volatile oil or essential oil, fixed oil, waxes, terpenes, alkaloids, glycosides, steriods etc.
- Examination of reactions*:* Reaction mixture can be examined by Thin layer chromatography to access whether the reaction is complete or not. This method is also used in checking other separational processes and purification processes like distillation, molecular distillation etc.
- Biochemical analysis*:* TLC is extremely useful in isolation or separation of biochemical metabolites or constituent from its body fluids, blood plasma, serum, urine etc.
- In chemistry*:* TLC methodology is increasingly used in chemistry for the separation and identification of compounds which are closely related to each other. It is also used for identification of cations and anions in inorganic chemistry.

- In pharmaceutical industry*:* Various pharmacopoeias have adopted TLC technique for detection of impurity in a pharmacopoeial chemical.
- Various medicines like hypnotics, sedatives, anticonvulsant tranquillisers, antihistaminics, analgesics, local anaesthetics, steroida have been tested qualitatively by TLC method.
- One of the most important application of TLC is in separation of multicomponent pharmaceutical formulations.
- In food and cosmetic industry , TLC method is used for separation and identification of colours, preservatives, sweetening agent, and various cosmetic products.
- This are some of the applications of Thin layer Chromatography (TLC)

4.4 Advantages of TLC technique

- TLC is very simple to use and inexpensive.
- Undergraduates can be taught this technique and apply its similar principles to other chromatographic techniques.
- There are little materials needed for TLC (chamber,watch glass, capillary, plate, solvent, pencil, and UV-light). Therefore, once the best solvent is found, it can be applied to other techniques such as High performance liquid chromatography.
- More than 1 compound can be separated on a TLC plate as long as the mobile phase is preferred for each compound.
- The solvents for the TLC plate can be changed easily and it is possible to use several different solvents depending on desired results.
- As stated earlier, TLC can be used to ensure purity of a compound. It is very easy to check the purity using a UV-light.
- Identification of most compounds can be done simply by checking Rf literature values.And can modify the chromatography conditions easily to increase the optimization for resolution of a specific component (ChemWiki,2015, http://chemwiki.ucdavis.edu)

4.5 Disadvantages of TLC technique

- TLC plates do not have long stationary phases. Therefore, the length of separation is limited compared to other chromatographic techniques.
- Also, the detection limit is a lot higher. If one would need a lower detection limit, one would have to use other chromatographic techniques.
- TLC operates as an open system, so factors such as humidity and temperature can be consequences to the results of your chromatogram (ChemWiki,2015, http://chemwiki.ucdavis.edu)

4.6 Common Problems in TLC

There are common problems in TLC that should be avoided. Normally, these problems can be solved or avoided if taught proper techniques.

- **Over-large Spot**s: Spotting sizes of sample should be not be larger than 1-2 mm in diameter. The component spots will never be larger than or smaller than sample origin spot. If the spot is large, this could cause overlapping of other component spots with similar Rf values on TLC plate. If overlapping occurs, it would prove difficult to resolve the different components.
- **Uneven Advance of Solvent Front**: Uneven advance of the mobile phase is a common problem encountered in TLC. Consequences would be inaccurate R_f values due to the uneven advance of sample origin spots. This uneven advance can be caused by a few factors listed below.
- *No flat bottom*. When placing the TLC plate into the chamber, place the bottom of the plate on the edge of the chamber (normally glass container (e.g. beaker)) and lean the top of the plate along the other side of the chamber. Also, make sure that the TLC plate is placed in the chamber evenly. Do not tilt the plate or sit it at an angle.
- *Not enough solvent.* There should be enough solvent (depends on size of chamber) to travel up the length of the TLC plate.

- *Plate is not cut evenly*. It is recommended that a ruler is used so that the plate is cut evenly.
- Rarely, water is used as a solvent because it produces an uneven curve front which is mainly accounted for by its surface tension.
- **Streaking**: If the sample spot is too concentrated, the substance will travel up the stationary phase as a streak rather than a single separated spot. In other words, the solvent can not handle the concentrated sample and in result, moves as much of the substance as it can up the stationary phase. The substance that it can not move is left behind. This can be eliminated by diluting the sample solution. To ensure that one has enough solution,one should use a short-wave UV light to see if the spot is visible (normally purple in color), as stated earlier.
- **Spotting**: The sample should be above the solvent level. If the solvent level covers the sample, the sample spot will be washed off into the solvent before it travels up the TLC plate (ChemWiki,2015, http://chemwiki.ucdavis.edu)

4.7 DPPH CHARRING PROCESS OF TLC PLATE

4.7.1 Materials Required

4% DPPH stock solution (1%), Methanol (9 ml), Test Tube, Pipette, Pipette filter, Petridish and Tweezers.

Procedure:

1. 0.4% solution of DPPH was prepared by adding 9 ml of methanol to 1 ml of 4% DPPH stock solution. The procedure was carried out in a dark room as DPPH is light sensitive.

2. By using tweezers the developed TLC plates would be dipped into this solution on the silica face down.

3. The plates were left in the dark room for 30 minutes for the color to develop after which they were observed for the formation of yellow, golden / brown color on the background of purple. This coloration indicates the presence of compounds that have antioxidant properties (Neeraj et al, 2013).

4.8 ANTI-OXIDANT TESTS

DPPH Test (1,1 -diphenyl-2-picrylhydrazyl radical)

4.8.1 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).

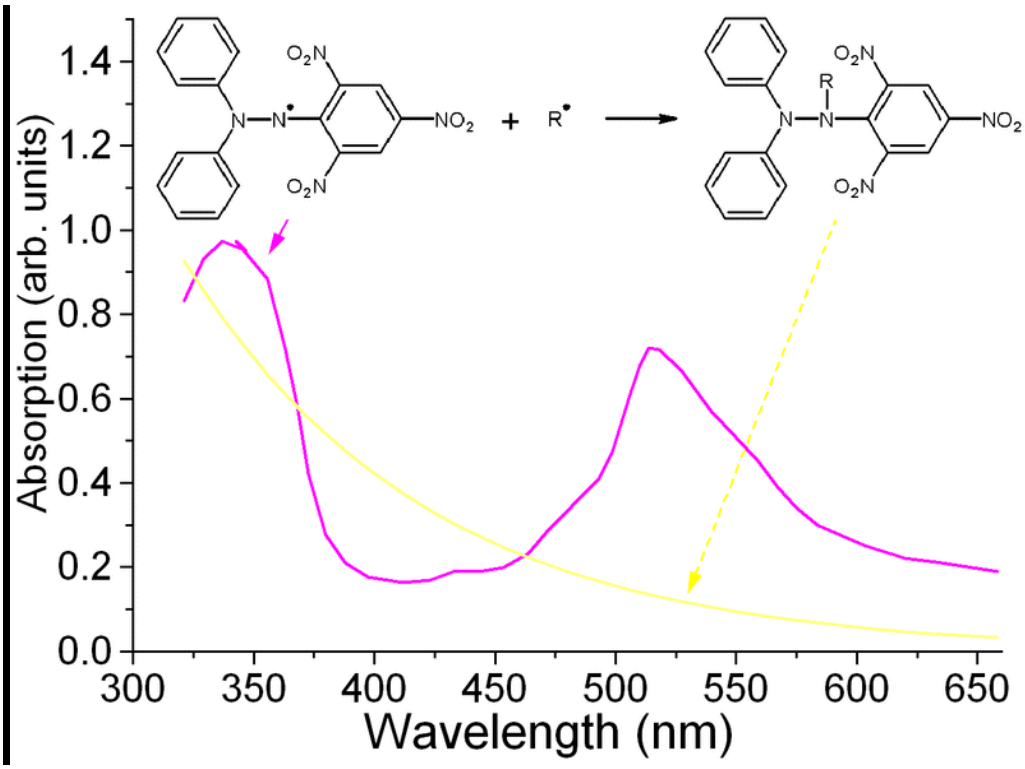


Figure4.8.1: Change in absorption spectrum (from magenta to yellow) upon reaction of DPPH with a radical (ABUIN, 2002:p.145-149).

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)

4.8.2 Apparatus

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

4.8.3 Reagents

- DPPH
- L-ascorbic acid
- Methanol
- Water

4.8.4 Procedure

4.8.4.1 Sample Preparation

- The methanolic extract of the *Tridax procumben 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.

4.8.4.2 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.

4.8.4.3 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.

4.9 IN VITRO ANTI-DIABETIC TEST

4.9.1 Introduction

Diabetes 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 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, *tridax procumben* 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).

4.9.2 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.

4.9.3 Procedure:

Antidiabetic activity of leaves of *Tridax procumben* 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).

4.10 Antimicrobial Screening of Methanolic Extract Using Agar Diffusion Method

The discovery of disease-causing pathogens is an important activity in the field of medical science, as many viruses, bacteria, protozoa, fungi, helminthes and prions are identified as a confirmed or potential pathogen. A Centers for Disease Control program begun in 1995 identified over a hundred patients, with life-threatening illnesses which were considered to be of an infectious cause, but could not be linked to a known pathogen. The association of pathogens with disease can be a complex and controversial process, in some cases requiring decades or even centuries to achieve (Day, 1997).There are many factors impairing identification of pathogens including Lack of animal models, Pre-existing theories of disease, Variable pathogenicity, Organisms that look alike but behave differently, Lack of research effort.

Control of many infectious diseases became possible with the pioneering work of Robert Koch and Louis Pasteur and the introduction of the germ theory of disease. With bacteriologic cultivation techniques came the first isolation and identification of etiologic agents; virus cultivation and identification became available some decades later. Reservoirs of microorganisms and their life cycles were identified; the epidemiology and natural history of many infectious diseases were described, and successful control measures were initiated. Water treatment, vector control, and rodent reduction programs followed. By the beginning of the 20th century, the principles of vaccination, established empirically by Edward Jenner more than 100 years earlier, began to be realized in earnest. Antibiotics were discovered, and disinfectants were developed. Collectively, these control measures dramatically decreased the incidence and prevalence of many infectious diseases and their fatality rates. The early part of this century is appropriately regarded as a golden age in public health (David Satcher, 2015; Emerging Infections: Getting Ahead of the Curve)

Death from infectious disease rankd 5th in 1981, has become the 3rd leading leading cause of death in 1992; an increasing of 58%. It is estimated that infectious disease is the underlying cause 58% of the death occurring in the US (Ahmed et al, 2011). The respiratory tract infection are increased and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosicomial and community acquired infections. Furthermore, the most increasr are occurring in the 25-44years old age group (Khosa, et al, 2011).

The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability estimated by disc diffusion (Kowti, et al, 2010).

Some investigator use the diameter of Zone of inhibition and the minimum weight of extract inhibit the growth of microorganisms/ However, a great number of factors, the extraction methods, inoculums volume, culture medium composition, pH and incubation temperature can presence the results (Britto ,et al, 2011).

Among the above mentioned techniques the disc diffusion is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity. It is essentially a quantitative and qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method.

4.10.1 Principle of disc diffusion Method

The agar diffusion assay is one method for quantifying the ability of antibiotics to inhibit bacterial growth. The agar diffusion test, or the Kirby-Bauer disk-diffusion method, is a means of measuring the effect of an antimicrobial agent against bacteria grown in culture. The bacteria in question is swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is

greater than or equal to the effective concentration. This is the zone of inhibition. This along with the rate of antibiotic diffusion are used to estimate the bacteria's sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that bacteria. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection (Mohanty A et al, 2010).

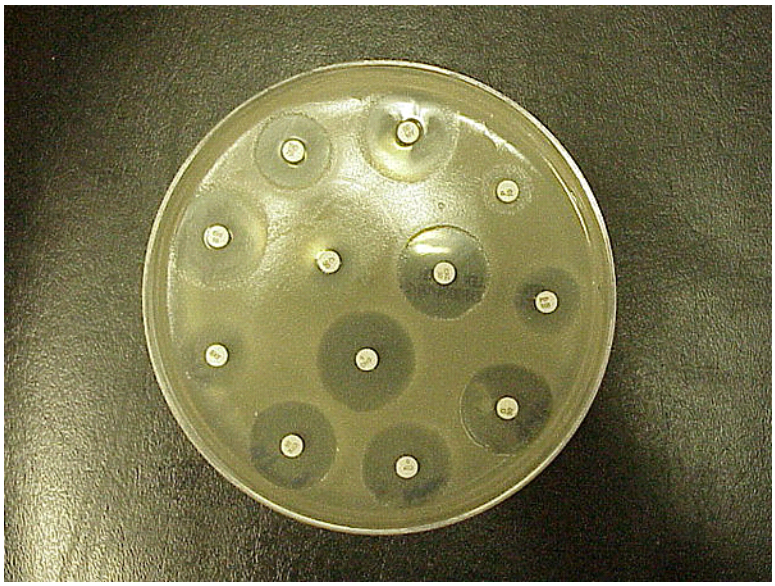


Figure 4.10.1:Discs containing antibiotics are placed on agar where bacteria are growing and inhibited.

4.10.2 Materials Required

- Filter paper discs
- Autoclave
- Nutrient Agar Medium
- Laminar air flow hood

- Petri dishes
- Spirit burner
- Sterile cotton swabs
- Refrigerator
- Micropipette
- Incubator
- Inoculating loop
- Ethanol
- Sterile forceps
- Nose mask and Hand gloves
- Screw cap test tube

4.10.3 Test Organisms

4.10.3.1 Gram Negative Bacteria

- *Salmonella typhi*
- *E.coli*
- *Pseudomonas*
- *Shigella dysentery*
- *Salmonella paratyphi*
- *Vibriomimicus*

4.10.3.2 Gram Positive Bacteria

- *Staphylococcus aureus*
- *Bacillus cereus*
- *Bacillus subtilis*

4.10.3.3 Fungi

- *Candia albicans*
- *Bacillus megaterium*
- *Aspergillus niger*

4.11 The Culture Medium and Its Composition

Nutrient agar was used to conduct the antimicrobial screening using the disc diffusion method. The nutrient agar was bought from the market. Nutrient agar contains the following substances:

4.11.1 Ingredients

- Bacto peptone - 0.5gm
- Sodium chloride - 0.5gm
- Bacto yeast extract - 1.0gm
- Bacto agar - 2.0gm
- Distilled water (Qs) - 100ml

4.11.2 Preparation of the Medium

First of all, The amount of nutrient agar needed was calculated and then added to distilled water in an agar bottle and mixed thoroughly. It was then autoclaved to dissolve the agar and sterilize it.

Figure 4.11.2: The autoclaved machine of East West University

4.11.3 Sterilization Procedure

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present in a specified region, such as a surface, a volume of fluid, medication, or in a compound such as biological culture media. 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. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121ºC and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Figure 4.11.3: The laminar hood of East West University

4.11.4 Preparation of the Test Plates

1. The test organisms were transferred from the subculture to petridish containing the required amount of melted and sterilized agar medium as required by the size of the dish.

2. The bacterial and fungal suspension was taken by a loop and mixed with normal saline with the help of vortex machine.

3. Then a sterilized cotton bud was taken and dipped into the bacterial/ fungal suspension. Then the bacterial/fungal sample is applied to the petridish with the help of this cotton bud.

4. The swabbing was done carefully so that the microorganisms would be spread out evenly on the dish.

Figure 4.11.4: The vortex machine of East West University

4.11.5 Preparation of Discs

4.11.5.1 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) standard disc was used as the reference.

4.11.5.2 Blank Discs

These were used as negative controls, which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves. Here the negative control used was methanol.

4.11.6 Preparation of sample discs with test samples

1. In a specific volume of solvent, Measured amount of each test sample was dissolved to obtain the desired concentrations in an aseptic condition.
2. For the each extract of husk, a stock solution of 10mg/ml was prepared and was used directly.

3. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of 10µl of test samples and dried.

4.11.7 Diffusion and Incubation

Here, incubation is done for maintaining controlled environmental conditions for the purpose of favoring growth or development of microbial or tissue cultures or to maintain optimal conditions for a chemical or immunologic reaction.

1.The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi.

2.The plates were then inverted and kept in an incubator at 370C for 24 hours.

Figure 4.11.7: Incubator for microorganisms at East West University

4.12 Determination of Antimicrobial Activity by using the Zones of Inhibition

The agar diffusion test, the size of the zone of inhibition indicates the degree of sensitivity of bacteria to a drug or testing agent. In general, a bigger area of bacteria-free media surrounding an antibiotic disk means the bacteria are more sensitive to the drug or the testing agent the disk contains .KB tests are performed under standard conditions, so the minimum inhibitory

concentration for a given antibiotic or testing agent can be calculated by comparing the observed zone of inhibition's size to known values.

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

RESULT

5.1 THIN LAYER CHROMATOGRAPHY(TLC)

TLCs were conducted on methanolic extract of the leaves of *Tridax procumben* by using all the three types of solvent systems, and the best results were obtained by using the non polar solvent system.

TLC was done in non polar solvent system which consist of Benzene 9ml, Ethanol 1ml.The naked eye view of the TLC was mentioned in the plate 1 which did not show any clear spot (1). Then the plate was observed under UV which is shown in the plate (2). 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 -3). In the crude extract layer three spot was observed.

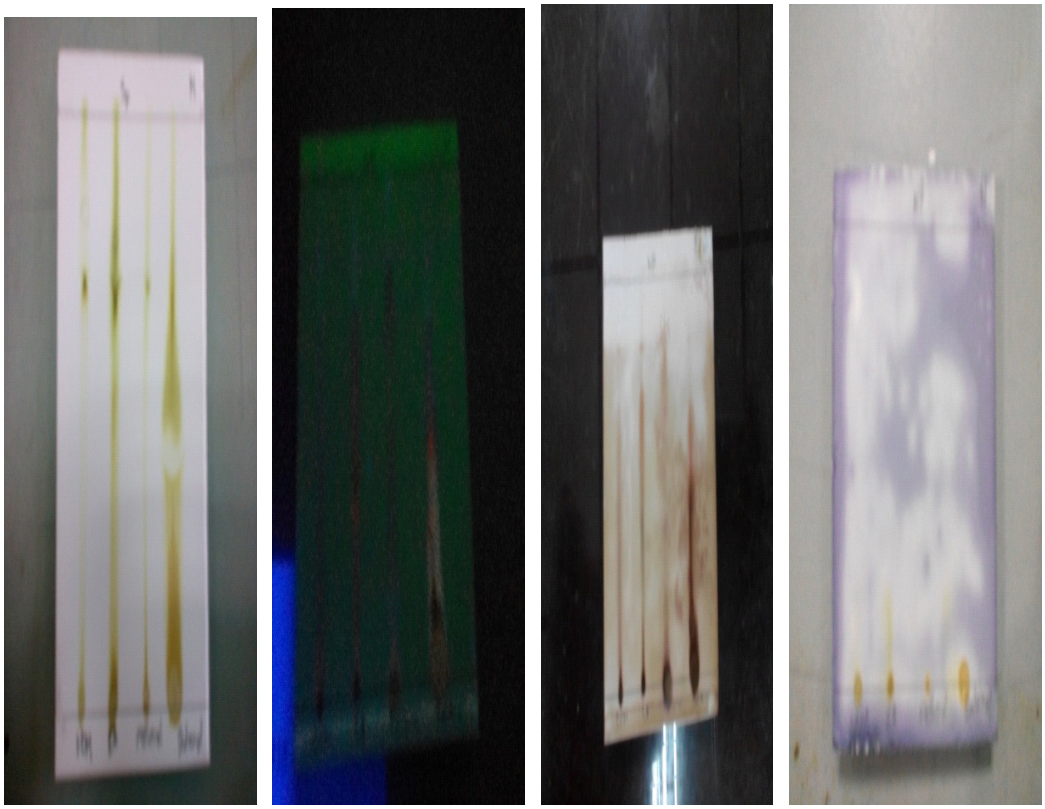
R.F (Retardation Factor) Value Calculation:

R_f = Distance spot travels/ Distance solvent travels

Table 5.1*:* R.F (Retardation Factor) Value Calculation of leave extract of for methanolic extract

Name of sample(Methanolic extract)	R_f value
1st spot	0.31
2^{nd} spot	0.50
3^{rd} spot	0.75

5.2 Thin layer Chromatography of Methanolic Extract of *Tridax procumben* leaves (Primary five fraction of VLC extract).



(1) (2) (3) (4)

Figure 5.2: (1)TLC plate in naked eye view, (2)TLC plate Under UV light,(3)TLC plate after charring with H_2SO_4, (4) TLC plate after application of DPPH.

TLC was done with primary five fraction of VLC. After TLC, it was found that the five fractions made some spots 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. There were some spot was found after TLC plates were dipped in DPPH solution (plate 4).

R.F (Retardation Factor) Value Calculation of primary five fraction of VLC

R_f = Distance spot travels/ Distance solvent travels

Table 5.2: R.F (Retardation Factor) Value Calculation of primary five fraction of VLC.

Name of sample	1st spot R_f value	2nd spot R_f value	3rd spot R_f value
DCM	0.53	0.77	0.89
Butanol	0.55	0.75	0.96
Ethyl Acetate	0.60	0.87	0.93
Methanol	0.2	0.5	

5.3 HYPOGLYCEMIC TEST

The result of hypoglycemic test of different fraction of methanolic extract of the *Tridax procumben* leaves are given below-

Table 5.3.2: Antidiabetic activity of DCM fraction of methanolic extract of *Tridax procumben* leaves

Sample Name	**Concentrations µg/ml**	**Absorbance (340nm)**	**% of inhibition**
	31.25	**0.280**	**7.68**
	62.5	0.305	15.25
DCM	125	0.352	26.56
	250	0.390	33.72
	500	0.479	46.03

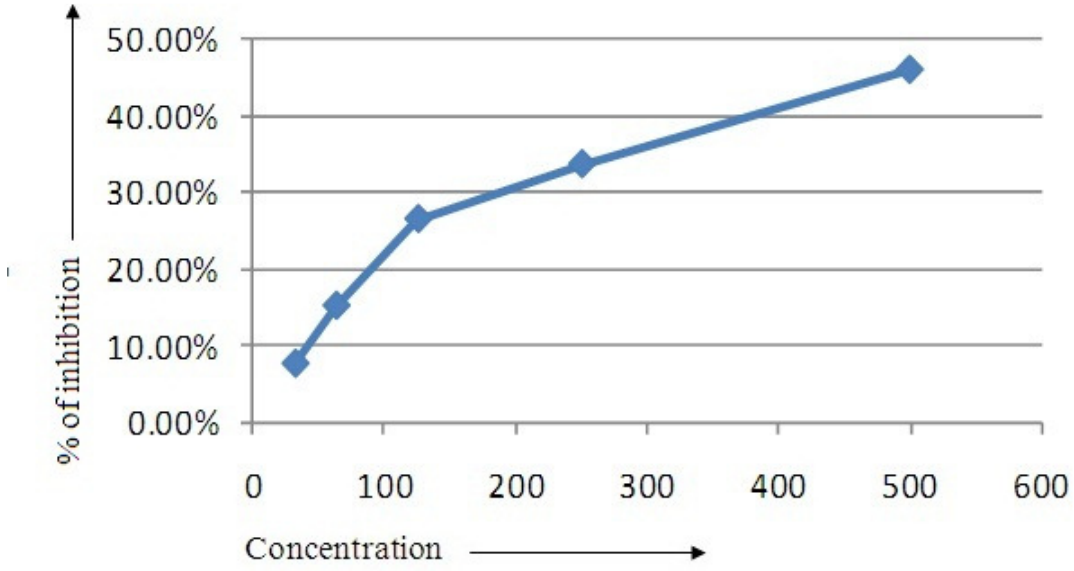


Fig 5.3.2 : Anti-diabetic activity of DCM fraction of *Tridax procumben* leaves.

Table 5.3.3: Antidiabetic activity of n-butanol fraction of methanolic extract of *Tridax procumben* leaves

Sample Name	**Concentrations µg/ml**	**Absorbance (340nm)**	**% of inhibition**
	31.25	0.294	12.07
	62.5	0.356	27.39
Butanol	125	0.540	52.13
	250	0.653	60.41
	500	0.694	62.75

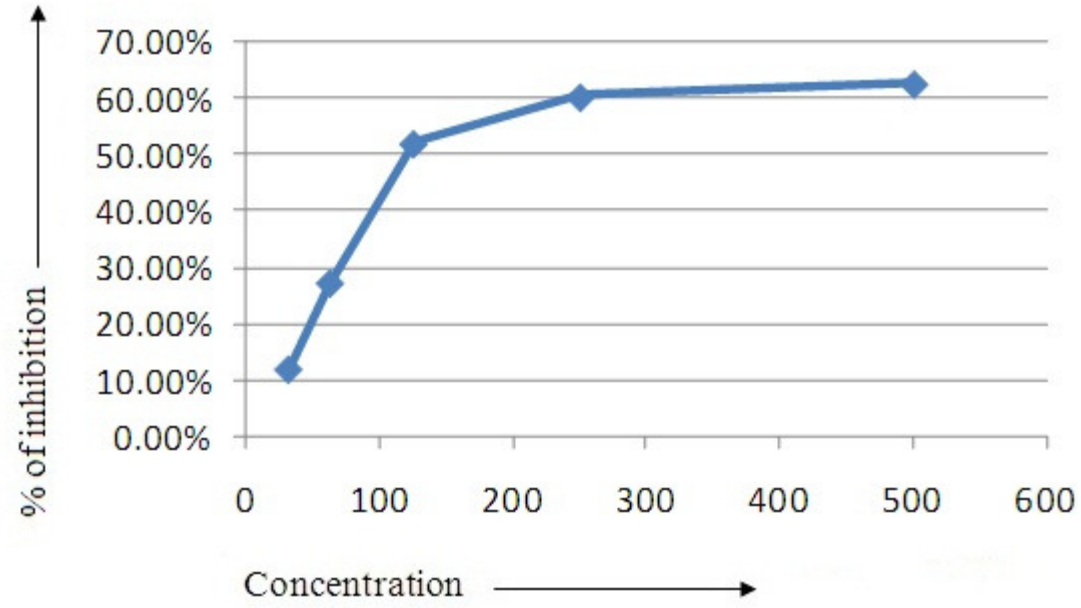


Fig 5.3.3: Anti-diabetic activity of butanol fraction of *Tridax procumben* leaves

Table 5.3.4: Antidiabetic activity of Ethyl acetate fraction of methanolic extract of *Tridax rocumben* leaves

Sample Name	Concentrations µg/ml	Absorbance (340nm)	% of inhibition
	31.25	0.300	13.83
	62.5	0.325	20.46
Ethyl acetate	125	0.423	39.01
	250	0.471	45.12
	500	0.622	58.44

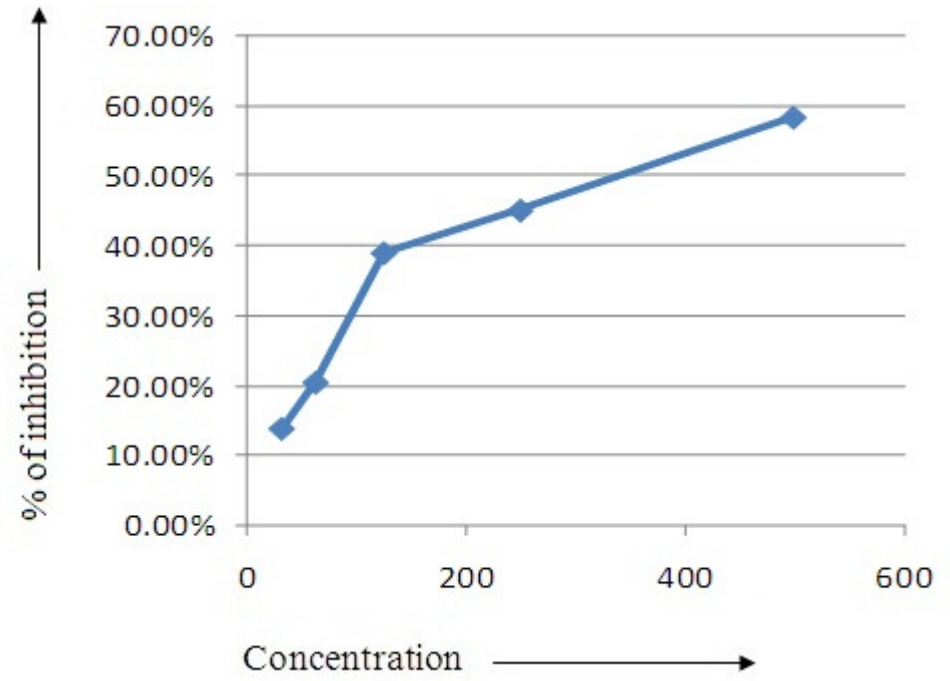


Fig 5.3.4: Anti-diabetic activity of Ethyl acetate fraction of *Tridax rocumben* leaves

Sample Name	**Concentrations µg/ml**	**Absorbance (340nm)**	**%of inhibition**
	31.25	0.294	12.07
	62.5	0.331	21.90
Methanol	125	0.343	22.89
	250	0.369	29.95
	500	0.530	51.22

Table 5.3.5: Antidiabetic activity of Methanol fraction of methanolic extract of *Tridax procumben leaves*

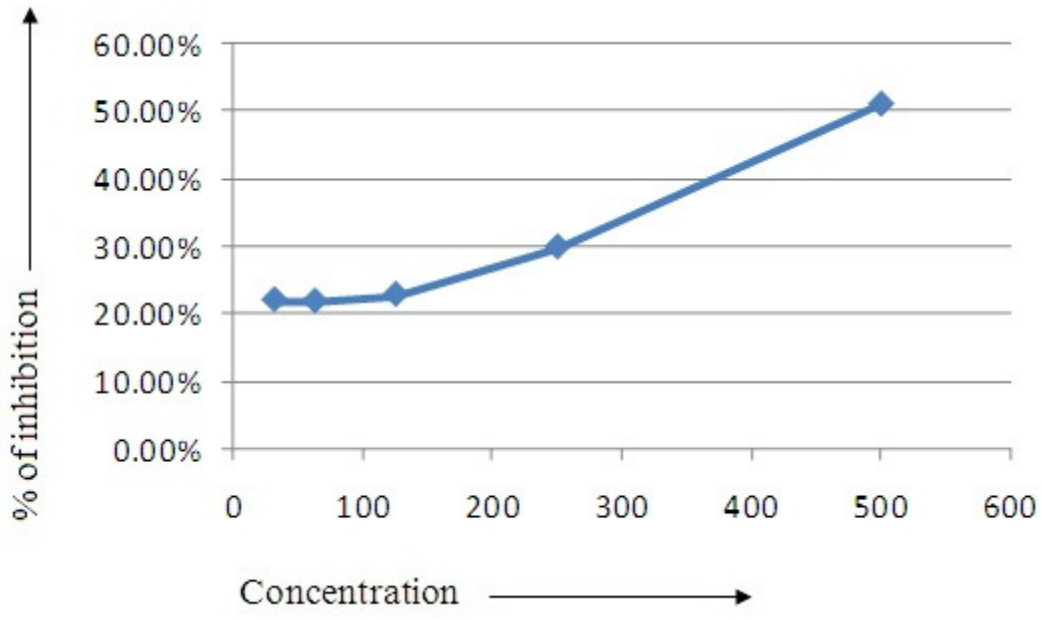


Fig 5.3.5: Anti-diabetic activity of Methanol fraction of *Tridax procumben* leaves.

Table 15.3.6: Antidiabetic activity of standard Metformin

Sample Name	**Concentrations µg/ml**	**Absorbance (340nm)**	**%of inhibition**
	31.25	0.333	22.37
	62.5	0.360	28.19
Metformin	125	0.387	33.20
	250	0.399	35.74
	500	0.429	39.74

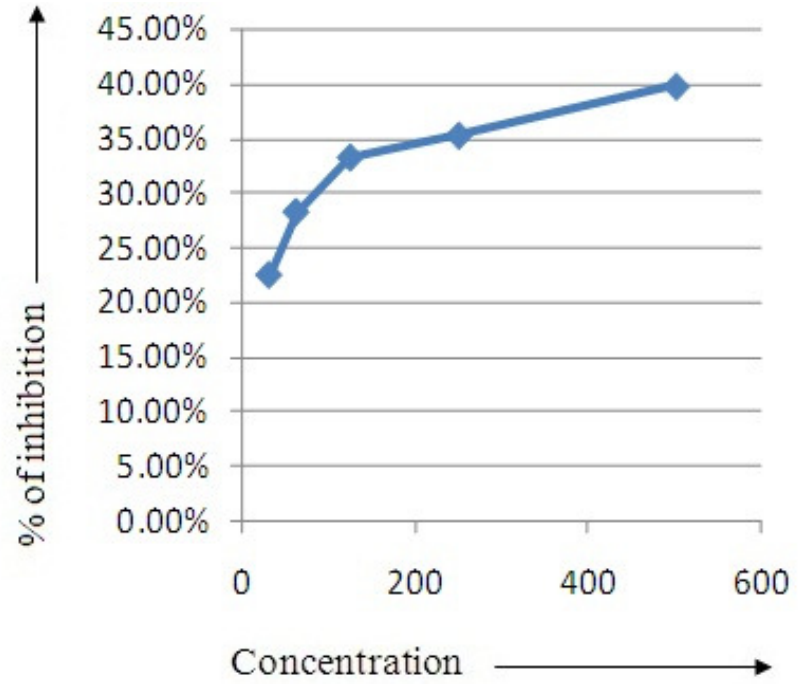


Fig 5.3.5: Anti-diabetic activity of Metformin

Anti oxidant activity

Table 5.3.8:Antioxidant activity of DCM fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**% of inhibition**
	31.25	0.523	1 2 . 6 1
	62.5	0.519	13.28
DCM	125	0.448	89.72
	250	0.355	40.68
	500	0.178	70.25

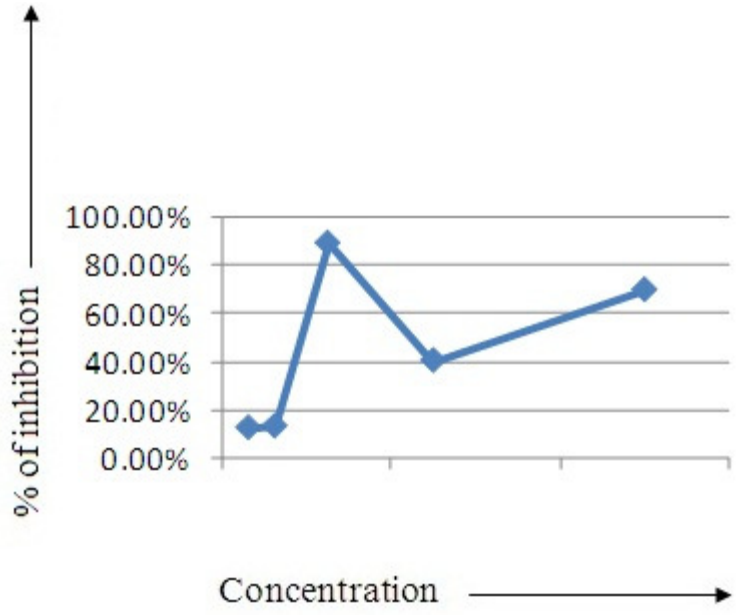


Fig 5.4.3 : % Free radical scavenging activity of DCM fraction of *Tridax procumben leaves*

Table 4.3.5: .Antioxidant activity of standard fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**% of inhibition**
	31.25	.246	5 8 . 8 9
	62.5	0.246	60.73
Standard	125	0.219	63.40
	250	0.199	66.75
	500	0.168	71.90

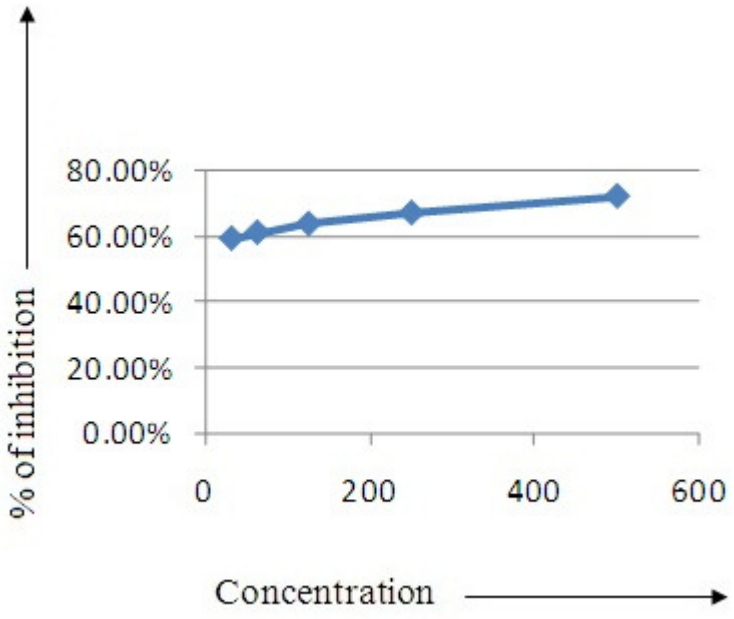


Fig 4.3.4 : % Free radical scavenging activity of Ascorbic acid

Table 4.4.5:Antioxidant activity of Ethyl acetate fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**% of inhibition**
	31.25	.226	6 2 . 2 3
	62.5	0.165	72.43
Ethyl acetate	125	0.115	80.78
	250	0.098	83.62
	500	0.092	84.62

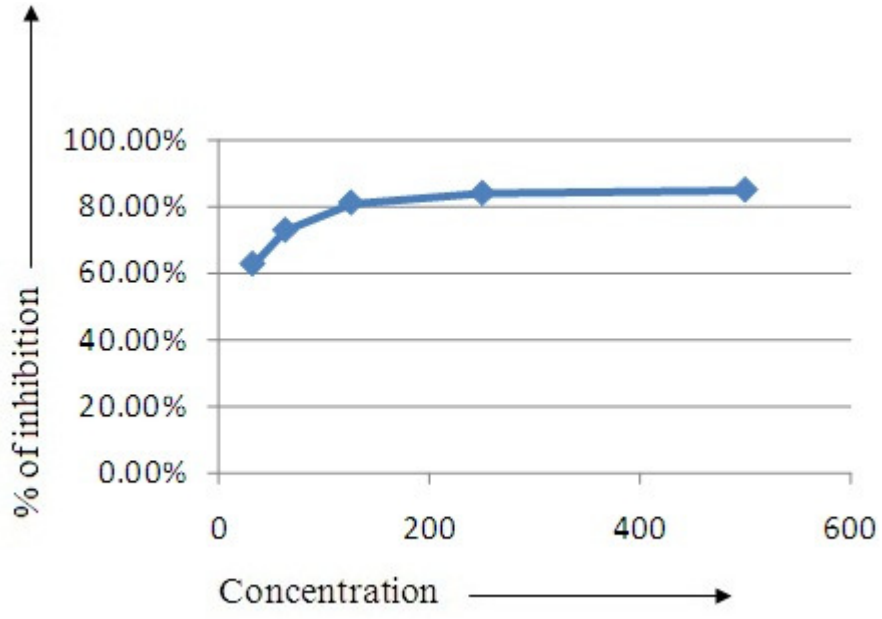


Fig 4.5.7 : % Free radical scavenging activity of Ethyl Acetate fraction of *Tridax procumben leaves ac*tivity

Table 4.4.6:Antioxidant of Methanol fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**%of inhibition**
	31.25	0.254	57.56
Methanol	62.5	0.178	70.25
	125	0.124	79.28
	250	0.108	81.95
	500	0.100	83.29

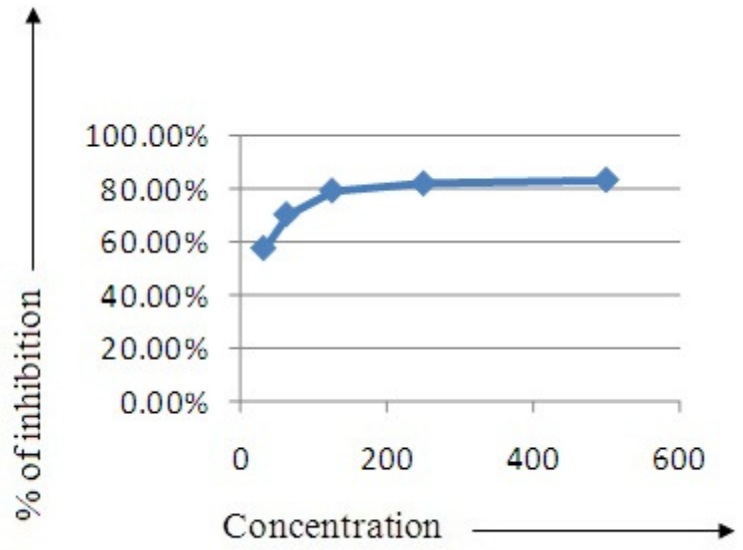


Fig 4.5.3 : % Free radical scavenging activity of Methanol fraction of *Tridax procumben leaves*

Table 4.4.7:Antioxidant of Butanol fraction of methanolic extract of *Tridax procumben leaves*

Sample Name	**Concentrations µg/ml**	**Absorbance (517nm)**	**% of inhibition**
	31.25	0.480	19.80
Butanol	62.5	0.344	42.52
	125	0.312	47.86
	250	0.288	51.89
	500	0.264	55.89

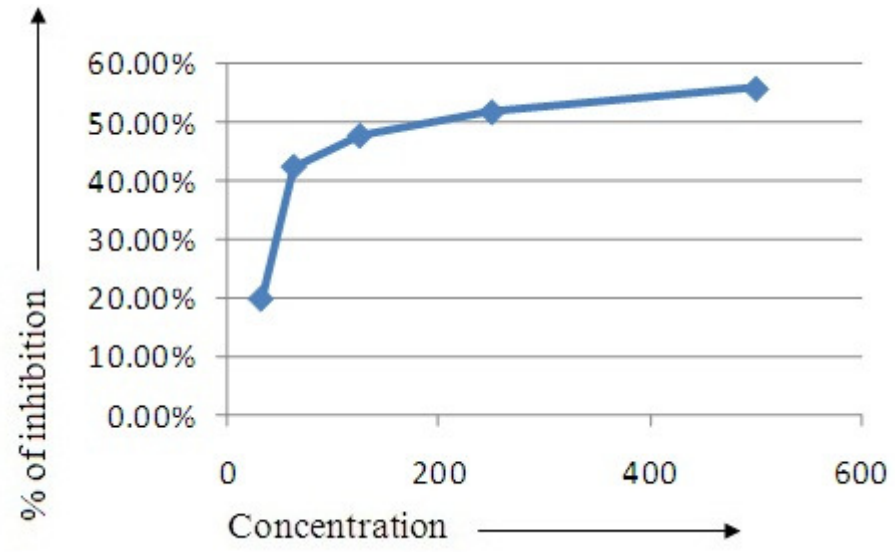


Fig 4.5.3 : % Free radical scavenging activity of Butanol fraction of *Tridax procumben leaves*

Antibacterial activity

Table 6.2: Antibacterial activity of the methanolic extract of Ethyl acetate ,Dichloromethane,Butanol, Methanol,standard and control test

Tested bacteria	**Zone of inhibition (mm)**					
	Methanol	**Ethyl acetate**	**DCM**	**Butanol**	**Standard**	**Control**
S. paratyphi	9	9	8	14	24	0
Bacillus sereus	8	9	7	7	23	0
Bacillus subtilis	13	11	8	9	26	0

Staphylocuccos aureas	13	12	9	21	30	0
Salmonela typhi	9	8	8	11	31	0
Shigella dysentriae	10	8	10	7	27	0
Vibrio mimicus	9	9	8	14	28	0
Candida albicans	9	10	9	11	27	**0**
Aspergillus niger	9	10	9	11	27	0
E.Coli	10	11	9	11	25	0
Vibrio parahemoliticus	11	9	8	13	30	0
Bacillus megaterium	13	11	8	9	27	0
Pseudomonas aurea	11	12	9	12	22	0

DISCUSSION

6.1 Thin layer chromatography

6.1.1 Discussion

TLC plates were developed with Dichloromethane, Butanol,ethyl acetate,Methanol crude using solvent system-1 (Benzene,Ethanol,Ammonium hydroxide) and 3 (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 (2). 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 3). 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 4). This provides us a preliminary idea of the various types of compounds that may be present in the methanolic extract of the leaves of *TridaxpProcumben* Further extractions and purifications from these crude drugs may lead to the possible isolation of these compounds from the crude extracts.

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,Dichloromeehane,Butanol, ethyl acetate,Methanol showed at least three spot.(plate-4)Some spots was found after TLC plates were dipped in DPPH solution

6.2 IN VITRO ANTI-DIABETIC TEST

6.2.1 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,Butanol and Ethyl acetate fraction of methanolic extract of give higher antidiabetic activity 62% and 58%.Whereas, Methanol and Dichloromethane fraction of methanolic extract of give 51%,46% lower antidiabetic activity .

6.3 DPPH TEST

6.3.1 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 methanolic extract of the *leaves of Tridax procumben* 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 Dichloromethane(DCM), Butanol, Ethyl acetate and Methanol fraction of *Tridax procumben* leaves give 89%,55% and 84%,83% antioxidant activity.

6.4 ANTIBACTERIAL TEST

6.4.1Discussion

Various strains of Gram positive, Gram negative bacteria and fungi were used in this test. The positive control used was ciprofloxacin (30µg/disc). For methanolic extract of,Ethyl acetate ,Dichloromethane,Butanol**,**was used to evaluate the activity against different types of microrganism. The zones of inhibition for the microbes were measured in centimeters using a transparent ruler after 24hrs of incubation.butanol extract was showed zone of inhibition up to 21cm antibacterial activity at the concentrations used against*, Staphylocuccos aureas* than other strain. A study showed that, The leaves of *Tridax procumben* possessing antimicrobial activity can be employed against human pathogens. The use of leaves of *Tridax procumben* might promote human health by preventing bacterial pathogenesis.

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 *Tridax procumben* 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 methanolic extract of the *Tridax procumben* 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.

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