East West University

Phytochemical and Biological Investigation of *Tridax procumbens* leaves

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

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Endorsement by the Chairperson

This is to certify that the dissertation, entitled **"Phytochemical And Biological Investifgation Of *Tridax procumbens* Leaves"** Is a thesis work done by Pipon Chandra Nandi (ID: 2010-3-70-050) in partial fulfillment of the requirements for the degree of B.pharm. We further certify that all the sources of information and laboratory facilities availed in this connection is duly acknowledged.

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Certificate by the Invigilator

This is to certify that the dissertation, **"Phytochemical And Biological Investifgation Of *Tridax procumbens* Leaves"** is a thesis work done by Pipon Chandra Nandi (ID: 2010-3-70-050), in partial fulfillment of the requirements for the degree of B.phrm. We further certify that all the sources of information and laboratory facilities availed in this connection is duly acknowledged.

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Declaration by the Candidate

I, Pipon Chandra Nandi (ID:2011-3-70-050), hereby declare that the dissertation entitled **"Phytochemical And Biological Investifgation Of *Tridax procumbens* Leaves"** , submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy is a genuine & authentic thesis work carried out by me during Fall 2014-Spring 2015 under the supervision and guidance of Dr. Repon Kumer Saha, Assistant Professor, Department of Pharmacy, East West University.

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ABSTRACT

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

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, pentacyclic triterpenes, fatty acids and polysaccharides. 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.[citation needed] its is also the best anti coagulant and used to treat the inflamation

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 procumbens* plant (family of Asteraceae) 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 procumbens* 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 procumbens* in the successful treatment of haemorroids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes.

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

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 pu rposes. 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 an 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 Tiliaceae

Tiliaceae is a botanical name for a family of flowering plants. Such a family is not part of APG II, but it is found all through the botanical literature and remains prominently listed by nomenclatural databases such as IPNI. All through its existence the family has had a very lively history, with various authors taking very different views on what should be part of this family. As a result it is recommended when this name is encountered to be careful to check what an author means when he uses this name. However, in the northern temperate regions the name is unambiguous as the only representative is Tilia, the lime or linden (Watson and Dallwitz, 1992)
The Tiliaceae are trees, shrubs, or rarely herbs comprising about 50 genera and 450 species that are further characterized by the presence of branched or stellate hairs. The leaves are simple and nearly always alternate, stipules are present. The flowers are actinomorphic and nearly always bisexual. The perianth consists of a valvate calyx with usually 5 distinct or basally connate sepals and a corolla of an equal number of petals or sometimes the corolla is sepaloid or absent. The androecium consists of usually many stamens that are distinct or basally connate or in fascicles. The gynoecium is a single compound pistil of 2-10 carpels, an equal number of stigmas, and a 2-10-loculed superior ovary with 1-several axile ovules in each locule. The fruit is variable (Watson and Dallwitz, 1992)

1.5 DESCRIPTION OF THE PLANT

Tridax procumbens Linn. belongs to the family Compositae. It is commonly known as 'Common button' or 'Coat button' and it is a weed found throughout India. A hispid, procumbent herb with woody base sometime rooting at the node, upto 60 cm high. Leaves are ovate-lanceolate 2 to7 cm and lamina pinnatisect, sometimes three lobed; flowers in small, long peduncled heads; achenes 1.5 - 2.5 mm long x 0.5 – 1 mm in diameter and densely ascending pubescent; persistent ; bristles of disc achenes alternately longer and shorter, 3.5 – 6 mm in length . Isolation of methyl 14 oxoacagaecunoate, methyl 14-oxononacosanoate, 3-methyl-non adecylbenzene, heptacosanyl cyclohexane carboxylate, 1-(2,2, dimethyl-3- hydroxypropyl) isobutyl phthalate, 12-hydroxytetracosa-15-one, 32-methyl-30- ozotetraatriacont-31-en-1-ol along with β-amyrin, β-amyrone, fucosterol and sitosterol, arachidic, behenic, lauric, linoeic, linolenic, myristic, palmitic and stearic acids have been isolated . The leaves are reported to be employed in bronchial catarrh, dysentery or diarrhea and for restoring hair. The leaf gel possesses antiseptic, insecticidal and parasiticidal properties. It is used to check haemorrhage from the cuts, bruises and wounds. An aqueous extract of plant produces reflex tachycardia and showed a transient hypotensive effect on the normal blood pressure. It is employed as an indigenous medicine for a variety of ailments including jaundice. The plant also has hepatoprotective activity and it is used in Ayurveda in various liver disorders. It is commonly used in Indian traditional medicine as anticoagulant, antifungal and insect repellant, in bronchial catarrh, diarrhea and dysentery . Moreover it possesses wound healing activity and promotes hair growth

1.5.2 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 (ବିଶଲ୍ୟକରଣୀ) in Oriya, kambarmodi in Marathi, gaddi chemanthi (గడ్డి చామంతి) in Telugu,vettukaaya poondu in Tamil, and kotobukigiku in Japanese,

1.5.3 BOARD 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 Human Use

Tridax procumbens is known for several potential therapeutic activities like antiviral, anti oxidant antibiotic efficacies, wound healing activity, insecticidal and anti-inflammatory activity. Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, to stop bleeding, as a hair tonic. Despite these known benefits, it is still listed in the United States as a Noxious Weed and regulated under the Federal Noxious Weed Act.[citation needed]

A study by Gamboa-Leon (2014) showed that a mixture of Tridax procumbens and Allium sativum extracts was a promising natural treatment for cutaneous leishmaniasis and that its healing effects made it a good candidate for a possible new phytomedicine. The mixture of Tridax procumbens and A. sativum extracts was better at controlling Leishmania mexicana infection while not being toxic when tested in the acute oral toxicity assay in mice.[citation needed]

Whole plant ethanolic extract of Tridax procumbens showed significant anti-arthritic effect, antidiabetic and antihyperlipidemic effects in rats using the Freund's Complete Adjuvant (FCA) model and streptozotocin-induced diabetic model.

Traditionally, Tridax procumbens has been in use in India for wound healing, as anticoagulant, antifungal and insect repellent. It is also used in diarrhoea and dysentery. Its leaf extracts were known to treat infectious skin diseases in folk medicines. It is a well-known ayurvedic medicine for liver disorders or hepato-protective nature besides gastritis and heart burn.

In humans, Tridax procumbens used as treatment for boils, blisters and cuts by local healers in Nalgonda and Warangal District of Telangana, Andhra Pradesh, India. A study had found anti-cancer properties of Tridax procumbens against human prostate epithelial cancer cell line PC 3.

A study was carried out to verify the claims wherein tribal inhabitants of Udaipur district, Rajasthan were using the plant for treatment of diabetes. It was concluded that the results were comparable to that of reference standard Glibenclamide and the Tridax procumbens flower extract showed antidiabetic properties.

Phatak et al., (1991) has investigated the hair growth promoting activity of Tridax procumbens and the petroleum ether extract of Tridax procumbens was found to be effective in promoting hair growth in male wistar albino rats.

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. 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)

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 procumbens* (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) **(**Navie, 2004).

Flower

Figure 1.2:Flower of *Tridax procumbens* (Forest and Kim Starr,2015).

1.8.4 Reproduction and Dispersal

This species mainly reproduces by seed, although suckers can be produced when plants are damaged.Seeds are mostly spread by water, and this dispersal mechanism is aided by the pods' ability to float considerable distances. They are also dispersed in mud attached to vehicles, machinery and animals **(**Navie, 2004).

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, pentacyclic triterpenes, fatty acids and polysaccharides. 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.[citation needed] its is also the best anti coagulant and used to treat the inflamation

2.1 PHYTOCHEMICAL REVIEW

2.1.1 NUTRITIVE COMPOSITION

The leaves of *Tridax procumbens* 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 procumbens* 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 procumbens* 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 procumbens 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 procumbens* 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 procumbens* 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 PHYTOCHEMICAL REVIEW

Phytochemical Analysis The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. Phytochemical test were carried out adopting standards procedure (Trease et. al 1983, Kokate et.al 1997, Hegde et.al 2010). Test were performed for Steroids, Tannin, Saponin, Anthocyanin, Coumarins, Emodins, Alkaloids, Proteins, Amino acids, Diterpenes, Phytosterol, Phenol, Phlobatannins, Leucoanthocyanin, Cardial glycosides and Flavonoids. Steroid 1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H2SO4 acid was added from the side of test tube. The upper layer turns red and H2SO4 layer showed yellow with green fluorescence .This indicates the presence of steroid. Tannin a) 2ml extract was added to 1% lead acetate a yellowish precipitate indicates the presence of tannins. b) 4ml extract was treated with 4 ml FeCl3 formation of green colour indicates that presence of condensed tannin Saponin 5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponin. Anthocyanin 2 ml of aqueous extract is added to 2 ml of 2N HCl & NH3, the appearance of pink red turns blue violet indicates presence of Anthocyanin. Coumarin 3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates coumarins. Emodins 2 ml of NH4OH and 3 ml of benzene was added to extract appearance of red colour indicates presence of emodins. Alkaloids A quantity (3 ml) of concentrated extract

was taken into a test tube and 1 ml HCl was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following test.

a) Wagner test: Filtrate was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.

b) Hager's test: Filtrate was treated with Hager's reagent, presence of alkaloids confirmed by the yellow colored precipitate.

Proteins Xanthoproteic test: Extract was treated with few drops of concentrated HNO3 formation of yellow indicates the presence of proteins.

Amino acids Ninhydrin test: To the 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes, formation of blue colour indicates the presence of amino acid.

Diterpenes Copper acetate test: Extract were dissolved in water and treated with 10 drops of copper acetate solution, formation of emerald green colour indicates presence of diterpenes. Phytosterol Salkowski's test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated H2SO4 and shakes, allow standing, appearance of golden red indicates the positive test.

Phenol Ferric Chloride test: Test extract were treated with 4 drops of Alcoholic FeCl3 solution. Formation of bluish black colour indicate the presence of Phenol Phlobatannins Deposition of red ppt when aqueous extract of each plant sample is boiled with 1% Aqueous HCl was taken as evidence for presence of Phlobatannins. Leucoanthocyanin 5 ml of isoamyl alcohol added to 5 ml of aqueous extract, upper layer appear red in colour indicates the presence of Leuanthocyanin. Cardial Glycosides Keller-Killani Test: Plant extract treated with 2 ml glacial acetic acid containing a drop of FeCl3 .A brown colour ring indicates the presence of positive test.

Flavonoid

a) Alkaline reagent test: Extract was treated with 10 % NaOH solution, formation of intense yellow colour indicates presence of Flavonoid.

b) NH4OH test: 3 ml of extract were 10 % NH4OH solution development of yellow fluorescence indicates positive test.

c) Mg turning test: Extract were treated with Mg turning and add conc.HCl to this solution add 5ml of 95 % ethanol, formation of crimson red colour indicates Flavonoid.

d) Zn test: 2 ml extract were treated with Zn dust and conc.HCl development of red colour indicates presence of Flavonoid.

2.2.1ANTIMICROBIAL ACTIVITY

The antibacterial activity of Tridax procumbens was determined against Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli and Bacillus cereus. All the organisms responded to both methanol and Ethyl acetate extracts by using Disc Diffusion and Agar Well Diffusion method.

The antimicrobial activities of ethanolic leaf extract of Tridax procumbens 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 procumbens*, Cassia fistula and Cassia tora were investigated for their antifungal activities on three pathogenic fungi (Microsporum gypseum, Trichophyton rubrum and Penicillium marneffei). Among 3 species, *S. alata* 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)

2.2.3 ANTIOXIDENT ACTIVITY

Many plants exhibit in vitro and an in vivo antioxidant property owing to their phenolics, proteins, vitamins and pectins contents. In the different literatures, it has been found that the antioxidant activity of plant extracts is responsible for their therapeutic effect against atherosclerosis, CVDs and cancer. Hence, Tridax procumbens plant extracts were evaluated for in vitro antioxidant activities. DPPH method provides a good assessment for evaluation of in vitro antioxidant activity. It is based on reaction between antioxidant (AH) with nitrogen centered free radical i.e. DPPH (1, 1-diphenyl, 2- picryl hydrazyl). The Ethyl acetate and n-Butanol fractions from methanolic extract have shown significant activity which is comparable to the activity of Ascorbic acid, as shown in Table No. 1 and Fig No.1. Fractionation of the parent extract reduced the complexity of material and provided more accurate idea related to the Phytochemicals, responsible for antioxidant activity of Tridax procumbens.

2.2.4 ANTIDIABETIC ACTIVITY

Adult, healthy male Wistar rats weighing between 180 ± 10 g were used for the experiment. The rats were divided into five different groups of six animals as follows.

Group I - Normal control rats

Group II - Diabetic control

Group III - Diabetic rats treated with glibenclamide (0.25 mg/kg)

Group IV - Diabetic rats treated with ethanolic extract of the whole plant of T. procumbens (250 mg/kg)

Group V - Diabetic rats treated with ethanolic extract of the whole plant of T. procumbens (500 mg/kg).

Diabetes was induced in overnight-fasted rats by a single intra-peritoneal injection of freshly prepared STZ 50 mg/kg b.w. followed by 120 mg/kg of nicotimanide in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg b.w. Diabetes was confirmed in the STZ-treated rats by measuring

fasting blood glucose levels after 48 hours of STZ injection using One-Touch Horison glucometer, with gluco-strips (Ortho-Clinical Diagnostics, Johnson and Johnson Company, USA). After 24 h of STZ + nicotinamide injection, animals were given 5% w/v of glucose solution (2 ml/kg b.w.) to prevent initial drug-induced hypoglycemic mortality. Rats with fasting blood glucose of more than 200 mg/dl were considered as diabetics.

The diabetic rats were divided randomly into group- II to group- V. The ethanolic extract of the whole plant of T. procumbens Linn. (Asteraceae) doses were selected form previously published reports (i.e., 250 and 500 mg/kg). The standard (glibenclamide) and investigational drugs were suspended in 0.5% w/w carboxymethyl cellulose (CMC) and administered once daily through oral gavage for 21 consecutive days. The blood sample (few drops) were collected on 1st, 7th, 14th, and 21st day from the tail vein of rats by pricking with sharp tip needle and immediately used for the estimation of blood glucose with glucometer. Weekly body weight variations were monitored for all the experimental animals. At the end of the experiment blood sample was withdrawn from all the experimental animals through retro-orbital plexus puncture, and serum was separated. The serum was used for estimation of the biochemical parameters.

2.2.5 ANTICANCER ACTIVITY

The effect of anti-cancer activity of Tridax procumbens flower crude extract in aqueous and acetone on prostate epithelial cancerous cells PC 3 was determined by measuring cell viability by MTT assay. The experiment consists of cleavage of the soluble yellow coloured tetrazolium salt MTT [3-(4, 5-dimethyl – thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide] to a blue coloured formazan by the mitochondrial succinate dehydrogenase. The flower extract of acetone showed 82.28% cancer cell death with in 24hrs and aqueous extract exhibited a very weak anticancer activity.

The present study reports the effects of 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 complex S. alata- 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 procumbens* (Ceasalpiniaceae)on the polyamines produced by Leukaemia cells).

Hepatoprotective Activity

The hepatoprotective activity of aerial parts of *Tridax* shows significant protection in alleviation of DGalactosamine/ Lipopolysaccharide (D-GalN/LPS) induced hepatocellular injury 7. D-GalN/LPS have been proposed to be hepatotoxic due to its ability to destruct liver cells. The multifocal necrosis produced by D-GalN and the lesion of viral hepatitis in humans are similar. This amino sugar is known to selectively block the transcription and indirectly hepatic protein synthesis and as a consequence of endotoxin toxicity, it causes fulminant hepatitis within 8 hr after administration.

Immunomodulatory Activity

Ethanolic extracts of leaves of *Tridax* have immunomodulatory effect on Albino rats dosed with *Pseudomonas aeruginosa* also inhibits proliferation of same 16. Also a significant increase in phagocytic index, leukocyte count and spleenic antibody secreting cells has been reported to ethanol insoluble fraction of aqueous extract of *Tridax*. Stimulation of humoral immune response was also observed along with elevation in heamagglutination antibody titer. Study also revels that *Tridax* influences both humoral as well as cell mediated immune system 15.

Wound Healing Activity Wound healing involves a complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma-derived proteins all coordinated by an array of cytokines and growth factors 19. *Tridax* antagonized antiepithelization and tensile strength depressing effect of dexamethasone (a known healing suppressant agent) without affecting anticontraction and antigranulation action of dexamethasone. Aqueous extract was also effective in increasing lysyl oxidase but to a lesser degree than whole plant extract. Further it has been shown that extract of leaves of this plant also

promotes wound healing in both normal and immunocompromised (steroid treated) rats in dead space wound healing model. The plant increase not only lysyl oxidase but also, protein and nucleic acid content in the granulation tissue, probably as a result of increase in glycosamino glycan content.

Miscellaneous

The cardiovascular effect of aqueous extract from the leaf of *Tridax* was investigated on anaesthetized *Sprague-Dawley* rat. The aqueous extract caused significant decreases in the mean arterial blood pressure in a dose-related manner, i.e. the extract caused greater decrease in the mean arterial blood pressure at higher dose than at lower dose also higher dose leads to significant reduction in heart rate where as lower dose did not cause any changes in heart rate.

It means that a leaf of *Tridax* has hypotensive effect 9. In one study, essential oils extracted by steam distillation from leaves *Tridax* were evaluated for its topical repellency effects against malarial vector *Anopheles stephensi* (*An. Stephensi*) in mosquito cages. All essential oils were tested at three different concentrations (2, 4 and 6%). Of these, the essential oils of *Tridax* exhibited relatively high repellency effect (>300 minutes at 6% concentration) and concluded that *Tridax* are promising as repellents at 6% concentration against *An. Stephensi* 14. *Tridax* also reported for its anti inflammatory and anti oxidant activity when DPPD (2,2 –diphenyl-1-picrylhydrazyl hydrate) and HET –CAM (Hen's egg chorioallanthoic membrane) assay were done 17. Leaves of *Tridax* are used for promotion of hair growth also it is reported for its preventing effect on falling of hairs but this part is open for research work 11, 12. Interestingly phytoremediation technology is used for the removal of Cr (VI) in industrial wastewater and *Tridax* used as bioadsorbent. Also *Tridax* has been used for bronchial catarrh, dysentery, diarrhoea and in the West Africa sub-region and tropical zone of the world, Traditional medical practitioners and the native peoples of these areas use the leaves of the plant as a remedy against conjunctivitis 17 Chromium (VI) is one of the highly toxic ions released into the environment through leather processing and chrome plating industries. 97 percent Cr (VI) removal in synthetic wastewater sample was achieved when 5g of the bioadsorbent was used. This method is also applied to the removal of Cr (VI) from tannery industry wastewater. Hence, it is recommended that, this bioremediation technology is a cleaner and useful methodology for the removal of Cr (VI) from the industrial wastewater 18

2.2.12 IMMUNO STIMULATING AGENT

The plant *Tridax procumbens* 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. Thus, the plant *Tridax procumbens* 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, Cassia alata (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).

2.2.16 ANTI-CORROSIVE EFFECT

The inhibition effect of *Tridax procumbens* 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 Cassia alata leaves extract on Mild steel in 1.0N Hydrochloric acid).

2.2.18 ANTI INFLAMMATORY ACTIVITY

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

2.2.19 HEPATOPROTECTIVE ACTIVITY

Aqueous extract of the leaves of *Tridax procumbens* has hepatoprotective activity (.Effraim KD et al. 1999, Antihepatotoxic activity of aqueous extract of *Cassia alata* (Linn) leaves against carbon tetrachloride induced liver damage in rats.) Tridax procumbens 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 *Cassia alata* in Rats Exposed to Carbon Tetrachloride. J Appl Sci Environ.)

2.2.20 ANTI-CRYPTOCOCCUS ACTIVITY

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

2.2.21 INSECTICIDAL ACTIVITY

Hexane extract of Tridax procumbens 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 *Cassia alata* against bio-molecules and enzymaticparameters of *Callosobruchuschinensis* L).

2.2.22 BRONCHORELAXANT EFFECT

Aqueous-ethanolic extract of *Tridax procumbens* produce relaxation of tracheal smooth muscles exhibits broncho relaxant effect(Ouédraogo et al, 2013, Evaluation of the Bronchorelaxant, Genotoxic and Antigenotoxic Effects of *Cassia alata* 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 procumbens* did not induce DNA migration(Ouédraogo et al, 2013, Evaluation of the Broncho relaxant, Genotoxic and Antigenotoxic Effects of *Cassia alata* L. Evidence-Based Complementary and Alternative Medicine).

2.2.24 ANALGESIC ACTIVITY

Kaempferol 3-O-sophoroside was isolated from the leaves of *Tridax procumbens* exhibited analgesic activity (Owoyale J A et al, 2005 ,Antifungal and Antibacterial Activities of an

Alcoholic Extract.The hexane, chloroform and ethyl acetate extract of the leaves of exhibites analgesic activity(Irene et al, 2002, Bioactivity studies on *Cassia alata*Linn. leaf extracts).

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 procumbens* 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 procumbens* for my research work to see whether the leaves have antioxidant and anti-diabetic and antimicrobial activity or not.

3.2 PLANT COLLECTION

After selection of plant it is must to collect the plant parts for the research purpose. But the plant *Tridax procumbens* is not available throughout the bangladesh. The plant sample was collected from Jhalokathi District, under Barisal division on 9th October, 2014.

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).

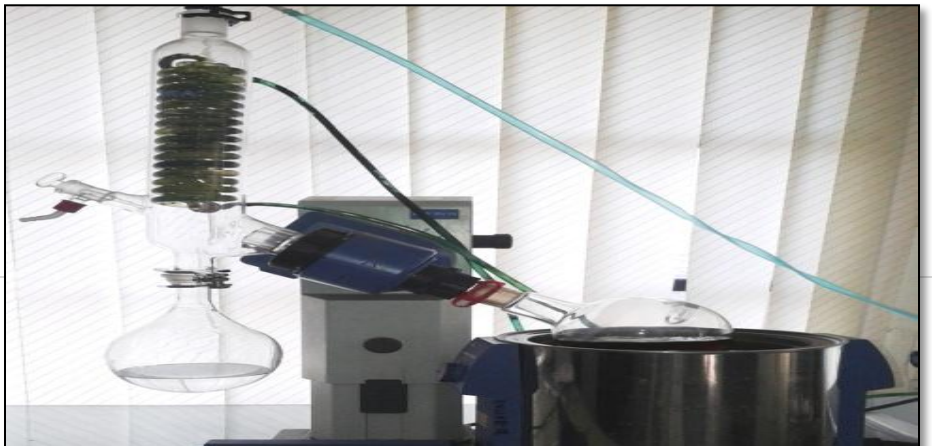


Figure 3.5.1: Rotary Evaporato

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 vacuum/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.

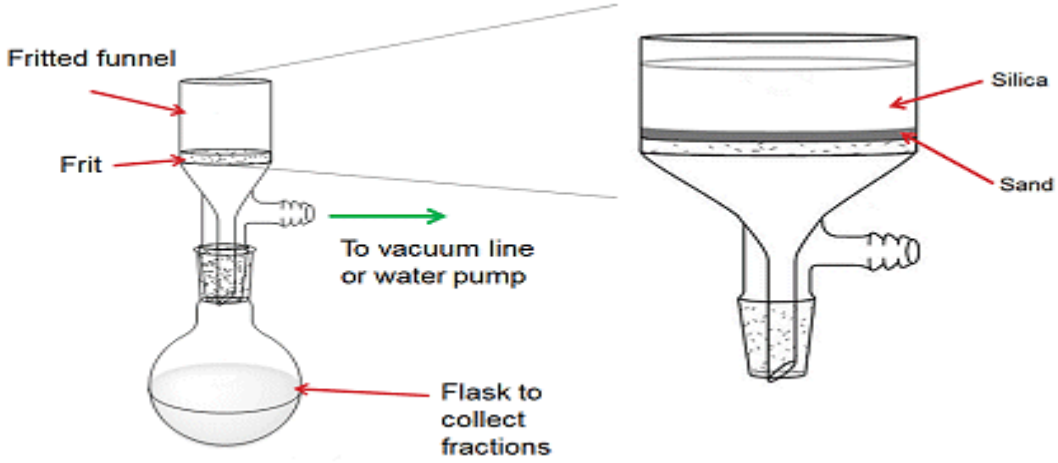


Figure 3.6.1: Vacuum Liquid Chromatography

3.6.2 Apparatus

- VLC chamber.
- Filter paper

3.6.3 Reagents

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

3.6.4 Procedure

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

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, n-Hexane, 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
- N-hexane
- Di-chloromethane
- N-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

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

1. 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.
2. 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.
3. 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.
4. Biochemical analysis*:* TLC is extremely useful in isolation or separation of biochemical metabolites or constituent from its body fluids, blood plasma, serum, urine etc.
5. 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.
6. In pharmaceutical industry*:* Various pharmacopoeias have adopted TLC technique for detection of impurity in a pharmacopoeial chemical.

7. Various medicines like hypnotics, sedatives, anticonvulsant tranquillisers, antihistaminics, analgesics, local anaesthetics, steroida have been tested qualitatively by TLC method.
8. One of the most important application of TLC is in separation of multicomponent pharmaceutical formulations.
9. In food and cosmetic industry , TLC method is used for separation and identification of colours, preservatives, sweetening agent, and various cosmetic products.
10. This are some of the applications of Thin layer Chromatography (TLC)

4.4 Advantages of TLC technique

1. TLC is very simple to use and inexpensive.
2. Undergraduates can be taught this technique and apply its similar principles to other chromatographic techniques.
3. 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.
4. More than 1 compound can be separated on a TLC plate as long as the mobile phase is preferred for each compound.
5. The solvents for the TLC plate can be changed easily and it is possible to use several different solvents depending on desired results.
6. 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.
7. 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 Disdvantages of TLC technique

1. TLC plates do not have long stationary phases. Therefore, the length of separation is limited compared to other chromatographic techniques.

2. Also, the detection limit is a lot higher. If one would need a lower detection limit, one would have to use other chromatographic techniques.
3. 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 Spots: 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.

1. 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.
2. Not enough solvent. There should be enough solvent (depends on size of chamber) to travel up the length of the TLC plate.
3. Plate is not cut evenly. It is recommended that a ruler is used so that the plate is cut evenly.
4. Rarely, water is used as a solvent because it produces an uneven curve front which is mainly accounted for by its surface tension.

5. 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.
6. 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:

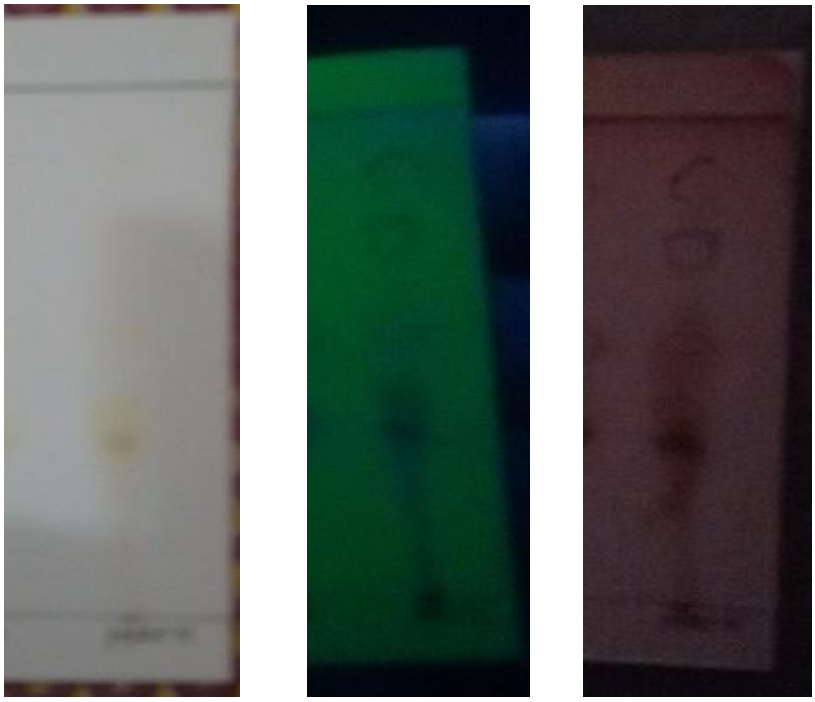
1. 0.4% solution of DPPH was prepared by adding 9 ml of methanol to 1 ml of 4% DPPH stock solution. The procedure was carried out in a dark room as DPPH is light sensitive.

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).

5.1 THIN LAYER CHROMATOGRAPHY(TLC)

TLCs were conducted on methanolic extract of the leaves of *Tridax procumbens* by using all the three types of solvent systems, and the best results were obtained by using the non polar solvent system. The pictures of the plates that were developed are displayed below:



1 2 3

Figure 5.1: (1)TLC plate in naked eye view,(2) TLC plate Under UV light,(3)TLC plate after charring with H_2SO_4.

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:

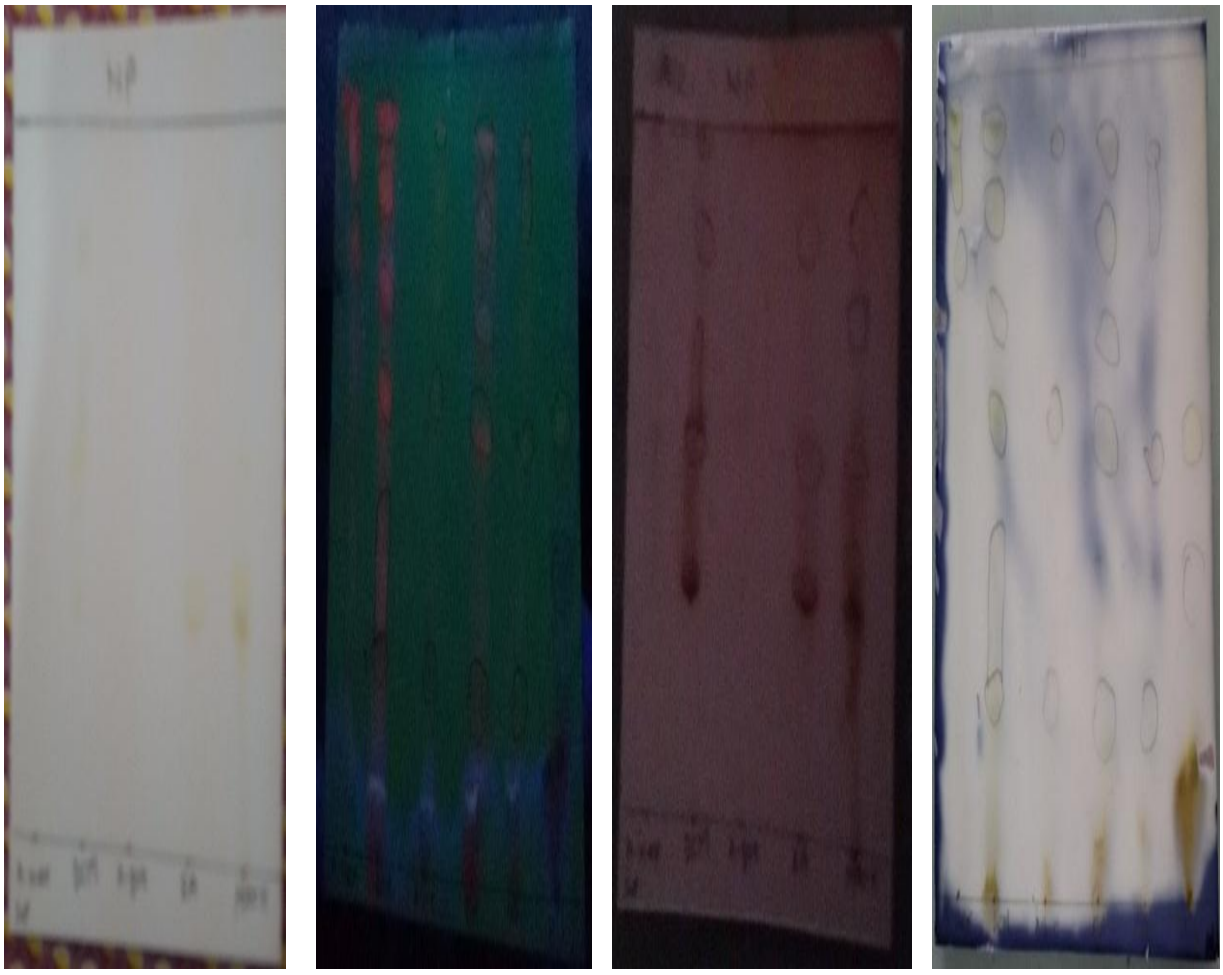
$$R_f = \frac{\text{Distance spot travels}}{\text{———————————}}$$

Distance solvent travels

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

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

5.2 Thin layer Chromatography of Methanolic Extract of *Tridax procumbens* 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

$$R_f = \frac{\text{Distance spot travels}}{\text{Distance solvent travels}}$$

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
N-hexane	0.84	0.94	
DCM	0.26	0.39	0.84
n-Butanol	0.24	0.57	0.92
Ethyl Acetate	0.21	0.55	0.68
Methanol	0.21	0.53	0.79

DISCUSSIONS

6.1 Thin layer chromatography

6.1.1 Discussion

TLC plates were developed with n-hexane,Dichloromeehane,n-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 *Tridax procumbens.*. 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(without n-hexane,which showed at least two spot),Dichloromeehane,n-Butanol, ethyl acetate,Methanol showed at least three spot.(plate-4)Some spots was found after TLC plates were dipped in DPPH solution

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 procumbens* ,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),Ethyl acetate and Methanol fraction of Tridax procumbens leaves give 84%,77% and 77% antioxidant activity.

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 procumbens* 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 procumbens* 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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