STUDIES ON THE LEAVES OF *Cardiospermum halicacabum* L. AND EXPERIMENTAL ASSESSMENT OF LIPID ABSORPTION INHIBITION AND CARDIO PROTECTION OF ITS BIOACTIVE INSULINOMIMETIC PINITOL

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for the award of the Degree of

MASTER OF PHARMACY

IN

BRANCH III - PHARMACOGNOSY

Submitted by

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MAY 2018

CERTIFICATE

This is to certify that the dissertation entitled "**STUDIES ON THE LEAVES OF *Cardiospermum halicacabum* L. AND EXPERIMENTAL ASSESSMENT OF LIPID ABSORPTION INHIBITION AND CARDIO PROTECTION OF ITS BIOACTIVE INSULINOMIMETIC PINITOL"** is a bonafide work done by **Mrs. R.SUGANYA. (Reg.No:261620709) DEPARTMENT OF PHARMACOGNOSY, COLLEGE OF PHARMACY, MADURAI MEDICAL COLLEGE, MADURAI-625 020** in partial fulfillment of the University rules and regulations for the award of **MASTER OF PHARMACY IN PHARMACOGNOSY** under my guidance and supervision during the academic year 2017-2018.

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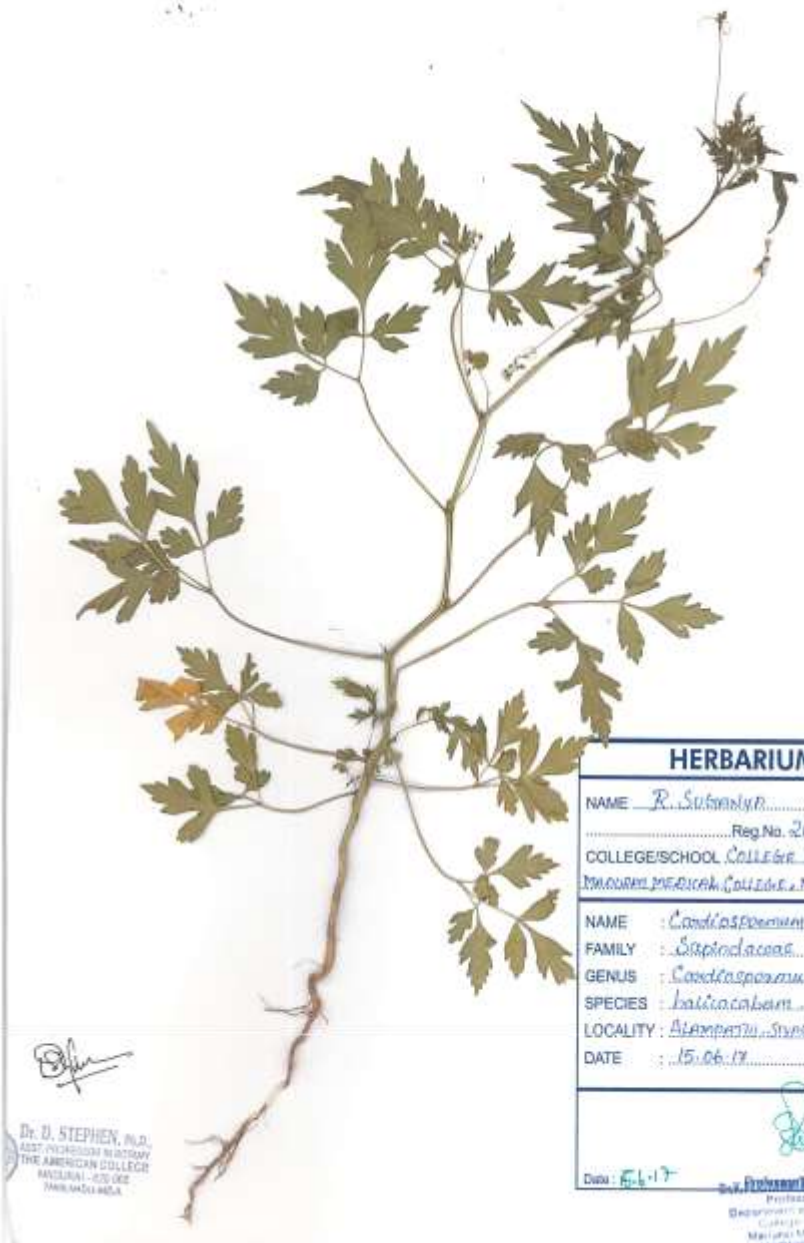

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DEDICATED TO MY PARENTS ,DAUGHTER AND GUIDE

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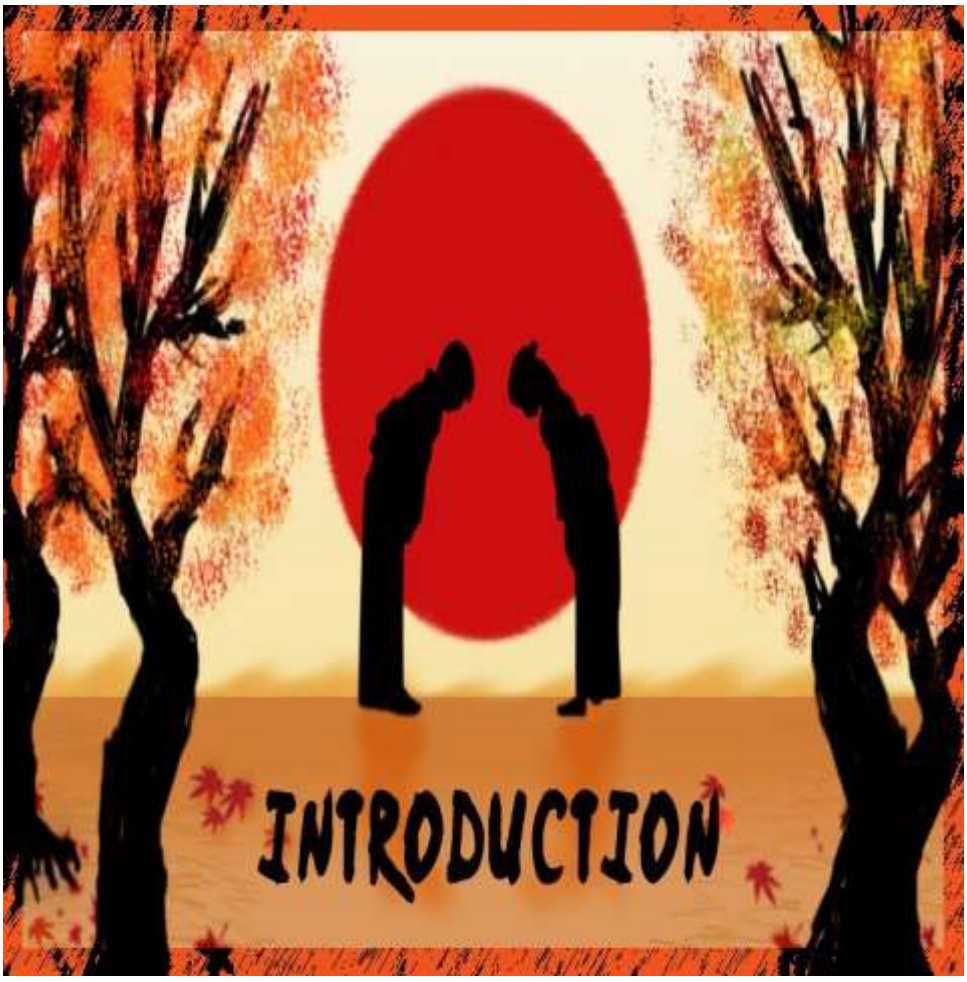

INTRODUCTION

INTRODUCTION

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to various culture, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, treatment or improvement of physical and mental illness (www.who.in/medicine.accessed). Traditional medicine (TM) is a term used to indicate both to systems such as traditional Indian Ayurveda, Chinese medicine and Arabic unani medicine, and to various forms of indigenous medicine. In countries where the outmost health care system is based on allopathic medicine, or where TM has not been implemented into the national health care system, TM is often termed **"complementary", "alternative" or "non-conventional" medicine** .

The connection between TM and biodiversity are exemplified as earth's natural system along with heritage of healing powers, whether this involve medicinal plants and animal species, the ambient invigorating air, spring water or the natural scenery. The pharmacopoeia of folk seties as well as other medical systems like Ayurveda, Chinese, biomedicine and Unani contains thousands of medicament from herbs, leaves, bark, roots, animal, mineral substances and other materials which are found in nature (Geslar, WN 1992).

TRADITIONAL MEDICINE ALL OVER THE WORLD:

Plants have been one of the important sources of medicines since the beginning of human civilization. For instance, the Chinese drug Mahung was being used for over 5000years for the treatment of different types of fever and respiratory tract disorders. African Traditional Medicine (ATM) provides holistic treatment. The medicament which was used in Africa can be administered in the form of decoctions, infusions

etc.(liquid), powder (solid), balsam (semisolid) or steam inhalation, incense etc. (gas). The only route of drug administration which is not present in TM in Africa is the intravenous route. E.g., *Ageratum conyzoides* is commonly used in TM for dressing wounds and ulcers, for cabbies and as an eyewash, *Zanthoxylum zanthoxyloides* a chewing sticks shows antimicrobial activity against oral micro flora, *Rauwolfia vomitora* roots in treating some mentally ill patients etc. Along with TM, the western or allopathic systems were followed in India and Pakistan. Their traditional medicine is divided into two types a) Unani and b) Ayurvedic. Both these systems have their from Mediterranean. Tibb is another system which was a combination of Hindu and Muslim system, was brought in to Unani systems. Hence this is known as Unani-Tibb or simply as Unani. '*Hakims*' are the traditional healers of Unani and have used St. John's wort in their remedies for run down conditions and depressions.

For thousand years ago, the knowledge of medicine on Indian subcontinent is termed as Ayurveda. Ayurveda still remains as the most important system of medicine and drug therapy in India. The Materia medica of all these medical systems consist of many herbs made into pills, syrups, confections and alcoholic extracts, and also some metals (Evans, WC 2009). Siddha is the system found with Dravidian culture of prevedic period which is largely therapeutic in nature. The causative factors of the disease is identified through pulse reading, body colour, voice study, examination of urine and tongue examination (Kokate, CK, *et al.,* 2006).

FUTURE OF TRADITIONAL MEDICINES:

By looking forward, it is very clear that rich collaboration of traditional and allopathic system of medicine benefits both disciplines. There is growing interest in this partnership, as shown by number international journals now dedicated to such research, such as Complementary Therapies in Medicine, CAM, Alternative and

Complementary Medicine (Singh seema, 2007). Even the WHO has taken note of the rich possibilities of the future of TM, having put out such documents as 2018 Who General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines. It is well established that TM plays a vital role in health care for a greater part of the population living indeveloping countries. For centuries, TM was the one and only health care system used to the prevention and treatment of diseases in various cultures. The interfaces among public health, TM and biodiversity conservation encompass a number of relevant and contemporary issues which are becoming increasingly apparent, as exemplified by WHO's aim in medicines: "**To help save lives and improve health by ensuring the quality, efficacy, safety and rational use of medicines, including traditional medicines, and by promoting equitable and sustainable access to essential medicines, particularly for the poor**."

USES OF HERBS IN TM

Numerous case series and reports of heavy metal poisoning associated with the use of Traditional Chinese Medicines (TCMs) have been published in WHO. WHO has insisted the usefulness of scientific investigations into indigenous herbal medicines, and many countries look into their native medicinal plants as possible additions to the WHO list of "essential drugs", once their value has been proven clinically. It is known that several infectious diseases can be transmitted from animals to humans (i.e. zoonoses). Hence, the possibility of transmitting infections or ailments from animal preparations to the patient should be considered seriously. Many tissues and organs including bones and bile can be a source of *Salmonella* infection which causes chronic diarrhoea and endotoxic shock. From the traditional scientific study of remedies, a large number of natural products have come to us and most of them

being plant derived. It is widely accepted that the presence of a biologically active constituent(s) in a plant is from folk or from ethno medical information. In other words, folk or ethno medical information represent 'leads' that could shortcut the discovery of modern medicines. A significant portion of the currently available natural and/ or semisynthetic pharmaceuticals in clinical use consist of drugs which is derived from higher plants followed by microbial, animal and mineral products, in that order (Remulo, RN 2007).

ETHNOBOTANICAL EVALUATION:

The primitive societies for their health management treatment of various diseases depended on herbal remedies from time immemorial, which was developed by observations, trail, error and experimentation methods. India with vast ethnic groups with rich biodiversity has centuries of old ethno botanical heritage for the promotion of health and treating minor illness. The scope, concepts and implication of ethno botany have been very fastly expanding and their knowledge has practical relevance for searching new sources of herbal medicines. During the last decades, a procession of so called wonder drugs eg. Reserpine, rescinnamine, quinine, ephedrine, cocaine, emetine, khellin, colchicine, digoxin, artemisinin, phodophyllotoxin, gugulipid, taxols, ajmalicine, vinblastin,vincristine etc. have been discovered from ethno medicinal plants with rich ethno botanical lore in tribal and aboriginal societies.

CONTRIBUTION OF MEDICINAL PLANTS:

Herbal drugs constitute the main stay of complementary medicines. As the cost of most of the western medicines lies beyond people's affordability, millions of world population in the developing countries solely depends on herbal medicines. Since the

population of herbal medicines is increasing in developing countries, herbal drugs are now entering the mainstream of medical care (Mukerjee, PK *et al.,* 2003).

Alternative therapies include homeopathy, acupuncture, energy healing, herbal medicines, folk medicines, and message to name only a few. Millions of people have used and will always use herbal drugs because they have faith in them and regard them as “their’ medicine, in contrast to the “allopathic” system of medicine brought in from “outside”. The traditional practitioners of medicine who are the part of community prescribe medicinal herbs which are locally available and the patient feels comfortable in their presence (Manuchari, EB *et al.,* 2001).

Herbal drugs which formed the fundamental of health care all over the world since the earliest days of mankind are still being used, and have considerable importance in international trade. Clinical, pharmaceutical and economic value of herbal drugs are still growing, although this differs widely between countries.

RESURGENCE OF HERBAL DRUGS:

Still now a large proportion of the rural population depends on herbs as medicine for the treatment of diseases because of its availability and affordability. The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted medical practice as an integral part of their culture (Farnsworth, NR *et al.,* 1991).

CREDITS OF HERBAL MEDICINES OVER MODERN SYSTEM OF MEDICINE:

Natural products like plants, animals and minerals have been the basis on the treatment of human diseases. Modern medicine or Conventional Western System of Medicine has gradually developed due to the efforts of scientists – however, the basis of its development remains in the roots of TM and therapies (Bhusan, P 1992). A large number of drugs have come out from Ayurvedic experimental base e.g. *Rauwolfia* alkaloids -hypertension, Psovalens - vitiligo, *Holarrhena* alkaloids -amoebiasis, Guggulsterons - hypolipidemic agents, *Mucuna pruriens* - Parkinson, Piperidines - bioavailability enhancers, Baccosides - mental retention, Picrosides- hepatic protection, Phyllanthins - antivirals, Curcumines - inflammation and so on.

DRUG DISCOVERY FROM MEDICINAL PLANTS:

Based on the information obtained from the traditional practitioners roughly 121 pharmaceutical products have been discovered during the last century. The best example is periwinkle, well known example of African plant used in African Traditional Medicine for diabetes. The traditional use of the plant had led to commercially marketed product Convinca in South Africa and Inclusion in England for treatment of diabetes. Based on the uses of *Catharanthus roseus* as a folk cure for diabetes, Eli Lily Company during 1950s discovered its well-known cancer alkaloids (Boer de, HJ 2008).

Considerable research on Pharmacognosy, phytochemistry, clinical therapeutics and pharmacology has been worked out on herbal plants. Several preclinical and clinical studies have examined cytoprotective, immunomodulatory and immuno adjuvant potential of Ayurvedic medicines. The development of TM with the

prospective of efficacy, safety, and quality will not only preserve the traditional heritage but also to use of herbal products in the health care.

HIGH-THROUGHPUT SCREENING (HTS) IN THE NEWER DRUG DEVELOPMENT:

HTS techniques are used mostly in the pharmaceutical industry in support of lead projects whose aim is to efficiently sort through enormous numbers of compounds for leads, the starting chemical structures for the drug development process. Organic small molecules from large libraries or natural products are batch-tested against biological targets in industry using standard 96- and 384-well plates, sometimes even higher density 1536- or 3456-well plates. As automation and robotics are increasing throughput ranges from thousands to ten lakhs samples tested per day which depend on the specific assay. Rate-limiting steps required for the assay often lie in the type of signal detection; simple fluorescence signals on the top end and cellular imaging assays at the bottom end of the throughput range. Although a lot of chemicals required to screen toxicity for lead generation is not in the range of the numbers of chemicals typically screened for drug discovery, the opportunity to broadly make an outline of compounds for toxicity with various assays efficiently and cheap cost makes an attractive approach for HTS. Therefore few chemicals investigated against a large number of assays, is the converse of the drug molecule discovery paradigm where **many molecules were examined against one biological target.** (Houck, KA 2008).

HIGH THOUGHPUT SCREENING *in Vivo* studies

In vitro screening method is based on simple interactions of chemicals with a drug target e.g. receptor binding or enzyme activity inhibition. As the complicated

physiological environment is not found in the *in vitro* systems, hence in vitro results poorly correlate with in vivo results. Even though cell-based assays provide reliable results, cultured cells do not give physiological environment and interaction between different cell types and tissues. Human tissues are now being used for drug discovery research. Even tissues provide an isolated ex vivo condition, it is not complete representative of *in vivo* response as the action of drug involves metabolism and they interplay among different tissues. For example, drug acting on muscle may involve intestinal absorption and metabolism by the liver.

Therefore results in animal studies are important to validate HTS (high-throughput screening) hits and exclude molecules with unfavorable ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties, which are responsible for more than half of compound attrition in expensive clinical trials.

Currently, *in vivo* studies are not usually done until or after the lead optimization stage. This is partly due to the low speed and high cost of animal models (typically rodents) and also due to high number of preliminary hits from HTS. As the small-animal models are emerging, it is now possible to perform *in vivo* testing .So researchers have developed animal model systems using both vertebrates like zebra fish and invertebrates like the fruit fly, *Drosophila melanogaster* and nematode like the *Caenorhabditis elegans* for drug screening. The small size, high fertility, and experimental tractability of these animals make a cost-effective and rapid screening of enormous compounds (Mac 2004).

BIOFLAVONOIDS:

Phenolic compounds constitute one of the main classes of secondary metabolites. They display a large range of structures and they are responsible

for the major organoleptic characteristics of plant-derived foods and beverages, particularly color and taste properties and they also contribute to the nutritional qualities of fruits and vegetables. The most important natural pigments are carotenoids which are tetrapyrrole derivatives of naturally occurring phenolic compounds ubiquitously distributed in plant kingdom.

Among these compounds, flavonoids constitute one of the most ubiquitous groups of all plant phenolics. So far, over 8,000 varieties of flavonoids have been identified1. Until ~50 years ago, information on the working mechanisms of flavonoids was scare. But it has been widely known for centuries that compounds of plant origin possess a broad spectra Like as phenolic acids, flavonoids are secondary metabolites of plants with polyphenolic structure. They are synthesized by the polypropanoid pathway and the startup component is phenylalanine molecule. The biological effects of these compounds vary. All flavonoids share the basic C6-C3-C6 structural skeleton, consisting of two aromatic C6 rings (A and B) and a heterocyclic ring (C) that contains one oxygen atom um of biological activity.

Flavonoids and phenolic acids make up one of the most pervasive groups of plant phenolics. Due to their importance in plants and human health, it would be useful to have a better understanding of flavonoid concentration and biological activities that could indicate their potentials as therapeutic agents, and also for predicting and controlling the quality of medicinal herbs. Plants and herbs consumed by humans may contain thousands of different phenolic acid and flavonoid components. The effect of dietary phenolics is currently of great interest due to their antioxidative and possible anticarcinogenic activities. Phenolic acids and flavonoids also function as reducing agents, free radical scavengers, and quenchers of singlet

oxygen formation. In addition, flavonoids and phenolic acids components play important roles in the control of cancer and other human diseases.

EFFECT ON CARDIOVASCULAR SYSTEM:

Vasorelaxant agent

The consumption of flavonoids may prevent endothelial dysfunction by enhancing the vasorelaxant process leading to a reduction of arterial pressure. Endothelial dysfunction represents a critical event in the development of cardiovascular diseases and the major complication of atherosclerosis and arterial thrombus formation. The consumption of flavonoids can prevent a number of cardiovascular diseases including hypertension and atherosclerosis. Recently, many experimental studies have shown that these polyphenolic compounds may reduce the arterial pressure in rats and enhance the vasorelaxant process. The endothelium-dependent relaxation induced by flavonoids has been well documented. Furthermore, Also investigators have demonstrated that *Anthocyanin delphinidin* exerts a significant endothelium dependent vasorelaxation.

Antiatherosclerotic effects

Oxidative modification of low-density lipoproteins (LDL) by free radicals is an early event in the pathogenesis of atherosclerosis. The rapid uptake of oxidatively-modified LDL via a scavenger receptor leads to the formation of foam cells. Flavonoids may directly scavenge some radical species by acting as a chain braking antioxidant. The ability of quercetin and the quercetin glycosides to protect LDL against oxidative modification has shown a significant protective demonstrated that *Anthocyanin delphinidin* exerts a significant endothelium dependent vasorelaxation.

Antithrombogenic effects

Platelet aggregation plays a pivotal role in the physiology of thrombotic diseases. Activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals which inhibit the endothelial formation of prostacyclin and nitrous oxide. It was shown in the 1960s that tea pigment can reduce blood coagulability, increase fibrinolysis, and prevent platelet adhesion and aggregation. Selected flavonoids such quercetin, kaempferol and myricetin were shown to be effective inhibitors of platelet aggregation in dogs and monkeys. Flavonols are particularly antithrombotic because they directly scavenge free radicals, thereby maintaining proper concentration of endothelial prostacyclin and nitric oxide. One study showed that flavonoids are powerful antithrombotic agents *in vitro* and *in vivo* because of their inhibition of the activity of cyclooxygenase and lipoxigenase pathways.

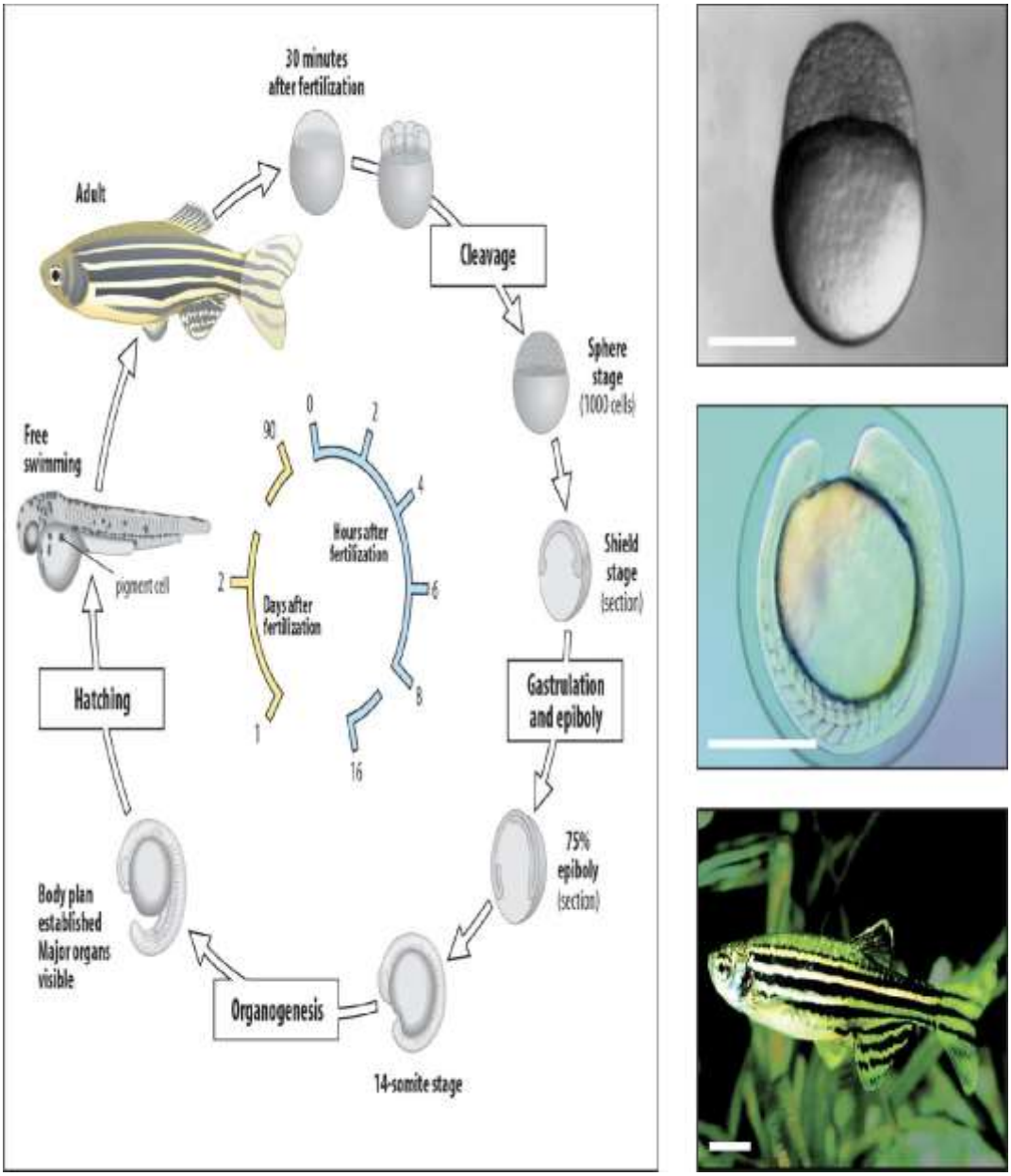
Cardio protective effects

Recent interest in flavonoids has been stimulated by the potential health benefits arising from the antioxidant activity of these ploy phenolic compounds. These are the result of their high propensity to transfer electrons, chelate ferrous ions, and scavenge reactive oxygen species58. Because of these properties, flavonoids have been considered as potential protectors against chronic cardio toxicity caused by the cytostatic drug doxorubicin. Doxorubicin is a very effective antitumor agent but its clinical use is limited by the occurrence of a cumulative dose-related cardio toxicity, resulting in, for example, congestive heart failure (negative inotropic effect). In a recent report, the cardio toxicity of doxorubicin on the mouse left atrium has been inhibited by flavonoids, 7- monohydroxyethylrutoside and 7',3',4'-trihydroxyethylrutosid.

ZEBRAFISH : A POWERFUL ANIMAL MODEL ORGANISM:

A powerful vertebrate model system is the zebra fish which has been used for the developmental pathways in genetic analysis and is only beginning to be exploited as an efficient model for human disease and clinical research. Hence this attribute have led to the usage of zebra fish as a preeminent embryological model, for forward and reverse genetic analyses which provides a unique way to uncover novel insights into the molecular genetics of cancer. The advantages of the zebra fish as an animal model system include fecundity, with each female capable of laying 200–300 eggs /week, external fertilization that permits manipulation of ***embryos ex utero****, and rapid development of* ***optically clear embryos***, which makes to observe the developing internal organs and tissues *in vivo* directly (Berghmans, S, *et al.,* 2005).

LIFECYCLE OF ZEBRAFISH



CAUSES OF VARIOUS CHRONIC DISEASES

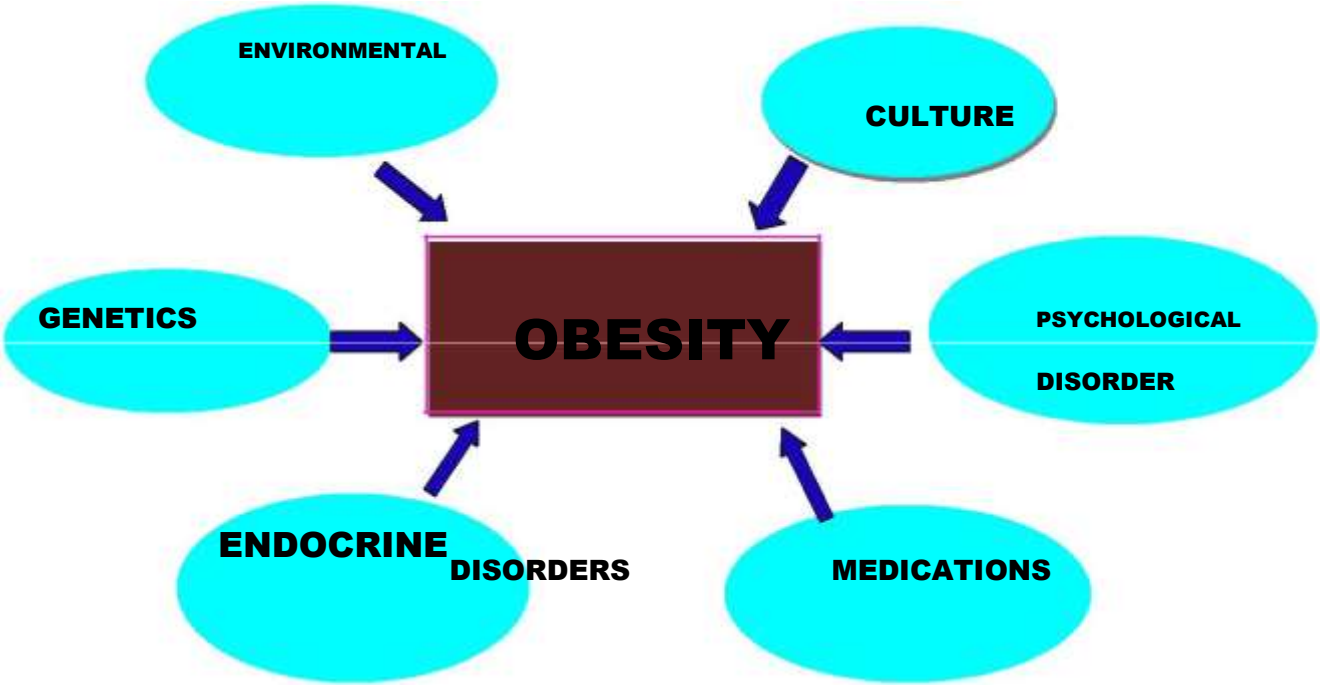
Various animal experiments have been developed to find out the effects of drugs on living organisms, dispersed cells and isolated tissues these give an idea about where and how a drug acts. The subject of ***'Drugs' is as old as disease***. From

the beginning of his existence illness has been his heritage and the search for remedies to combat it is equally old (Satoskar, RS *et al.,* 1997). For the survival of every individual and species, adequate nutrition is a fundamental requirement which remains the dominant activity in the lives of most creatures. In recent decades, economic improvement has been rewarded by plentiful nutrition unknown to previous generations, which has led to a pandemic of obesity with its serious consequences of health. Famine and malnutrition is still present for the less economically successful which is a huge global burden. Quantity as well as quality of food influences one's health. Inappropriate dietary intakes have been linked with various diseases like coronary heart disease and cancer mainly in developed countries.

According to law of thermodynamics, ***energy balance is achieved when energy intake is equal to energy expenditure****.* The Basal Metabolic Rate defines the obligatory energy expenditure which is required to maintain all metabolic functions in tissues and hence to sustain life (Nicholas, A, *et al.,* 2006).

COMPLICATIONS OF OBESITY:

Obesity is discouraged by the present modern generation for social as well as medical reasons. The incidence of ***diabetes mellitus, angina pectoris, hypertension, and myocardial infarction is higher among the individuals with obesity*** has increased dramatically in recent years for unclear reasons. Possible reasons include the automobile, the internet, television, a decrease in school based physical activity programs, fast food etc.



A growing number of medications cause weight gain in some or most of those patients from whom they are prescribed. Obesity gives rise to increased CVD risk by aggravating some of the CVD risk factors; including high blood pressure, low HDL cholesterol, insulin resistance and hypertriglyceridemia.

UNMODIFIABLE CARDIOVASCULAR DISEASE RISK FACTORS

Several CVD risk factors are essentially immutable, including older age, male gender, and a family history of CVD. Nonetheless, these risk factors are important to consider in evaluating risk in an individual patient.

RISK FACTORS

1. Cigarette smoking

Cigarette smoking along with hypertension and dyslipidemia, is Considered as one of the three major risk factors for Coronary Heart Disease (CHD) thromboembolic stroke and peripheral arterial disease (PAD).

2. Dyslipidemia

Dyslipidemia is probably a better term than hyperlipidemia because it includes all lipid and lipoprotein abnormalities, such as high level of low-density lipoprotein (LDL) and cholesterol, which can be a potent risk factor for CVD, with a strong dose-response relationship that is exponential at higher levels of cholesterol. Even though much of the inter individual variability in cholesterol is genetic, dietary intake of cholesterol, saturated fat, and trans-fatty acids increases the blood cholesterol. Total cholesterol is carried on three lipoproteins in the blood, resulting in three separate cholesterol fractions with differing prognostic significance:

1) Very low-density lipoprotein (VLDL) cholesterol
2) Low-density lipoprotein (LDL) cholesterol
3) High-density lipoprotein (HDL) cholesterol

LDL cholesterol is positively related to CVD incidence and HDL cholesterol is inversely related.

3. Hypertension

Blood pressure can be increased by a number of mechanisms. Increased relaxation and increased cardiac output may all contribute to hypertension in obesity. Renal sodium absorption is increased in patients with hyperinsulinemia which has been

proposed as a causative to hypertension in obesity via increased circulating blood volume. Abnormalities of vascular resistance may also contribute to the pathophysiology of obesity related hypertension. Under some experimentation conditions, elevated FFA levels have been found to cause increased vasoconstriction and reduced NO-mediated vasorelaxation, similar to that seen in the metabolic syndrome. It has also been suggested that there is an increased activity of the sympathetic nervous system in some obesity phenotypes, and that this contributes to obesity related hypertension (Goldman, L *et al.,* 2004).

TRADITIONAL MEDICINE TO MEET REQUIREMENTS OF BOTH DEVELOPED AND DEVELOPING COUNTRIES

Traditional medicine can provide new inputs into the drug development strategy. For example the Medical Research Council of South Africa carried out an investigation on traditional medicine used by communities for the treatment of fevers with the aim of invention of new drug leads to treat malaria.

One such strategy of CSIR involves a method called as “reverse pharmacology” in which starting with natural products and work backward to identify its active ingredients. So it is vital to consider the relative effectiveness of hese approaches to generating cost effective, safe herbal products for poor people.

REASON FOR THE STUDY AND SELECTION OF THE PLANT:

Based on the above discussions we focus our study to utilize the vast economic potentiality of a crop which can be fully established by its vast consumption, then employment generation for an agriculture worker throughout the year. It is evident that

there is good level of experimental evidence to support claims and advantages of various medicinal herbs used in our traditional diet and medicines. In this view we selected the widely available and neglected plant “Balloon vine” leaf *Cardiospermum halicacabum*. for our study.

The Plant is popularly known as Heart pea, Puff-ball, Heart seed Vine, Love in a puff. The scientific name of balloon vine is *Cardiospermum halicacabum* belong to the family Sapinadaceae.

C.halicacabum L. a green healer from backyard traditionally used in various diseases including Rheumatism, Lumbago, Cough, Hyperthermia, Nervous disease, Stifness of limbs and snake bite, etc., Though there is traditional and experimental evidences to support various claims and benefits of these plants still it needs proper evaluation and exploitation.

A study aims to scientifically explore its important medicinal uses which have not been fully studied is inevitable. These initiated us to investigate the leaves of this plant with strict scientific protocols so that the vast economic potentiality of this crop can be adequately established by its consumption, opportunity for an employment to an agricultural worker throughout the year. Literature survey revealed lacunae in the pharmacognostic, phytochemical and pharmacological studies in this plant.


LITERATURE REVIEW

CHAPTER II

LITERATURE REVIEW

PHARMACOGNOSY

TAXONOMICAL CLASSIFICATION (Anonymous 2011 Floras: Flora of China, Ndatabase (version 2011)

Kingdom	:	Plantae –plantes,planta,vegetal,plant
Subkingdom	:	Viridiplantae
Infrakingdom	:	Streptophyta-land plants.
Superdivision	:	Embryophyta
2011Division	:	Tracheophyta-vascular plants, tracheophytes.
Subdivision	:	Spermatophytina-spermatophytes, seed plants phanerogames.
Class	:	Magnoliopsida.
Superorder	:	Rosanae.
Order	:	Sapindales
Family	:	Sapindaceae –Soapberries
Genus	:	*Cardiospermum*
Species	:	*halicacabum*

SYNONYMS (Subramanyam R *et al*., 2007, Krishna Murti *et al.,2*010).

Synonym of *C.halicacabum C. corundum* Lam; *C. glabrum* Schum. & Thonn.; *C. inflatum* Salisb.; *C. microcarpum* H.B. & K.; *C. microspermum* E. Mey; *C. villosum* Mill. ex DC

VERNACULAR NAMES (Krishna Murti *et al*.,2010, G.Ponmari *et al*.,2011, Raza S *et al.*,2013)

English	:	Balloon vein,Heart's pea.
Sanskrit	**:**	Sakralata,Indravalli.
Hindi	:	Kanphuti,Kapalphoti.
Bengali	:	Lataphatkari
Marathi	:	kanphuti, shibjal, kakumardanika
Gujarthi	:	Ghisoda.
Telugu	:	buddakakara, ekkudutige
Tamil	:	Mudakattan

ENTIRE PLANT:

ETHNOMEDICAL USE:

- *Cardiospernum halicacabum* decoction used in rheumatism, nervous diseases, pain and as diuretic. (Pillai.N.R and Vijayamma.N.,1985).
- It is used in Rheumatism, Lumbago, Nervous disease, Demulcent inorchitis and Dropsy.(Patil *et al*.,2011).(G.Ponmari *et al*.,2011).
- Traditionaly *C.halicacabum* highly useful in Ayurveda, Sidha, Homeopathy, Unani indian system of medicine to treat Rhumatoid Arthritis, GI disease, Respiratory disease, Inflammatory disease in India and China.(Shekhawat.M.S *et al*.,2012).

- This plant juice is useful in Amenorrhoea,Gonorrhoea,Asthma and Nervous system problem.The herbal decoction used for Rheumatism, Nervous illnesses, pain and diuretic.(Raza S *et al.,* 2013, Jayabalan.,2006)
- This plants are used in traditional medicine for treatment of Rheumatism, Lumbago, Cough, Hyperthermia, Nervous disease, Stifness of limbs and snake bite.(Jeyadevi R *et al.,* 2013).
- It is remedy for joint pain.young shoots are used as vegtable, fooder, diuretic, stomatchic and Rubefacient. (Shobana Devi.S *et al.,*2016). (shobana devi.S *et al* .,2016).

FORMULATIONS:

- Herbal products from this plant like Gel, Cream, Shampoo,Spray availble in market. These are all use to dry itching skin and scalp. Allergic liquid used for Hay fever allergies,sneezing,watery eyes and for skin disease such as inflammation, scaling, burning and pain. Cream for uing of Rheumatism, back ache, Ear ache and fever.(Patil A.G *et al* .,2010).
- This plant one of the ingredient in Allergy Relief Liquid and Bioforce pollinoson tabs marketed by Bioforce USA.Florasone cardiospermum cream for skin ailments by US based company.(Patil A.G *et al.,*2010).

PHARMACOGNOSY:

- *Cardiospermum* is the combination of the Latin words cardio, meaning heart, and sperma, meaning seed and refers to the white heart-shaped pattern on the seed .(Plants For A Future, 1996 -2003. Last modified: June 2004).
- Synonym of this plant *C. corundum* Lam; *C. glabrum* Schum. & Thonn.; *C. inflatum* Salisb.; *C. microcarpum* H.B. & K.; *C. microspermum* E. Mey; *C.*

villosum Mill. ex DC.(Subramanyam R *et al*., 2007,Krishna Murti *et al.,2*010).

- Balloon-vine is a perennial creeper at its base, the plant's stem is only approximately 3mm thick, but it can reach a height of up to 2 metres. The stem forms internodes of between 5-10 cm in length.The grooved stem carries alternate double triad leaves, 3 to 5 cm long, which are hairless or covered in a soft down of hairs. The oval or lanceate leaves have a deeply serrated or lobated edge. The leaflets at the side are smaller. The tiny radiate flowers are white,standing in rolls on long flower stems of 5 to 10 cm in length.(Krishna Murti *et al*.,2010) .
- Common names of this plant is Heart pea, Puff-ball, Balloon vine, Heart seed Vine, Love in a puff.(Krishna Murti *et al*.,2010).
- *C.halicacabum*, commonly known as Mudakathan in Tamil is also called as Kanphuti, Kapalaphoti in Hindi,Uzinja in Malayalam and Balloon wine in English which annually spread with tendril hooks.(G.Ponmari *et al*.,2011).
- *C.halicacabum L.* commonly known as "Balloon vine" , belonging to the family Sapindaceae.It is dioeecious, hairy climbing vine with balloon cluster of white flower and delicate foliage.(Patil e*t al 2010.,*).(G.Ponmari *et al.,*2011).
- *C.halicacabum* grows in plains of Africa,America,Bangaladesh,India and Pakistan.(Raza S *et al.,* 2013).(Selvarani S,Vinayagamoorthi and Rohini R 2015),(Dhayabaran D *et al.,* 2012).
- This plant is pubertal or almost glabrous yearly perpectually having slim twigs that climb by tendrillar hooks.(Raza S *et al.,*2013).
- It contains 16 species present in Brazil and 12 species are found in South America.This plant is an herbaceous climber 2-4m length,heart shaped,

commonly known as balloon vein.(Raza S *et al.,*2013).

- In England (Heartpea), Philippines (parol-paralon),China(jiahu gua), Tamilnadu-India(Mudakathan keerai).(Raza S *et al.*,2013).
- *Cardiospermum halicacabum* L. locally known as peria bulan in Malaysia . (Mohad Norfaizal *et al.,*2017).

PHYTOCHEMISTRY:

- The Pet ether extract of *C.halicacabum* contained sterols, carbohydrates, tannins and triterpenes.(Venkat Rao. N, Chandra Prakash.K,Shanta Kumar.SM.,2006).
- Sodium was not detected in the shoots, but other minerals such as Ca (1.30%), K (4.01%), Mg (0.43%), P (0.83%), Organic-N (5.19%), Total-N (7.16%), and C (48.1%) were present. (Subramanyam R, *et al*., 2007).
- Fingerprint profile of the bioactive Ethanolic extract of *C.halic*acabum by using HPLC , DAD method.In HPLC the mobile phase consist of water and Acetonitrile with 1% TFA Gradient program Acetonitrile 5% -25%-30% in 0-10-30-60 min. flavanoids and phenolic acids were detected by UV at 280 nm.(Ling cheng *et al.,*2013, Krishna Murti, *et al.,*2010).
- *C.halicacabum* extracts possessed high phenolic content, and exhibited strong free radical scavenging activity and ferric reducing proper(Annamalai A, Ponmari G, Sathishkumar Rand Lakshmi P.T.V.,2O11).
- Beta-Arachidic acid,apigenin,apigenin 7-o glucronides,Chrysoerial-7 Glucronide and 80 Luteolin 7-o glucronide two crystalline compounds beta-sistosterol and beta d-glucoside. Acetic acid,1,6,10-dode catriene,7,11 methyl-3-methylene ,phenol,2,6 bis methyl methyl carbamate,3-0-methyl-d-glucose 1,14-Tetra decanediol, 3,7,11,15,Tetramethyl-2 hexadecan

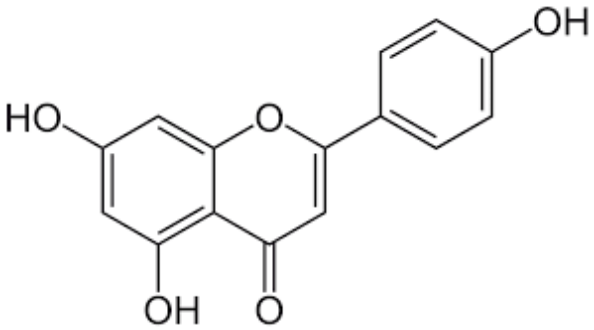
1-ol, Phytol,pseudo ephedrine,2-propenamide are presence in ethanolic extract.(Raza S *et al*.,2013).

- Rutin also present in this plant,which is a flavanols,quercetin and the diasaccharide rutinose.the biological activity of the plant was accounted for by saponins, alkaloids (+)-pinitol, apigenin, luteolin, chrysoeriol, quebrachitol, proanthocyanidine, stigmasterol, fatty acid, 11 eicosenoic acid triterpenoids, glycosides, sterols, tannins and flavanoids. (Raza S *et al*.,2013).
- RP- HPLC analysis done by photo iodide arrat Detcetors .Reverse phase column 4.6 X 250 nm. 5 micro meter particle size.The mobile phase used as water with 0.1 % formic acid(solvent -A) and 100% Acetonitrile(solvent-B) for identifying the phenolic compound.(Jeyadevi R *et al*.,2013).
- Now 18 Phenolic compounds identified in *C.halicacabum* using UPLC-ESI-Q-TOF-MS/MS.LC/MS/MS indicate presence of phenolic acids such as Chlorogenic acid,Caffeic acid, Coumaric acid and flavanoids like Luteoline 7-o glucronide , apigenine 7-0 glucronide and chrysoeriol.(Jeyadevi R *et al*.,2013).
- 17-compounds are present in ethanolic extract of *C. halicacabm* 1) quercetin 3-0-alpha - Rhamnoside 2)kaempferol 3-o - alpha-l-Rhamnoside 3)Apigenin 7-o-beta-d glucronide 4)Apigenin 7-0- beta d-glucronide methyl ester 5)Apigeni 7-o - beta d glucronide ethyle ester 6)Chrysoeriol 7)Apigenin 8)Kaempferol 9)Luteolin 10)Methyl 3,4 dihydroxy benzoate 11)p- coumaric acid 12)4-hydroxy benzoic acid 13)quarcetin 14)Protocathehuic acid 15)Gallic acid 16)Indole 3-carboxylic acid 17)Hydroquinone.(Ling cheng *et al.,*2013).
- The solvents such as butanol,acetone,methanol and petroleum ether of *C.halicacabum* was reported by using preliminary phytochemical

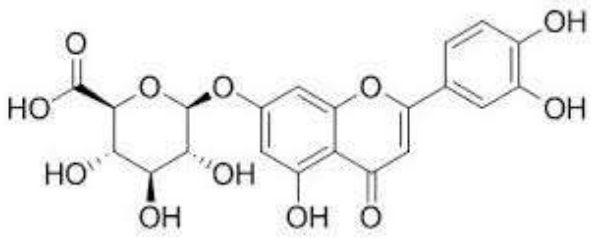
analysis,presence of alkaloids,phenols,tannins,saponins,terpenoids and glycosides.(Harborne J.B 1998,Sagadevan P *et al.,*2013).

- Diethyl ether, chloroform, acetone extracts of leaf, stem and seed coat of *Cardiospermum halicacabum L.* was analysed for the compounds such as tannin, saponin, flavonoid, steroid, terpenoids, cardiacglycosides,alkaloids, anthraquinones. (Annadurai.A,Elangovan .V,Velmurugan .S and Ravikumar R.,2013).
- Apigenin and Luteolin (secondary metabolites) are present in this plant.It was reported by using RP-HPLC method.(Selvarani s,Vinayagamoorthi P,and Rohini R 2015).
- *C.halicacabum* contains tannins,saponins ,flavanoids,glycosides and Cardiac glycosides.Aglycones,Triterpenoids,Varitey of fatty acids ,volatile ester confirmed by phytochemical screening.(shobana devi.S.,2016).
- The phytochemical screening of the plant was reported a positive test for presence of various phytoconstituents like alkaloids,flavanoidsand tannins.The amount of total phenol was found to be between 29.697±0.232 to 187.372 ±0.615 mg pyrogallol eqivalent/g in various extracts.(Mohaddesi B,Dudhrejiya A,Chauhan M 2016).

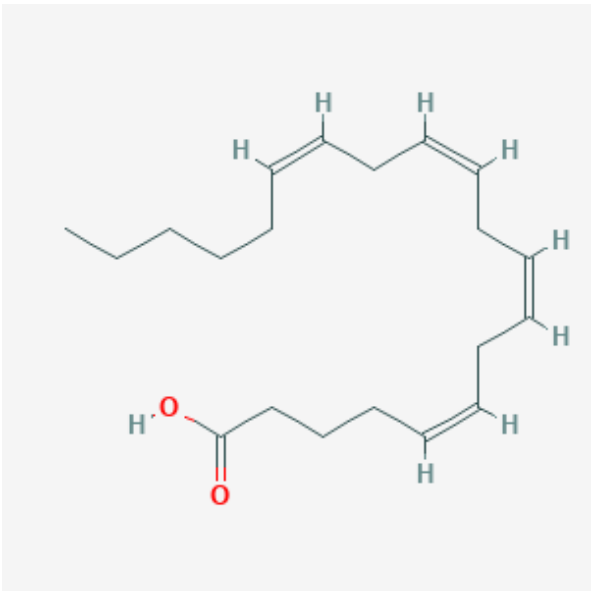
APIGENIN



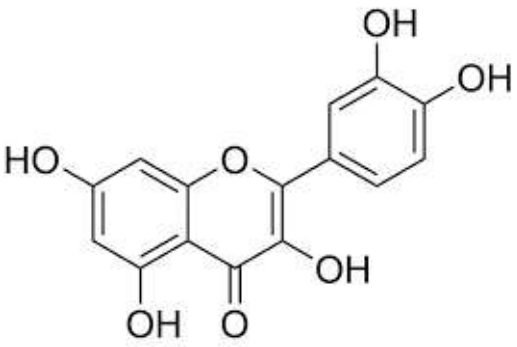
APIGENIN 7-O GLUCRONIDE



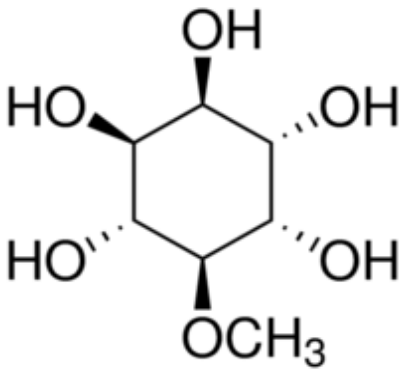
BETA-ARACHIDIC ACID

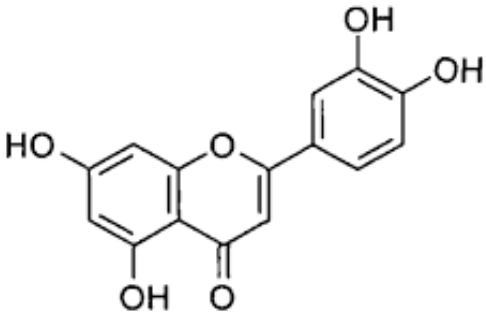


QUERCETIN

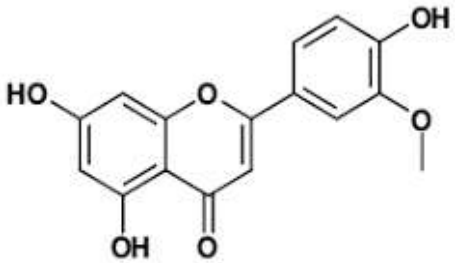


PINITOL



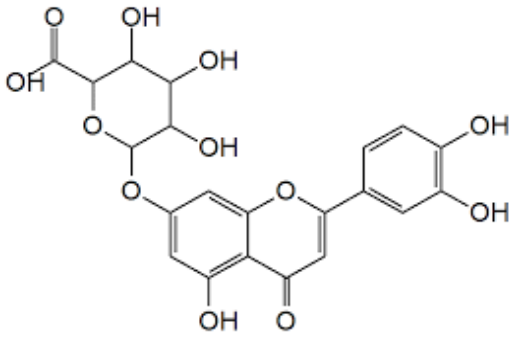


Luteolin

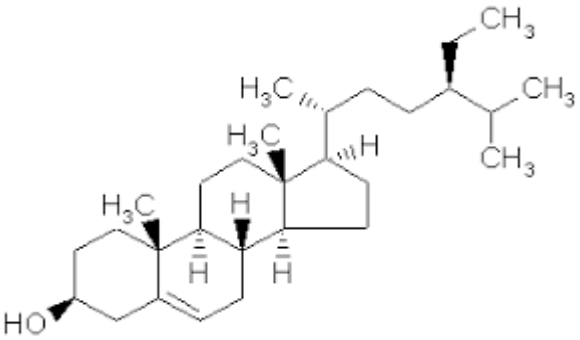


Chrysoeriol

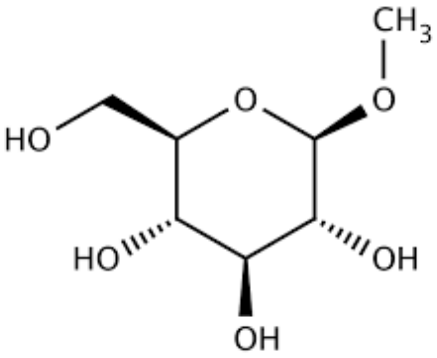
CHRYSOERIOL- 7 GLUCRONIDE



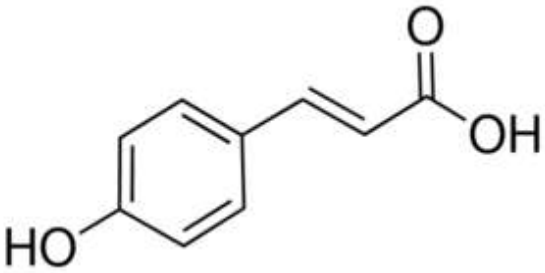
BETA SITOSTEROL



D-GLUCOSIDE

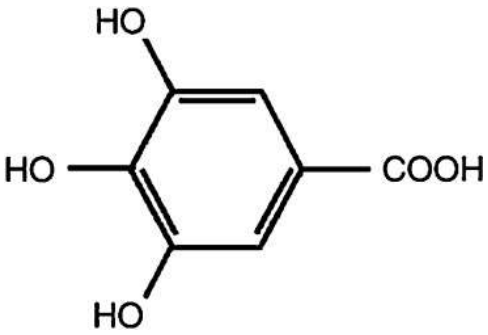


P-COUMARIC ACID



HYDRO QUINONE

GALLIC ACID



PHARMACOLOGY:

- Ethanolic extract of this plant affect the adult worm damage.It affect the motility of the microfilariae.(Khunkitti w *et al.,2000).*
- *Cardiospermum halicacabum* L. was reported it is a traditional Chinese herb (Ë¦a¹a) and found inhibiting histamine release and nitric oxide production.(Subramanyam R *et al.,*2007).
- The whole plant, roots and leaves of this plant are traditionally used as anxiolytic and as anticonvulsant.(Malaviya.S *et al.,2009).*
- *C.halicacabum* was reported to possess Antidiarrhoeal activity ,Antimalarial activity, Antiulcer activity, Anti-inflammatory activity.(Krishna Murti *et al*., 2010).
- *C.halicacabum* entire plant was reported to possess anti-inflammatory, antibiotic against many bacteria, antiparasitic,antipyretic and analgesic.It also used to reduce swellings and hardened tumors.It has non toxic Antifertility activity.(Patil *et al.,*2010).
- Extract of *C.halicacabum* used as antiulcer analgesic and vasodepresent activities.(Chang *et al*.,2013).(G.Ponmari *et al*.,2011).
- *C.halicacabum* extract was reported to possess high phenolic content, and showed strong free radical scavenging activity and ferric reducing property. Large quantity of phenolic compounds in *C.halicacabum* extract create it a strong free radical scavenger, which indicates that the extract has good potential as a source for natural antioxidants to prevent free radical mediated oxidative damage.(G.Ponmari *et al*.,2011).
- The hepatoprotective activity for the flavanone from *C.halicacabum* against liver injury induced by a toxic dose of acetaminophen.(Rupeshkumar .M, Kavitha.K, Basu.S.K.,2012).

- This Plant is reported to possess Anti- inflammatory activity by reducing PLA2 activity that is concerned with inflammatory process.The methanolic extract of the herb reduced the Hepatitis B surface Antigen (HBsAg).In vitro tissue experiment the herb shows Antispasmodic activity.(Raza S et al.,2013).
- Ethanolic extract of this herb is effective against Gastric ulcer in balb-c rats the herbal tea increases the blood levels of theophylline, so avoided the herbal tea for treated with theophylline because increases the bioavailability of the recomendation of the drug.(Raza S et al.,2013).
- On central nervous system the decoction of this plant showed sedative effect. It exhibited significant analgesic and anti-inflammatory activities. (Babu, Dr.Suthakaran R, Srinivas Reddy CH.,2014).

ANTIFUNGAL ACTIVITY :

- *C.halicacabum* extract inhibit against the fungi *C.albicans* .Alcoholic extract has high antifungal activity than chloroform.(Raza S et al.,2013)

ANTIPYRETIC ACTVITY:

- This plant active against yeast induced pyrexia in rats.(Asha and Ushpangadan, 1999).

ANTIFILARIAL ACTIVITY:

- Ethanol and aqoues extrct of this herb used as Antifilarial activity on mature worm and microfilariae of Brugiapahangi.(Khunkitt et al., 2000).
- In vitro effects of ethanol and aqoues extract of the medicinal plant of *C.halicacabum* on adult worm microfilariae of Brugia pahangi.Aqoues extract of this plant was reported adult worms were damaged but did not affect the motility of the microfilariae.(Veeramani.C *et al.,*2015)

ANTI DIARRHOEAL ACTIVITY:

- The whole plant extracts (i.e. petroleum ether, alcohol and aqueous) of *C. halicacabum* (Linn) contain tannins, flavonoids, saponins, sterols and triterpenes, which could have contributed to the antidiarrhoeal activity. (Venkat Rao. N, Chandra Prakash.K, Shanta Kumar.SM., 2006, Kurian,1995)

ANTI-INFLAMMATORY ACTIVITY:

- The anti-inflammtaory activity of ethanol extract against carrageenan-induced rat paw edema has been established . The ethanol extract of the plant suppresses the production of TNF-_ and nitric oxide in human peripheral blood mononuclear cells (Sheeba.M.S, Asha.V.V.,2009, Selloum et al., 2003)

ANTIMICROBIAL ACTIVITY:

- Acetone, alcohol, benzene, chloroform and aqueous extracts of leaf and stem of C.halicacabum have antimicrobial activity. (Viji.M, Murugesan.S., 2010).

- The antimicrobial activity of different fractions and oil of *Cardiospermum halicacabum* against twelve Gram positive and negative bacterial strains. *Cardiospermum halicacabum* represents strongest antibacterial activity against *Salmone paratyphi B.* (Shareef.H *et al.,*2012).
- The herbal leaf extract possesses antimicrobial activity against certain bacterial species i.e. *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* and more useful against *E. coli* (Deepan et al., 2012).
- Diethyl ether, chloroform and acetone extracts of seed coat of this plant have maximum inhibitory zone and chloroform extract of this plant stem

have a maximum inhibitory zone in pseudomonas. (Annadurai.A *et al.,*2013).

- *C.halicacabum* used as antimicrobial,anticancer,antiparasitic,antimalarial and antifilarial activity.Aqoues extract of this plant has antimicrobial activity,it active against Escherichia coli.Ethanolic extract of this plant has antimicrobial activity, it active against Staphylococcus areus (Gram positive bacteria). (Selvarani.S,Vinayagamoorthi.P,and Rohini.R *et al*., 2015).
- Now days its used as a antibacterial activity.It was reported active against clinical human bacterial pathogens.
    - Butanol extract → stem and root
    - Acetone extract → leaf and root
    - Methanol extract → seed
- Acetone extract has the highest antibacterial activity compare than methanol and petroleum ether.(Selvarani.S *et al*., 2015).

ANTI HYPERGLYCEMIC ACTIVITY:

- Anti-hyperglycemic effects against streptozotocin induced diabetic male Albino rat.Ethanollic extract of C.halicacabum supress the production of TNF alpha and NO in human pheripheral blood mono nuclear cells.This plant has antioxidant activity because of presence of phenolic compounds.(jeyadevi R *et al*.,2013).
- The juice of the herb is used to cure ear-ache and to reduce hardened tumours. . (Veeramani.C *et al.,*2015).

AERIAL PART

PHARMACOLOGY:

ANTI-INFLAMMATORY ACTIVITY:

- By rat- paw edema method(500 mg/kg) transudative exudative and proliferative components supression of chronic inflammation.(Raza S *et al.,* 2013).

ANTIMICROBIAL ACTIVITY:

- aerial parts have reported different biological activities like antimicrobial and antibacterial properties.(Mohaddesi.B,Dudhrejiya.A,Chauhan.M *et al.,*2016).

LEAF:

ETHANOMEDICAL USE:

- Decoction of leaf with jaggery and pepper is used for fever associated with cough (Sheeba.M.S, Asha.V.V.,2009).
- *C.halicacabum* leaf juice cures the ear ache and Haemorrhoids .Leaves and stalks of the plant are used as Diarrhoea and Dysentry .(Raza S et al 2013).

PHARMACOGNOSY:

MACROSCOPY:

- Leaves are alternate and have axillary tendrils.(Krishna Murti *et al*.,2010).
- Leaves are ternate biocomponent and leaflets acuminate at top. The leaflets are mostly 3-part and pinnately lobed having narrowed stalks.Leaflets are variable in shape ovate or narrowly ovate.Leaf stalk is long, ridged, slim and with tiny stipules at the base.(Raza s *et al .,*2013).

- Texture: Sparsely pubsecent .leaves alternated and compound, deltoids, 2-ternate, serrate, very acute appex and narrow base. This plant leaf has bitter taste (Patil *et al.,*2010).

MICROSCOPY:

- *C.halicacabum* dorsiventral leaf. Tissues are present in the midrib and lamina (shows upper epidermis).Xylem towards the dorssal surface phloem towards the ventral surface. (Patil *et al.,2010).*
- hypostomatic and anomocytic stomata, has non-glandular and glandular trichomes, and also secretory cells present in mesophyll sponge cells in the *C.halicacabum* leaves. Cuticle on the epidermal of leaf surface of the *C.halicacabum.* .(Mohd. norfazil.G *et al*.,2017,Patil *et al.,2010).*

MIDRIB:

- Outline: adaxial: protruding structure form into hump; abaxial side:U-shaped. closed system; consisting of adaxial and abaxial vascular bundles. Sclerenchyma cells: few layers of sclerenchyma cells present at the outer layer of phloem tissue. Parenchyma cells: parenchyma cells at the medullary área have are larger than other parenchyma cells found in the midrib. Collenchyma cells: layers of collenchyma cells present underneath epidermis at the adaxial side. Cells inclusión: druses, secretory cells present found in medullary and parenchyma cortex area. Trichome: simple unicellular, mainly at the abaxial side.(Mohd. norfazil.G *et al*.,2017).

LEAF LAMINA:

- Cuticular layer: relatively thick. Adaxial epidermis: single layer with *height:width* ratio – 1:2. Abaxial epidermis: single layer with *height:width* ratio – 1:1. Chlorenchyma cells: mesophyll palisade: single layer filling ½

part of the height of leaf lamina. Spongy mesophyll: 4-5 layers of spongy mesophyll. Vascular bundles: simple vascular bundles. Parenchyma cells: single layer encircles each vascular bundle. Trichome: simple, unicellular,mainly present on adaxial leaf surface, papillae (dome-shaped) densely covering the adaxial leaf surface.(Mohd. norfazil.G *et al*.,2017, Patil *et al*., 2010).

PETIOLE:

- Outline: rounded, 45º recurved downwards to the abaxial side.Adaxial side: presence of protruding structure (wings) on the left and right of adaxial side; abaxial side: U-shaped. Epidermal cells: ratio height: width (3:1). Main vascular tissue system – closed system, consists of one continous vascular tissue in “0” shape. (Mohd. norfazil.G *et al*.,2017).

POWDER MICROSCOPY:

- The characterstic of powder leaf shown covering unicellular trichomes, spiral thickening, stomata and fibres.(Patil *et al*.,2010).
- The powder of leaves of *C.halicacabum* shows acicular crystal,rosette crystals of calcium oxalate. Acicular crystals are needle like, slender long pointed at the end clusters. fragmented oil cells are also present.Glandular and watry unicellular trichomes are present. paranchyma cells contain mucilage and sclerenchyma also present.(Suhas.S.N,Vidya vilas.,2014).

PHYTOCHEMISTRY:

- The *C.halicacabum* leaves contain total phenolic contents 38.04 ± 0.50, total flavonoid contents 1.05 ± 0.01 and total tannin content 4.99 ±. 0.33.(Senthilkumar.S and Vijayakumari.K.,2012).
- *C.halicacabum* ethanolic extract of leaves of LC/MS/MS indicate presence

of Anti inflammatory compounds Luteolin-7-o-glucronide,Apigenin 7-0-glucronide and Chrysoeriol.(Jeyadevi R *et al.,*2013).

- Flavonoid, Terpenoids and cardiac glylosides were predominantly found in the Acetone, Chloroform,Diethyl ether solvent extracts of leaf of *C.halicacabum*.(Annadurai A,*et al*.,2013).
- It was reported steroids present in dried leaf powder of *C.halicacabum* .(Suhas.S.N,Vidya vilas.,2014).
- *C.halicacabum* leaves contain considerable amounts of saponins , alkaloids, (+)-pinitol, apigenium, luteolin. minerals such as Ca (1.30%), K (4.01%), Mg (0.43%), P (0.83%), Organic-N (5.19%), Total-N (7.16%), and C (48.1%) are present. The major cyano lipid (49%) is a diester having two fatty acid moieties esterfied with 1-cyano-2- hydroxymethyl-prop-2ene-l-ol followed by a diester derived from 1-cyano- 2-hydroxymethyl-prop-2-ene-3-ol (6%). other chief components of the oil include oleic acid (22%), arachidic acid (10%), linolenic acid (8%), palmitic acid (3%) and stearic acid (2%) including small proportions (1- 2%) of a low-molecular weight acid, and several C22 acids.(Prabakaran.S, Pugazhendy.K and Revathi.A.,2014).

PHARMACOLOGY:

Acute toxicity studies

Acute toxicity studies were carried out on rats for EECH at doses of 50, 100, 500, 1000 and 2000 mg/kg body weight. The acute toxicity of extract showed no mortality and morbidity even under high dose levels (2000mg/k b.w.) indicating high margin of safety of the plant extract. (Vijayakumari K, Senthilkumar S., 2017).

- Leaves of this plant rubefacient and used in the treatment of rheumatism.(Malaviya S et al.,2009).

- *In vitro* studies reported ethanolic crude extract of the leaf of *C. halicacabum* inhibit TNF-α and nitric oxide in human peripheral blood mononuclear cells (Swaminathan.P, Saleena.L *et al.,*2017).

ANTIMICROBIAL ACTIVITY:

- The leaf and stem extracts of *C.halicacabum* were reported for their antimicrobial activity against S. aureus, B.Subtilis, C. freundii, E. coli, P. aeroginosa, S typhi, K. pneumonia.(Viji.M,Murugesan.S.,2010).
- The antimicrobial activity of the leaf extract was assayed by Cup plate or cylinder plate method against 3 bacterial species *S. aureus, B. substilis* and *E.coli.(* Deepan T, Alekhya V, Saravanakumar P and Dhanaraju M D., 2012).

ANTI-ARTHRITIC ACTIVITY:

- The ethanolic extract of leaves give Anti- arthritic effect.The leaf extract treated in Diabetic rats reduce the hyperglycemic and reduce the Glycoprotines in plasma,liver and kidney.It increases the serum albumin phosphate, creatinine, blood urea nitrogen. In rats methanolic extract improved result than petroleum ether extract.(Patil *et al.,*2010).

ANTI DIABETIC ACTIVITY:

- This plant leaf possesses several flavonoids such as apigenin, pinitol and luteolin which are reported as the antidiabetic activity.(Veeramani C et al.,2012.).
- The *C.halicacabum* leaf of methanol extract at a concentration of 50g plant was found to be more potent than other extracts with the lowest mean glucose concentration of 201±1.69 mg/dl at the end of 27 hrs. A Decoction of Cardiospermum halicacabum leaves was screened for hypoglycaemic

activity on alloxan-induced diabetic rats.. (Stalin.C, Vivekanandan.K and Bhavya.E.,2013).

- The antihyperglycemic effect of cardiospermum halicacabum leaf extract may be attributed to activation of glucose uptake, inhibition of intestinal glucose transporter and decreasing the expression of genes that control gluconeogenesis.(Veeramani.C *et al.,* 2015).
- CHE is potent inhibitor of cardiac dysfunction and improve the membrane fluidity on STZ-induced diabetic rats. antihyperglycemic, antioxidant and antihyperlipidemic properties of CHE could be helpful to maintain the levels of membrane-bound ATPases in STZ-induced diabetic rats showing the membrane stabilizing property of extract. (Veeramani.C *et al.,* 2015).

CNS ACTIVITY:

- The 95% ethanol extract of the leaves has been reported to produce central nervous system depression. (Rupeshkumar .M, Kavitha.K, Basu.S.K.,2012).

RADICAL SCAVENGING ACTIVITY:

- It was reported The ethanolic leaf extract of C.halicacabum contain phenol and flavones it has free radical scavenging activity.(Senthilkumar.S and Vijayakumari.K *et al.,*2012).

ANTIHYPERLIPIDIMIC ACTIVITY:

- Elevated level of plasma total cholesterol, phospholipids, triglycerides, and free fatty acids has been observed in the STZ-induced diabetic rats but treatment with the plant brings them to their normal level. It also elevated plasma low-density lipoprotein (LDL-C) and very low-density lipoprotein (VLDL-C) and lowered high-density lipoprotein (HDL-C). Hence, it has been

shown that in these rats the plant play antioxidant and hypolipidemic activity, and the existence of flavonoids, like apigenin and luteolin are responsible for this activity (Veeramani *et al*., 2010).

- Cardiospermum halicacabum aqueous leaf extract (0.01-0.625 mg/mL) showed significant inhibition on LDL glycation in a dose-dependent manner. (Stalin.C, Vivekanandan.K and Bhavya.E.,2013).

ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITY:

- Ethanolic extract of C.halicacabum leaves have the Anti-inflammatory activity against carragenan induced paw- edema method.This extract of leaves are also useful to the Antipyretic activity against yeast in rats.(Jeyadevi R *et al.,* 2013).

SPERM MOTILITY:

- Aqueous leaf extract (ALE) of Cardiospermum halicacabum for 30 days was reported produced a significant dose dependent increase in the sperm counts and sperm motility in both caput and cauda regions.(Dinithi.L *et al*., 2015).

ANTIULCER ACTIVITY:

- The leaves of *C*. *halicacabum* possess significant anti ulcer activity may probably due to the presence of interesting bioactive compounds like flavonoids, tannins, phenols, saponins and caffeic acid.(Vijayakumari K and Senthilkumar S.,2017)

ADULTICIDAL ACTIVITY:

- Leaf extract have extraordinary Adulticidal role.(Raza S *et al.,* 2013).

ANTIOXIDANT ACTIVITY:

- The ethanolic extract of *C.halicacabum* leaves (EECH) has Antioxidant and Antirheumatic activity in wristar rat.Orally dose 250 and 500 mg.Daily for 20 days.Reference –Diclofenac.(Kumaran and Karunakaran, 2006).
- The combined petroleum ether extract of *Cardiospermum halicacabum L.* and *Delonix elata L.* leaves (CPCD) possess antioxidant activity. (Ravichandran.S and Panneerselvam.IP.,2013).(Scartezzini P, Speroni E., 2000).

ANXIOLYTIC AND MEMORY ENHANCING ACTIVITY:

- The methanolic extract of *Cardiospermum halicacabum* leaves have anxiolytic activity and memory enhancing activity, that was reported. (Mahmood.R *et al*.,2015).

STEM:

PHARMACOGNOSY:

- Stem is 5-grooved,slim and hairless to sparingly hairy.(Raza S et al., 2O13).

PHYTOCHEMISTRY:

- Petroleum ether extract of stem of this plant was reported presence of carbohydrates, steroid, terpenoid and trace amount of alkaloid and glycosides. Chloroform extract of this plant was repotred presence of carbohydrates, steroid, terpenoid, glycosides, alkaloid, phenols and tannins. Ethyle acetate extract of this plant has carbohydrate, steroid, terpenoids, phenols, tannins, proteins, amino acids, glycosides, flavanoids, and alkaloids and methanolic extract of this plant has carbohydrates, amino acids, phenols, tannins,alkaloids,glycosides andaqoues extract of this plant

was reported carbohydrates, flavanoids, amino acids, proteins, steroids, terpenoids, phenols, tannins, glycosides, alkaloids.(Ara.A,Srinivasa reddy. K,C.S Reddy., 2009).

- Tannins, Flavonoid, Terpenoids and cardiac glylosides and anthrquinonus were predominantly found in Acetone, chloroform, diethyle ether extracts of the stem.(Annadurai A *et al.*,2013)

PHARMACOLOGY:

ANTIMICROBIAL ACTIVITY:

- Acetone extract of stem of this plant active against micrococcus luteus(35 mm),proteus vulgaris and staphylococcus areus (32 mm).(Selvarani .S et al.,2015).

FLOWER:

PHARMACOGNOSY:

- Flowers are white in colour and are small.These are unisexual,obliquely zygomorphic having stright pedicel.(Raza S *et al.,* 2013).

FRUITS:

PHARMACOGNOSY:

- Fruits are membranous, depressed pyriform caring branched at the angles.It is a spherical caring, inflate 3 lobed and 3- celled.(Jeyadevi R *et al.,* 2013).(Krishna Murti *et al*.,2010).

SEEDS:

ETHNOMEDICAL USE:

- Seeds are used as a tonic for fever and as a diaphoretic.(Dhayabaran D *et al*.,2012).

- The seeds of *C.halicacabum* is used as oral pain relievers or applied to aching joints as a paste.(Krishna murthy naik.V,*et al*.,2014**).**

PHARMACOGNOSY:

- Seeds are black in colour.It has large white heart formed aril.(Raza S *et al.,* 2013).
- The *C.halicacabum* seed is round, black and lined, roughly 1/3 of the surface, with a white heart shaped “finely porous,chordate spot at the micropyle” . The strength of the seed coat is hypothesized to extend the viability of the seed over time.(Matthew Anthony Dempsey, B.A.,2011).

PHYTOCHEMISTRY:

- seeds of this plant contains 33% of fatty acids, and of these fatty acids, about 55% are cyano lipids. Cardiospermum halicacabum seed protein content (35.9% by dry weight) and amino acids in g/100g protein (Asp 8.3%, Thr 4.1%, Ser 6.0%, Glu 15.9%, Pro 3.2%, Gly 9.2%, Ala 6.6%, 1/2Cys 1.1%, Val 6.3%, Met 0.9%, Ile 3.9%, Leu 5.7%, Tyr 1.8%, Phe 3.8%, His 2.7%, Lys 3.9%, Arg 5.1%).(Subramanyam R *et al.,* 2007).
- seed coat contains Tannins, Flavonoids and Terpenioids were predominantly found in Acetone,Diethyle ether,chloroform extracts of *C.halicacabum.*(Annadurai.A,Elangovan.V, Velmurugan.S.,2013)

PHARMACOLOGY:

- Seed oil was reported to posses anticancer activity.
- seeds are used as a tonic for fevers and as a diaphoretic.((Sheeba.M.S, Asha.V.V.,2009).

ROOT:

ETHANOMEDICAL USE:

- Root of this plant helpful for nervous disease and it also used as emetic, laxative.(Dhayabaran D *et al* .,2012).
- The root is mucilaginous and considered emetic, laxative and antirheumatic. (Sheeba.M.S, Asha.V.V.,2009).

PHYTOCHEMISTRY:

- Alcoholic extract of root of this plant contains fixed oils,fats,protiens,flavanoids,phenolics,tannins,carbohydrates,saponins,phyt osterols and triterpenoids.(Dhayabaran D *et al*.,2012).

PHARMACOLOGY:

ANTIANXIETY ACTIVITY:

- In a study conducted in mice using alcoholic and aqueous root extracts of cardispermum halicacabum reported to be helpful in investigating the anti anxiety effects.(Malaviya.S *et al*.,2009).

ANTIEPILEPTIC ACTIVITY:

- Alcoholic root extract of *C.halicacabum* L used in epilepsy.It was reported in swiss albino mice dose(30,100 and 300 mg/kg).Root of this plant also used as anxiety.(Dhayabaran D *et al*.,2012).

PINITOL:

- Pinitol is a very common cyclitol present in many leguminous plants and it is the mono methyl ether of d – inositol and commercially very important anti-diabetic molecule proven to have insulinomimetic action and is non-toxic with no side effects. (Shubashini K.*et al*.,2011).

- Pinitol is a completely non-toxic. pinitol has been reported hypoglycemic action and anti-hyperlipidemic effect well established. Clinical trials on the effect of pinitol on glycemic control and lowering cardiovascular risk factors in patients with type II diabetes mellitus have been proved. Pinitol is reported safe and nontoxic as an anti-diabetic agent even at high levels.(Pavithra *et al*.,2017).
- The name pinitol is derived from “pine,” as it was first isolated from a pine tree.(International Journal of Basic & Clinical Pharmacology.,2015.)
- The D-pinitol also lowered significantly (p ¡ 0.05) LDL andVLDL cholesterol levels and increased significantly (p ¡ 0.05) HDL cholesterol levels in the serum of diabetic rats. Thus, the present study clearly showed the antihyperlipidemic effect of D-pinitol in STZ-induced type II diabetic rats.

  Synonyms:

  -O-Methyl-D-chiro-inositol;

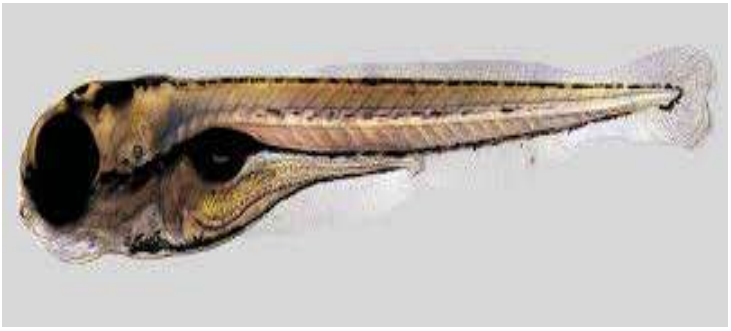
  D-(+)-chiro-Inositol;D-Pinitol;

  Inzitol;

  D-(+)-Pinitol; (+)-Pinitol (Gobato R,*et al*.,2016).

ZEBRAFISH

ZEBRAFISH LARVAE



ZEBRAFISH - AN *In Vivo* MODEL FOR DRUG SCREENING

Zebrafish (*Danio rerio*) as a model organism for investigating endocrine disruption. Study was carried out on endocrine-disrupting compounds (EDCs) which are widespread in the aquatic environment will cause alterations in development, physiological homeostasis and health of vertebrate. When the morpholino technique is used to knockdown cyp19a1 in the zebrafish embryo, translating EDC induced molecular responses into organism toxicity and disease was found to be getting more complicated for older life stages. (Senger, H 2009).

Studies has been carried to assess pharmacological effects of drugs on the optic nerves, motor neurons, and dopaminergic neurons and performed whole mount immunostaining were performed and visualized different neuronal cell types in vivo. Showed that compounds that induce neurotoxicity in humans caused similar neurotoxicity in zebrafish (Parng, C *et al.,* 2007).

An *in vivo* transgenic model of zf was used to screen two known antiangiogenic compounds SU4312 and AG1478 in which additionally indirubin-3-monoxime was identified which showed that each of the hit compounds had dose dependent antiangiogenic activity (Sandbeg, E 2007).

A novel system has been developed in which zf is amenable to transgenic and both forward and reverse genetic strategies that can be used to identify or generate zf models of different type of cancer. Ultimately, high-throughput modifier screens based on zf cancer models can lead to the identification of chemical or gene involved in the suppression or prevention of the malignant phenotype (Berghmans, S *et al.,* 2005).

A data on behavioral responses of adult zebrafish to a wide spectrum of putative anxiolytic and anxiogenic agents was carried out . Using the novel tank test as a sensitive and efficient behavioral assay, zebrafish anxiety-like behavior can be bi-directionally modulated by drugs affecting the gamma-aminobutyric acid, monoaminergic, cholinergic, glutamatergic and opioidergic systems (Stewart, A *et al.,* 2010).

Work has been carried out to evaluate the teratogenic effect of the human antiepileptic drug valproic acid which generates malformation such as oedema, brain deformities, a shortened and bent tail and bipartite axiation of the posterior trunk on zebrafish larvae (Herrmann, K 1993).

A survey has taken on the stock of the available zebrafish assays in the context of alternative mammalian cell-based assays, and of the validation outcomes to date and the survey data indicate that the preferred way forward would be a collaborative effort between the pharmaceutical/biotechnology industry and the zebrafish contract research companies, alongside expert input from academia and regulatory authorities (Redfern, WS *et al.,* 2008).


AIM & OBJECTIVE

CHAPTER III

AIM AND OBJECTIVE

The number of diseases associated with hyperlipidemia is rapidly increasing. Current experimental studies of hyperlipidemia often use genetically modified mice, rabbit and hamster fed high fat, high-cholesterol diets, which rapidly induce extreme hyperlipidemia and lipid accumulation in the artery wall. In addition, mammalian hyperlipidemia models are often time-consuming, labor intensive and expensive.

Inhibition of dietary lipid is an emerging strategy for the treatment of lipid metabolitic complications of cardiovascular system. class of pharmacological agents which commonly used are orlistat and ezetimibe which improve the serum lipoprotein profiles of patients those are at high risk of stroke, acute coronary syndrome and sudden death, and therefore it can be used as an adjuvant to HMG co-reductase inhibitors (statins).

Cell culture models and in other *in vitro* systems of drug screening has been carried out, but due to lack of organ structures, extrapolation of these results to the whole organism is often challenging. Live animal *in vivo* model that allows a detailed analysis of lipid metabolism would be highly valuable for lipid metabolism studies and for lipid-lowering drug screening.

Given the prevalence of lipid metabolism disorders lead compound identification is desirable and that can be developed into new formulations which then inhibit the absorption of lipids via novel mechanisms. Here we report the utility of zebrafish for this study. Because of their **small size, optical**

transparency of zf larvae which are well suited for chemical library screens using morphological assays. Compound efficacy can be rapidly assessed and toxicity studies can be performed *in vivo* which makes it a great advantage in chemical screens. This study is aimed primarily at phytochemical and bioactivity of EECHL from the leaves of *C.halicacabum* as potential biological and pharmacological resources in the above aspects.

AIM:

To study the pharmacognostical, preliminary phytochemical and *in vivo* effect on the hyperlipidemic zebrafish larvae and cardiovascular system of the ethanolic extract of the leaves of *Cardiospermum halicacabum L*. family Sapinadaceae.

OBJECTIVE:

The objective of the study was divided into 3 parts.

Part 1**: Pharmacognostic study**:-

Collection and authentification of plant.

Macroscopy of the leaf

Microscopy :-

1.Anatomical studies.

2.Microscopic schedules.

3.Scanning Electron Microscopic study (SEM).

4.Powder microscopy of the leaf

Physico-chemical parameters:-

1.Ash values.

2.Loss on drying.

3.Extractive values.

Part 2: **Preliminary phytochemical screening:-**

Qualitative analysis of the leaves for the presence of various phytoconstituents.

Determination of trace elements present in the leaves by Energy Dispersive Spectrum analysis (EDS) preparation of Ethanolic extract of the leaves of *C.halicacabum* (EECH) Determination of flavonoid content, total phenolic content of the leaves of *C.halicacabum.* HPTLC profile of the EECHL to identify and quantify pinitol.

Part 3: **Pharmacological screening**:-

The 3R's ethical principle (**R**eduction, **R**efinement, **R**eplacement) was implemented that help to minimize harms to vertebrate animals used in science.

Collection of zebra fish (zf) larvae.

To study the preliminary toxicological studies of the EECHL on the early development of zf

1.Whole embryo culture toxicity study

2.Larval toxicity study

Efficacy assessment of EECHL on *in vivo* hyperlipidemic zf larvae model

To determine the effect EECHL on the CVS (cardiac morphology, blood circulation) of zf larvae.


MATERIALS
&
METHODS

4.1. PLANT COLLECTION AND AUTHENTIFICATION:

Leaves of the plant ***Cardiospermum halicacabum*** **L**. selected for our study was collected from **Alampattu, Sivagangai District**, Tamil Nadu, India during the month of June 2017 and was authenticated by **Dr. D. Stephen,** Department of Botany, American college, Madurai.

LEAF DRYING AND PULVERIZING:

The leaves were collected and shade dried. It was powdered in a mixer. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

4.2. PHARMACOGNOSTICAL STUDIES:

Morphological and micro morphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies. Botanical identity of the plants is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many useful pharmacological active properties in the plant. If the botanical identity of the plant happens to be dubious or erratic, the entire work on the plant becomes invalid. Thus it is needless to stress the botanical identity of the crude drug is the threshold in the processes of pharmacological investigations. The researchers should be equipped with all possible diagnostic parameters of the plant on which the researchers plan to work.

4.2.1. MORPHOLOGICAL STUDIES OF *C.halicacabum L*:

Aerial part, leaf and petiole, flower, fruits and root were studied individually for its morphological characters by organoleptic test.

4.2.2. MICROSCOPICAL STUDIES ON THE LEAF OF *C.halicacabum*:

COLLECTION OF SPECIMEN:

Care was taken to select healthy plants and for normal organs. Leaf, Petiole specimens were collected from a healthy plant by making a cut with petioles.The materials were cut into pieces and immediately immersed in fixative fluid FAA (Formalin – 5ml + Acetic acid – 5ml +70% Ethyl alcohol – 90ml).

DEHYDRATION:

After 24 hours of fixing , the specimens were dehydrated with graded series of ethyl alcohol and tertiary-butyl alcohol (Sass, JE 1940). The specimen is kept is in each grade of the fluid for about 6 hrs.Every time the fluid is decanted and immediately the specimen were flooded with next grade of fluid.

INFILTRATION WITH PARAFFIN WAX:

After dehydration, the shavings of paraffin wax were added to the vial containing the plant material with pure TBA. The paraffin shavings are added every 30mts at about 40-45ºC four or five times. Then the vials were filled with wax without damaging the tissues. The vial filled with wax is kept open in warm condition to evaporate all TBA, leaving the specimen in pure molten wax. The specimen filled with pure molten wax for 2 or 3 times by decanting the old wax every time.

CASTING TO MOLD:

A boat made out of chart board, by folding the margin, is used to prepare a mold of wax containing specimens. The paraffin along with the leaf and petiole specimen was poured into the boat. With the help of heated needles, the specimen were arranged in parallel rows with enough space in between the specimens. The block was then immersed in chilled water and allowed to cool for few hours.

SECTIONING:

The paraffin embedded specimens were sectioned with the help of microtome**.** The thickness of the sections was 10-12µm. Dewaxing of the sections was by customary procedure. The sections were stained with **Toluidine blue** as per the method published by O'Brien, TP *et al*., (1964).

Since toluidine blue is a poly chromatic strain, the straining results were remarkably good and some **cytochemical reactions** were also obtained. The dye rendered pink colour to the **cellulose** walls, blue to the lignified cells, dark green to **suberin**, violet to the **mucilage**, blue to the **protein** bodies etc. Where ever necessary sections were also stained with **safranin** and **fast-green** and potassium iodide (for starch). For studying the stomatal morphology, venation patttern and trichome distribution, **paradermal sections** (sections taken parallel to the surface of leaf as well as **clearing** of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of differentparts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured. (Sass, JE 1940).

PHOTOMICROGRAPHS:

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken.with Nikon labphot 2 Microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light were employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifigations of the figures are indicated by the scalebars. (Johansan, DA, 1940, Purvis, MJ *et al*., 1966).

4.2.3. MICROSCOPICAL STUDY OF LEAF USING SCANNING ELECTRON MICROSCOPE:

Movement of beam of foccussed electrons across an object forms a 3D image on a cathode - ray tube in a Scanning Eectron Microscope and it reads both the electrons scattered by the object and the secondary electrons produced by it. The electromagnetic lenses are used in SEM and focussing is done by the current. On photographic plate of screen the image is projected which gives comprehensive, quasi 3-D representation of the objects gives the ultra structure of plant cells. In addition , shows the unsuspected details and any undescribed characters. In other words the micrograph from SEM, shows the best possible structural details of the specimens. (Robards, 1970).

USAGE:

SEM info was handled as conventional character (or) character complexes as “pure” information without being broken down (or) interpreted as individual character using computer processing. The SEM information can be used some what at the

superficial level just described to assist in solving taxonomic problem by confirming, changing (or) other grounds. It is also used often as diagnostic feature to avoid misleading by over simplified descriptions and one may find new kinds of microstructures not previously recognised and apprently simple structures may be extremely complex. Remarkably, poor conventional descriptions enabling taxonomic process of reducing a complex pattern to a few simple characters (Heywood, VH, 1971).

SEM plays a vital role when a specimen need to be satisfactorily defined in terms of characters. For most biological materials, maximum information is obtained by employing light and electron microscopy jointly and an attempt was made by applying SEM to the leaf of *C.halicacabum* , to pinpoint the positions of specific characters with in the cell, which can be easily seen in final image.

SEM SAMPLE PREPARATION:

Sample for SEM analysis were mounted on the specimen stub using carbon adhesive sheet. Small sample were mounted with I sq. cm glass slide and kept in carbon adhesive sheet.. Samples were coated with gold to a thickness of 100 AO using hitachi vacuum evaporator. Coated sample were analysed in a Hitachi Scanning electron Microscope 3000 H model.

4.2.4. POWDER MICROSCOPY:

MACERATION TECHNIQUE:

Maceration is the process of separation of individual cells by selectively dissolving the pectic middle lamella between the cells. The middle lamella binds the cells with each other forming different tissues. The middle lamella is dissolved by

employing a chemical that dissolves the lamella to free the cells to obtain their three dimensional view.

MACERATION FLUID:

Jaffrey's maceration fluid is one that is commonly used for maceration (Johansan, DA, 1940). The fluid consists of equal volumes of 5% chromic acid and 5% nitric acid. The plant material is cut into small pieces and immersed in the maceration fluid. The fluid with the materials is kept at 55ºC for 3-5 hrs. Then the material is washed thoroughly with water and placed on a glass slide in a drop of Safranin (0.5%) for 15-20 min. The stain is drained carefully and mounted with a drop of dilute glycerine. The cells are spread well with a needle and the material is covered with cover slip. The slide so prepared is examined under the microscope to study different components of the macerate.

4.2.5 MICROSCOPIC SCHEDULES: (Wallis, TE. 1953, Wallis, TE,

1965, Iyengar, MA, 1994, Anonymous, 2001)

The vein islet number, vein terminal number, stomatal number and stomatal index were determined on fresh leaves using standard procedures.

A. VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER:

The term vein islet in used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of vein islets per sq.mm. Area is called vein islet number.

Vein terminal number may be defined as the number of vein terminals present in one sq., mm. area of the photosynthetic tissue.

B. DETERMINATION OF VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER:

Small square portion from the lamina region of the leaf was cleared in chloral hydrate, stained and mounted on a slide. A camera Lucida is set up and by means of a stage micro meter the paper is divided into squares of $1mm^2$ using a 16mm objective. The stage micro meter is then replaced by the cleared preparation and the veins are raced in four continuous squares, either in a square 2mm x 2mm (or) rectangle 1mm x 4 mm.

When counting, it is convenient to number each vein-islet on the tracing. Each numbered area must be completely enclosed by veins, and those which are incomplete are excluded from the count if cut by the top and left-hand sides of the square (or) rectangle but included if cut by the other two sides. Ten readings for vein islet and vein termination number were recorded.

C. STOMATAL INDEX:

It is the percentage, which the numbers of stomata from the total number of epidermal cells, each stoma being counted as one cell.

I. **Stomatal index = S/S+E x 100**

Where, S = Number of stomata per unit area.

E = Number of epidermal cells in the same unit area.

D. DETERMINATION OF STOMATAL INDEX:

The procedure adopted in the determinations of stomatal number was observed under high power (45 X).The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula.

4.2.6 PHYSICOCHEMICAL PARAMETERS: (Anonymous, 1996, 1998, 2001)

DETERMINATION OF ASH VALUES:

ASH VALUE:

The ash values were determined by using air dried powder of the leaf as per the official method.

TOTAL ASH:

Two grams of the air dried leaf powder was accurately weighed in a silica crucible separately. The powder was scattered into a fine even layer on the bottom of the crucible and incinerated by gradually increasing the temperature not exceeding 450^{o}c, until free from carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried powder was calculated.

WATER SOLUBLE ASH:

The ash obtained from the total ash procedure was boiled with 25ml of water for 5 minutes and the insoluble matter was collected on an ash less filter paper and washed with hot water. Then it was ignited for 15 minutes at a temperature not exceeding 450^{o}c. The weight of the insoluble matter was subtracted from the weight

of the total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried powder.

ACID INSOLUBLE ASH:

The ash obtained from the total ash was boiled for five minutes with 25ml of dilute hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, dried and weighed. The percentage of acid insoluble ash with reference to the air dried drug was calculated.

DETERMINATION OF LOSS ON DRYING

For the determination of loss on drying, the method described by Wallis was followed. One gram of dried powdered leaf was accurately weighed in a tarred Petri dish, previously dried under the conditions specified in IP'96. The powder was distributed as evenly as practicable, by gentle sidewise shaking. The dish was dried in an oven at 100 – 105˚C for 1 hour. It was cooled in desiccators and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

EXTRACTIVE VALUES

- **PETROLEUM ETHER SOLUBLE EXTRACTIVE VALUE**

Five gram of the coarsely powder was macerated separately with 100ml of petroleum ether in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of petroleum ether. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The

percentage of the petroleum ether soluble extractive value was calculated with reference to the air dried powder.

- **ETHANOL SOLUBLE EXTRACTIVE VALUE**

Five gram of the coarsely powder was macerated with 100ml of ethanol in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethanol. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the ethanol soluble extractive value was calculated with reference to the air dried powder.

- **WATER SOLUBLE EXTRACTIVE VALUE:**

Five gram of the coarsely powder was macerated separately with 100ml of chloroform water in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of chloroform water. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the chloroform water soluble extractive value was calculated with reference to the air dried powder.

4.3 PHYTOCHEMICAL STUDIES:

(Anonymous, 1998, Chaudhri, RD, 1999, Kokate, CK, 2005, Agarwal, SS 2007, Horbone, JB, 1973).

4.3.1 PRELIMINARY PHYTOCHEMICAL SCREENING:

TEST FOR ALKALOIDS:

VARIOUS PROCEDURES TO LIBERATE ALKALOIDS:

- Powdered drug was mixed thoroughly with 1ml of 10% ammonia solution and then extracted for 10 minutes with 5 ml methanol, under reflux. The filtrate was then concentrated.
- Powdered drug was mixed thoroughly with 1ml of 10% sodium carbonate solution and then extracted for 10 minutes with 5ml methanol, under reflux. The filtrate was then concentrated.
- Powdered drug was ground in a mortar for about 1 minute with 2ml of 10% ammonia solution and then thoroughly mixed with 7 gram basic Aluminium oxide. The mixture was then loosely packed into a glass column and 10ml chloroform was added, eluted, dried and methanol was added.
- Powdered drug was shaken for 15 minutes with 15ml of 0.1 N sulphuric acid and then filtered. The filter was washed with 0.1 N sulphuric acid to a volume of 20ml filtrate; 1ml concentrated ammonia was then added. The mixture was then shaken with two portions of 10ml diethyl ether. The ether was dried over anhydrous sodium
- Sulphate, filtered and evaporated to dryness and the resulting residue was dissolved in methanol.
- Powdered drug was mixed with one gram of calcium hydroxide and 5ml of water, made into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. 20ml of 90% alcohol was added, mixed well and then refluxed for half an hour on a water

bath. It was then filtered and the alcohol was evaporated. To that dilute sulphuric acid was added.

- The above made extracts were tested with various alkaloid reagents as follows.

1. **MAYER'S REAGENT**
2. **DRAGENDORFF'S REAGENT**
3. **HAGER'S REAGENT**
4. **WAGNER'S REAGENT**

TEST FOR PURINE GROUP (Murexide Test)

- The residue obtained after the evaporation of chloroform was treated with 1ml of hydrochloric acid in a porclain dish and 0.1g of potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapours of dilute ammonia solution.

TEST FOR CARBOHYDRATES:

MOLISCH'S TEST:

- The aqueous extract of the powdered material was treated with alcoholic solution of α- naphthol in the presence of sulphuric acid**.**

FEHLING'S TEST:

- The aqueous extract of the powdered material was treated with Fehling's I and II solution and heated on a boiling water bath.

BENEDICT'S TEST:

- The aqueous extract of the powdered drug was treated with Benedict's reagent and heated over a water bath.

TEST FOR GLYCOSIDES:

GENERAL TEST:

- **TEST A:**

200 mg of the powdered drug was extracted with 5ml of dilute sulphuric acid by warming on a water bath, filtered and neutralized with 5% sodium hydroxide solution. Then 0.1ml of Fehling's solution I and II were added, until it becomes alkaline and heated on a water bath for 2 minutes.

- **TEST B:**

200g of the powdered drug was extracted with 5ml of water instead of sulphuric acid. Boiled and equal amount of water was added instead of sodium hydroxide solution. Then 0.1ml of Fehling's solution I and IIwere added, until it becomes alkaline and heated on a water bath for 2 minutes.

ANTHRAQUINONES:

- **BORNTRAGER'S TEST:**

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The inorganic layer was separated and ammonia solution was added slowly.

- **MODIFIED BORNTRAGER'S TEST:**

About 0.1gram of the powdered leaf was boiled for two minutes with dilute hydrochloric acid and few drops of ferric chloride solution was added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well.

TEST FOR CYANOGENETIC GLYCOSIDES:

Small quantity of the powdered leaf was placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place.

TEST FOR CARDIAC GLYCOSIDES:

- **KELLER KILLIANI'S TEST:**

About 1gram of the powdered leaf was boiled with 10ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3ml of glacial acetic acid containing a trace of ferric chloride. To this 3ml of concentrated sulphuric acid was added along the sides of the test tube carefully.

- **RAYMOND TEST:**

To the alcoholic extract of the leaf, hot methanolic alkali was added.

- **LEGAL'S TEST:**

To the alcoholic extract of the powdered drug, pyridine and alkaline sodium nitro prusside solution were added.

TEST FOR PHYTOSTEROLS:

The powdered leaf was first extracted with petroleum ether and evaporated. The residue obtained was dissolved in chloroform and tested for sterols.

- **SALKOWSKI TEST:**

Few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside.

LIBERMANN – BURCHARD'S TEST:

- To the chloroform solution few drops of acetic anhydride was added and mixed well. 1ml of concentrated sulphuric acid was added through the sides of the test tube and set aside for a while.

TEST FOR SAPONINS:

About 0.5gram of the powdered leaf was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5ml of the filtrate was then diluted with water and shaken vigorously.

DETERMINATION OF FOAMING INDEX:

One gram of the coarsely powdered leaf was weighed and transferred to 500 ml conical flask containing 100 ml of boiling water. The flask was maintained at moderate boiling, at 80-90^{o}C for about 30 minutes. Then it was cooled and filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100 ml (V1).

Ten Stoppard test tubes were cleaned (height 16 cm, diameter 1.6 cm) and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3ml up to 10ml and adjusted the volume of the liquid in each tube with water to 10ml. Then the tubes were Stoppard and shaken lengthwise for 15 seconds, uniformly and allowed to stand for 15 minutes and measured the length of the foam in every tube.

TEST FOR TANNINS:

To the aqueous extract of the powdered leaf, few drops of ferric chloride solution were added.

- **GOLD BEATER'S SKIN TEST**:
- 2% hydrochloric acid was added to a small piece of gold beater skin and rinsed with distilled water and placed in the solution to be tested for five minutes. Then washed with distilled water and transferred to a 1% ferrous sulphate solution.

TEST FOR PROTEINS AND FREE AMINOACIDS:

- **MILLON'S TEST:**

The acidulous alcoholic extract of the powdered leaf was heated with Millon's reagent.

- **BIURET TEST:**

To the alcoholic extract of the powdered leaf 1ml of dilute sodium hydroxide was added. Followed by this one drop of very dilute copper sulphate solution was added**.**

- **NINHYDRIN TEST:**

  To the extract of the powdered drug, Ninhydrin solution was added, and boiled.

TEST FOR MUCILAGE:

To the aqueous extract of the powdered leaf, Ruthenium red solution was added.

TEST FOR FLAVONOIDS

- **SHINODA TEST:**
- A little amount of the powdered leaf was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added, and boiled for 5 minutes.
- **ALKALINE REAGENT TEST:**

  To the alcoholic extract of the powdered leaf, few drop of sodium hydroxide solution was added.

- **ZINC HYDROCHLORIDE TEST:**

  To the alcoholic extract, mixture of zinc dust and concentrated Hydrochloric acid was added.

TEST FOR TERPENOIDS:

The powdered leaf was shaken with petroleum ether and filtered. The filtrate was evaporated and the residue was dissolved in small amount of chloroform. To the chloroform solution tin and Thionyl chloride were added.

TEST FOR VOLATILE OIL:

About 100gram of fresh leaves, were taken in a volatile oil Clevenger apparatus and subjected to hydro distillation for four hours.

TEST FOR FIXED OIL:

A small amount of the powdered leaf was pressed in between in the filter paper and the paper was heated in an oven at 105^{o}C for 10 minutes.

4.3.2. FLUORESCENCE ANALYSIS OF POWDERED LEAF:

Powdered leaf material of *C.halicacabum* was subjected to analysis under UV light after treatment with various chemical and organic reagents like Ethanol, Ethyl acetate, Chloroform, Water, 50% sulphuric acid, 10% sodium hydroxide, 50% nitric acid and dried leaf powder. (Horbone, JB, 1973).

PREPARATION OF ETHANOLIC EXTRACT

The Leaf Powder was sieved in a no.60 sieve and refluxed with ethanol for 4hrs and filtered. The filtrate evaporated under vacuum. The dark green residue obtained (EECH) was stored in the refrigerator until further use.

4.3.3. ESTIMATION OF FLAVONOID CONTENT:

(Chang, CC *et al*., 2002, Mabry, TJ *et al*., 1970 and Siddiquie, MA *et al.,* 2010).he flavonoid content of plant extract was estimated by aluminium chloride method. In this method, aluminium chloride complexes with flavonoids of C3-C5 hydroxyl group and to produce intense colour in acidic medium. The intensity of the color is proportional to the amount of flavonoids and can be estimated as quercetin equivalent at wavelength of 415nm.

MATERIALS REQUIRED:

- Ethanolic extract of leaves of *C.halicacabum* (EECHL)
- 10%w/v aluminium chloride
- 1M Potassium acetate
- 95%v/v ethanol

PROCEDURE:

0.5ml of the extract (1mg/ml) was transferred to a test tube. To this solution, 0.1ml of aluminium chloride, 0.1ml of potassium acetate, 1.5ml ethanol were added and made up to 5ml with distilled water. The mixture was allowed to stand for 30 min with intermittent shaking. The absorbance was measured at 415nm. The calibration curve was generated using quercetin as a standard at different concentrations (5-50µg/ml). The reaction mixture without aluminium chloride was used as a blank. The flavonoids content was expressed as mg of quercetin equivalent per gram of extract.

4.3.4. ESTIMATION OF TOTAL PHENOLIC CONTENT (Singleto, VL, *et al*., 1979, Gouthamchandra, K, *et al.,* 2010)

PRINCIPLE:

The total phenolic content of the extract was determined by Folin & Cio-calteu's phenol reagent. This reagent consists of phosphotungstate and phosphormolybdate mixture which is reduced to mixture of blue molybdenum and tungsten oxides while phenolic content of the extract was oxidized. The intensity of color is proportional to the amount of phenolic content of the extract and which was measured at 765nm. The total phenolic content in the extract was expressed as milligrams of gallic acid equivalent (GAE) per gm of extract.

MATERIALS:

- Ethanolic extract of leaves of *C.halicacabum* (EECH)
- 10%w/v sodium carbonate solution
- Gallic acid
- Folin&Cio-21021calcateu's phenol reagent

PROCEDURE:

0.5ml and 1ml of extract was transferred into separate test tube. To this solution, FCR 0.5ml and 1ml of sodium carbonate were added and final volume made up to 10ml with distilled water. The mixture was allowed to stand for 1hr with intermittent shaking. The absorbance was measured at 765nm. A calibration curve was generated using Gallic acid as a standard at different concentrations (2, 4, 6, 8, 10µg/ml). The reaction mixture without sample was used as a blank. The total Phenolic content was expressed as milligrams of Gallic acid equivalent (GAE) per g of extract.

4.3.5 DETERMINATION OF TRACE ELEMENTS IN THE LEAF OF *C.halicacabum* BY ENERGY DISPERSIVE X-RAY SPECTROMETER (EDS):

The SEM allows the observation of materials in macro and submicron ranges. SEM is capable of generating 3-D images for analysis of topographic features. When SEM is used along with EDS the analyst can perform an elemental analysis on specimens of microscopic sections or contaminants that may be present.

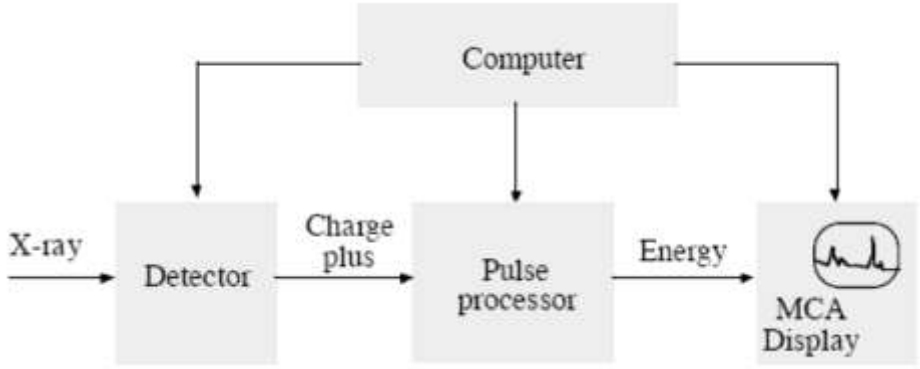
EDS ANALYTICAL CAPABILITIES:

Backscattered electron images in the SEM display compositional contrast that results from different atomic number elements and their distribution. EDS is used to find particular elements are and their Atomic %. The Y-axis shows the counts (number of X-rays received and processed by the detector) and the X-axis shows the energy level of those counts (Bob Hofner).

By Viewing 3-D images of specimens solves some of the problem in an analysis and it is also necessary to detect different elements associated with the specimen. This is accomplished by using the “built-in” spectrometer called an Energy Dispersive X-ray Spectrometer.

EDS SYSTEM COMPRISES OF 3 BASIC COMPONENTS:

- An X-ray Detector - detects and converts X-ray into electronic signals.
- A Pulse Processor - measures the electronic signals to find out energy of each X-ray detected; and
- A Multiple Channel Analyser - interprets and displays analytical data.



EDS is an analytical technique in which the specimen emits X-rays due to the bombardment of electron beam on it which is used to identify the elemental composition of the specimen due to the ejection of electrons from the atoms on the

specimen surface. To explain further, when the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the specimen's surface. A resulting electron vacancy is filled by an electron from a higher shell, and an X-ray is emitted to balance the energy difference between the two electrons. The EDS X-ray detector measures the number of emitted X-rays emitted versus their energy. The energy of the X-ray is characteristic of the element from which the X-ray was emitted. A spectrum of the energy versus relative counts of the detected x-rays is obtained and evaluated for the determinations of the elements.

4.3.6 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY OF THE EECH LEAF:

High performance thin layer chromatography (HPTLC) is a modern adaptation of TLC with improved versatility, separation efficiency and detection limits. HPTLC is a useful tool for identification of plant extract because each plant species produces a distinct chromatogram, with unique marker compounds used for the plant identification. It is used as a quality control tool since comparison of chromatograms of different lots can demonstrate the similarities and differences between the test samples and their standard chemical markers. HPTLC is a reliable method for quantification of nano gram level even when present in complex formation. HPTLC fingerprint analysis is used for rapid identity Check, for monitoring purity of drugs, for detection of adulterants, for determining whether material is derived from a defined botanical species and also to know whether the constituents are clearly characterized.(Wagner,H e*t al,*1996)

HPTLC PROFILE	:	
Instrument used	:	CAMAG make HPTLC.
Software	:	winCATS 1.4.3
Sample Applicator	:	Linomat 5.
Detection	:	@254nm in Densitometry TLC Scanner
Sample preparation	:	100mg per ml of sample was prepared in Ehanol.
Stationary Phase	:	HPTLC plates silica gel 60 F 254.
Mobile Phase	:	Chloroform:methanol:water. (6:3.5:0.5).
Sample Solution	:	5µl sample is applied. (1µg/ml)
Standard solution	:	5µl sample is applied. (1µg/ml)
Drying device	:	Oven
Temperature	:	60°C
Time	:	5minutes
Scanning speed	:	20mm /s

MEASUREMENT TABLE

Wavelength : 254

Lamp : D2 & W

Measurement type : Remission.

Measurement mode: Absorption.

4.4 PHARMACOLOGICAL STUDIES

4.4.1. ACUTE TOXICOLOGICAL STUDIES :

Toxicology through intensive studies has traditionally focused on the effects of chemicals on living organisms which was done by one chemical at a time. Such approaches show the mode of action of many chemicals and provide a detailed mechanistic understanding of the molecular targets of toxicity for some as the cost of this approach is high. Toxicology studies rely on the utility of vertebrate animals which is an expensive undertaking in both time and cost with debatable predictive power in case of safety aspects for human (Bucher, JR 2002).

Role of zebrafish in high throughput screening

In the last two decades safety pharmacology has become a most important part of the non-clinical safety assessment in finding new chemical entities (Bass, A *et al.,* 2004).

The relative novelty of this discipline has granted it the flexibility to incorporate new experimental tools (Claude, JR and Claude, N 2004).

In addition to requiring small amount of compounds, the time and cost effectiveness of *in vitro* assays have led to their use by the pharmaceutical industry for high or medium throughput safety screens (Suter, W 2006).

One of the limitations encountered with *in vitro* studies is that they are not fully representative of *in vivo* models. Therefore, safety pharmacology needs an *in vivo* model with the capacity for higher throughput screening. The zebrafish model system is done from medium to high throughput because of many advantages, as they are small in size, cheap to maintain and fecundity as a single spawning produces 100–

200 eggs. Larvae, which are only 1–4-mm long, can live for seven days in a single well of a standard 96-or 386-well microwell titer plate by the support of nutrients stored in the yolk sac.

Administration of drugs in zebrafish

Larvae of zf can absorb small molecules diluted in the surrounding water through their gills and skin. Drugs can be given orally after this stage because zebrafish begin to swallow at 72 (hpf). Drugs can also be delivered by oral intubation in case of adult zf. Compared to testing in other animal models, statistically significant numbers of zebrafish can be used for each assay and small amounts (mg) of drug are required. In addition, the transparency of zebrafish larvae for several days of post fertilization (dpf) enables *in vivo* observation of live or whole mount fixed specimens, including the visualization of vital dyes, fluorescent tracers, antibodies and riboprobes. By 120 hpf, zebrafish develop discrete organs and tissues, including brain, heart, liver, pancreas, intestines, bone, muscles, nerve systems and sensory organs. These organs and tissues have been shown to be similar to their mammalian counterparts at the anatomical, physiological and molecular levels. Although conventional in vitro assays using cultured cells can be used to evaluate potential drug toxicity effects, results are frequently not predictive of results in vivo which involve drug absorption, distribution, metabolism and excretion (ADME). To streamline the drug development time-line, prioritize drug candidates for animal testing and reduce unnecessary costs for mammalian studies, drug-screening assays using zf are becoming increasingly popular (McGrath P and Chun-Qi Li 2008).

Morphology of zf larva

Fig-1

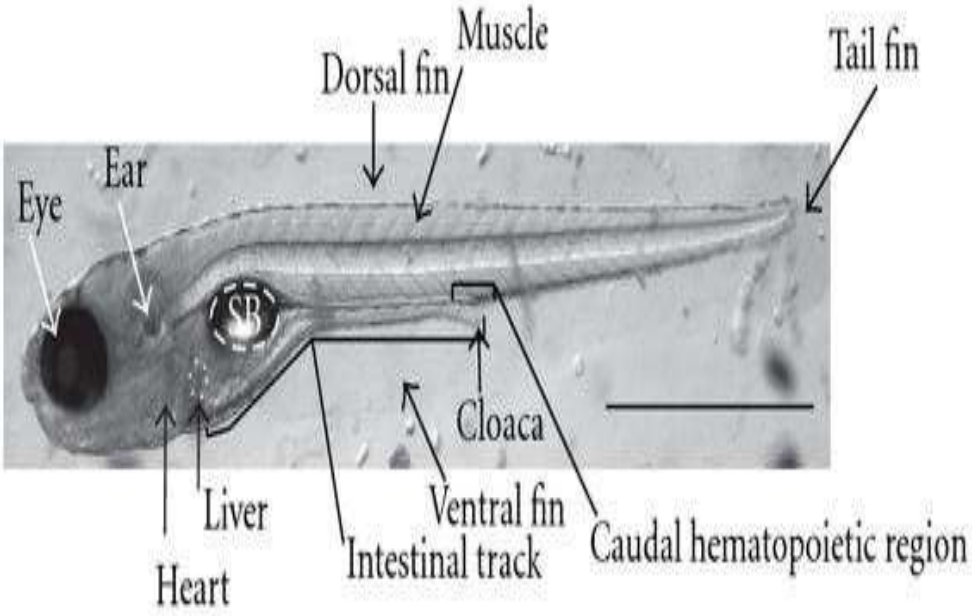


Fig-2

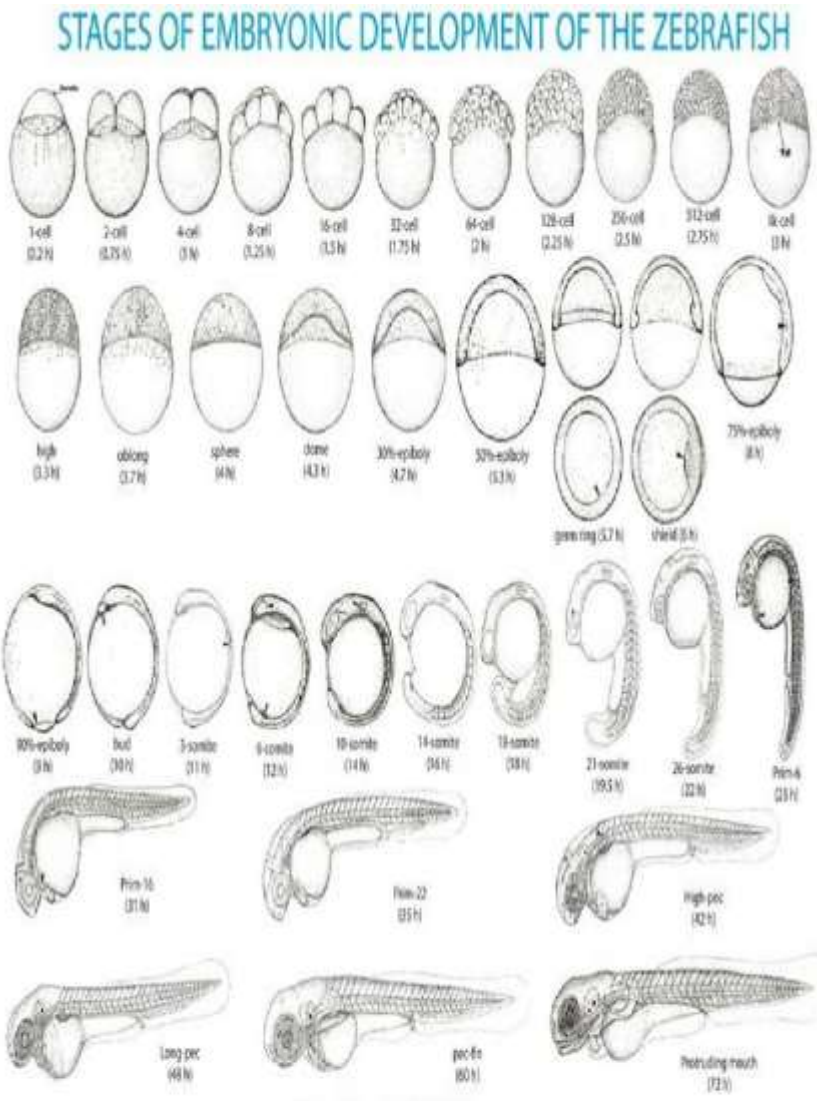


Fig. 2. Schematic drawing of the early development of the zebrafish. The stages and the stage-specific structures are given according to Hisaoka and Battle (1958) with the corresponding time after fertilization at 26°C. Abbreviations: B, blastomere; C, chorion; DC, deep cells; EL, enveloping layer; ES, embryonic shield; H, head anlage; MY, myomere; N, notochord; O, oocyte; OP, optic placode; OT, otic placode; PF, perivitteline fluid; S, somites; T, tail; Y, yolk; YSL, yolk syncitial layer.

Digestive system anatomy of zf larva

The zf intestinal epithelium is comprised of absorptive cells (enterocytes), endocrine cells and goblet cells. When zf larvae consume a lipid rich diet it must be broken down by luminal lipases into free fatty acids and cholesterol in the intestine

before entering the specialized absorptive cells (enterocytes) that line the gut (Pack, M *et al.,* 1996).

Zebrafish cardiovascular development and anatomy

As in all vertebrates, the heart is the first organ to function in zf. It develops rapidly and is fully formed by 2dpf, compared with 12dpf in the mouse and 35dpf in the human embryo at 3 weeks gestation. zf hearts, like those of humans, start out as a linear heart tube and start to loop 24-36 hour post fertilization (hpf).

Zf cardiac contraction begins at 22hpf initially as a peristaltic wave which then develops into co- contractions of the ordinate atrium and ventricle by 36hpf. Blood is first sent from the heart to the gills where it is oxygenated. It then passes to the head before moving posteriorly to the rest of the body. Despite the lack of a pulmonary circulation and its two chambered heart, there is significant conservation between mammalian and zf cardiovascular development at morphologic, physiologic and genetic level, making it an useful model for studying cardiac development (Dooley, K and Zon, LI 2000).

Collection of ZF larvae:

Zf spawn in the morning and the embryos are collected from the bottom of the tank and transferred into a petridish and incubated *in vitro* using standard medium. Embryos hatch from the chorion at around 48 hour post fertilisation (hpf) and the larvae are incubated at 28ºC. By 5-6dpf the yolk has been depleted and larvae must now eat to acquire nutrients (Munoz,G *et al.,* 1990).

4.4.1.1 WHOLE EMBRYO CULTURE TOXICITY STUDY

Materials

Fertile eggs, E3 embryo medium (standard medium), Dimethyl Sulphoxide (DMSO), glass petridishes, research microscope (laboscope microscope with microphotography), incubator, micropipette, EECH of *C.halicacabum* and standard podophyllotoxin.

Collection of eggs

Eggs were collected from natural spawning and reared in embryo medium at pH 7.2, and kept in an incubator at 28±0.5°C for our assay. The developmental stage of the embryos was determined using microscope. (Hisaoka, KK and Battle, HI 1958). (Fig - 2)

At around 2– 4 h postfertilization, only the fertilized eggs (blastula stage) were selected. The fertilized eggs were collected and rinsed several times with tap water.

Experimental design

The eggs were transferred to each of the glass petri-dishes (3 per dish) containing different concentrations of EECHL (0.5, 0.75, 1 and 2 µg/ml) dissolved in 1% DMSO at 28°C as well as DMSO control . Embryo medium served as the over-all control .Standard podophyllotoxin of concentration 10 µg/ml was taken as positive control. Occasional stirring was done to ensure even distribution of the chemical. The maximal acceptable toxicant concentration (MATC) was calculated according to (Dave, G *et al.,* 1987) by scoring the malformations. The development of blastula eggs was monitored at specified time points (12, 36, 60 & 80 hrs) under microscope. Endpoints used for assessing the effect of drug during the major organ is visible

included edema, eye malformation, bent tail, undulated notochord, twisted notochord and death. Malformations were also noted and described among the juveniles from the control 1% DMSO treated and standard podophyllotoxin .

4.4.1.2 LARVAL TOXICITY STUDY

Materials

Zf larvae of 5dpf, E3 embryo media (standard medium), 1% DMSO, glass petridishes, research microscope (laboscope microscope with microphotography), incubator, micropipette, ethanol extract from the leaves of *C.halicacabum* and standard podophyllotoxin.

Experimental design

Healthy 5dpf zf larvae were selected and used for larval toxicity study. About 5 larvae were released in the embryonic medium (10 ml) taken in a petridish, in triplicate. Various concentration of EECHL (0.5 and 0.75, 1 and 2 mg/ml) dissolved in 1% DMSO in the embryonic medium were tested. Two controls were used DMSO and embryonic medium respectively. Podophyllotoxin (10 µg/ml) was used as standard toxin .

Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control vials. Graph was plotted, concentration versus percentage lethality. Podophyllotoxin was used as a positive control in the bioassay.

The percentage lethality was calculated from the mean survival larvae of various concentrations of EECH treated dishes and control. The corrected(%) mortality was calculated by using Schneider-Orelli's formula.

Corrected %= Mortality(%) in Treated – Mortality (%) in Control x 100

100 –Mortality (%) in Control

Even though lipids play a vital role in the cellular function, it is not surprising that defects in lipid metabolism underlie many human diseases. More than a third of adults and 17% of children are currently classified as obese. This study was focussed on the inhibition of absorption of dietary lipid in the intestine of zf that provides an *invivo* environment as well as the potential for high throughput drug screening.

Observation Of Feeding Habit

5dpf zf larvae was fed with yolkmass in a petridish. After allowing time for its absorption (2-3 hrs), larvae from the petridish was collected and examined under research microscope to see the presence of intestinal yolkmass. Yolkmass within the intestinal lumen was detected and the expulsion of unabsorbed food material was also seen, which shows that the nutrient has been absorbed in the intestine and reaches the blood circulationZEBRAFISH HANDLING (Zhou, J *et al.,* 2015,Cliton D *et al.,*2010)

200-300embryos were maintained at 28°C in fish water (0.2% salt in demineralized water, pH 6.9-7.2) and the embryos were collected.

4.4.2 ASSESSMENT OF DRUG EFFECT ON ZEBRAFISH BLOOD LIPIDS

Zf larvae were fed with egg yolk for 48hr, followed by drug treatment for 24hr and 48hr at 3 concentrations. At the end of treatment, ORO was used to stain the lipids of Zf larvae. Fifteen to twenty Zf larvae from each group were randomly chose for ORO image acquisition. Zf larvae were immobilized in 3% methyl cellulose and

images were acquired in the identical lighting intensity under light microscope installed with a high speed video camera linked with computer system. When viewed dorso-laterally, the blood vessel of an 8 or 9 dpf Zf larvae was situated posterior to clocal pore and predominantly anterior to the tail fin. Quantitative image analysis of ORO was performed and Integrated Option Density (IOD) data were expressed as mean ±SEM. The effect of test drug EECHL was calculated based on the following formula.

Drug effect on lipids lowering (%) = (1-IOD compound/IOD vehicle)x100%.

To determine the statistical significance One way Anova is used to compare the differences among groups using SPSS 16.0 software.

4.4.3. EFFECT OF EECH ON THE CVS (HEART RATE, CARDIACMORPHOLOGY AND BLOOD CIRCULATION) OF ZEBRAFISH LARVAE

Cardiac function assessment

Hiperlipidemia zf larvae of 5dpf were placed in 96 well plates, at 1 larvae per well which different concentration of EECH (0.5, 0.75, 1 and 2mg/ml) (test). The larvae fed with normal diet (paramecia) in embryonic medium serve as the control. The cardiac function assessment was performed for normal diet and yolk mass fed larvae. All zf larvae were exposed for 3 hrs. Then they were immobilized in methylcellulose (3% w/v) and the heart rate per minute for each larva was recorded by eye under a research microscope. Cardiac morphology and blood circulation were observed. The test was performed in duplicate

PLATE-16

EMBRYO AND LARVAE OF ZEBRA FISH

EMBRYO OF ZEBRAFISH

LARVAE OF ZEBRAFISH 3dpf

LIPID UNFED ZEBRAFISH LARVAE 7dpf

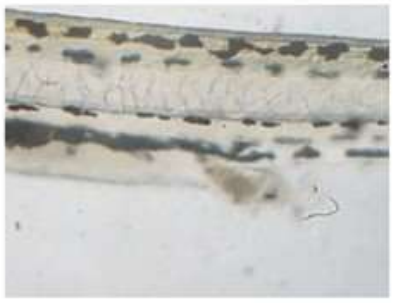
PLATE-17

ZEBRAFISH LARVAE FEEDING AND DIGESTION OF LIPID RICH DIET

ZF LARVAE EATING YOLK MASS

ZF LARVAL INTESTINE WITH YOLK MASS

ZF LARVA-EXPULSION OF DIGEST FOOD




RESULTS

CHAPTER V

RESULTS

5.1 PHARMACOGNOSY

5.1.1 MORPHOLOGICAL FEATURES OF *Cardiospermum halicacabum* (Plate1, Fig-1).

Balloon-vine is a perennial creeper at its base, the plant's stem is only approximately 3mm thick, but it can reach a height of up to 2 metres. The stem forms internodes of between 5-10 cm in length.The grooved stem carries alternate double triad leaves, 3 to 5 cm long, which are hairless or covered in a soft down of hairs.

LEAF: A pubescent or nearly globrous annual or perennial with slender branches climbing by means of tendriller **(Plate-2,3)**

Arrangement	: Ternately bicompound ,biternate essentially
Shape	: Ovate –lanceolate
Size	: 2-4cm in length
Colour	: Pale or light green
Margin	: Dentate irregularly deeply incised
Apex	: Acute
Base	: Obtuse-turncate beneath

STEM

Colour	: Green colour
Shape	: stems with minutely puberulous,sometimes slightly woody; Tendrils present,5 or 6-sulcate, slender,glabrous or sparesly hairy

PLATE-1

HABIT AND HABITATE OF

Cardiospermum halicacabum

FIGURE-1

DIAGRAMATIC REPRESENTAION OF

C.halicacabum

PLATE-2
LEAF ARRANGEMENT OF *C.halicacabum*

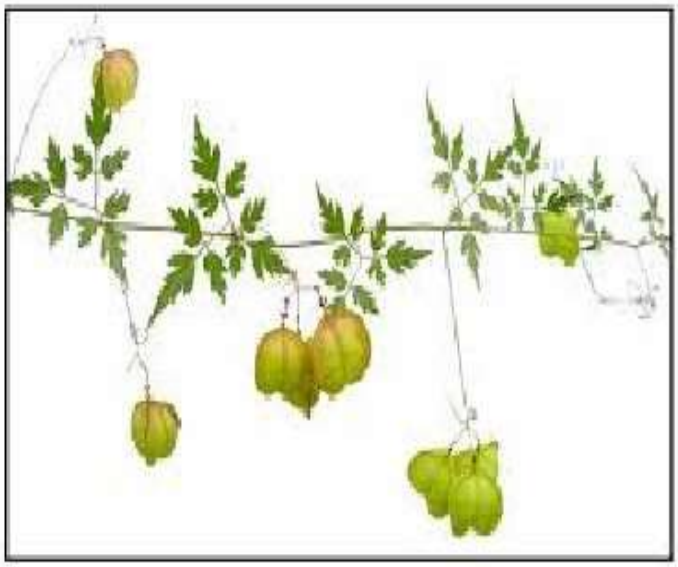


PLATE-3

DORSAL VIEW OF LEAF

VENTRAL VIEW OF LEAF

FLOWERS(Plate-4)

Description: Axillary heads usually 3 flowerd by abortion, white with yellowish centre Irregular flowers are borne in panicles (3, 6). Each flower bears four sepals, two large and two small, four whitish petals,. Petaloid appendages are at the base of each flower.

Size : 4mm long

Colour : Milky white

Ovary : 3-celled ovary bears one ovule per cell

Stamens : 8 stamens present

FRUITS(Plate-5)

Shape : Inflated , papery capsule

Size : 3 chambers, 3-4.5 cm in diameter

Colour : Before ripe- green colour ,After ripe-s light yellow colour

Ribes : 8-10 prominent longitidunal ribes not covered with spines or Papillae

SEEDS (Plate-6)

Shape : Opaque,finely porous heart shape

Colour : Black,smooth with white, Size : 5mm diameter

PLATE-4

FLOWER OF *C.halicacabum*

PLATE-5

FRUITS OF *C.halicacabum*

PLATE-6

SEEDS OF *C.halicacabum*

5.1.2 MICROSCOPY OF THE LEAF

Leaf is dorsiventral and prominent.

LEAF MIDRIB (Plate-7,Figure-2)

It is projecting both adaxial and abaxially. The adaxial part is thick and pyramid like but abaxial part is semicircular with undulate outline. The inner part of the adxial cone includes a cluster of angular collenchyma cells. The palisade is extended up to the shoulders of the cone. The vascular strand is fairly prominent and is triangular in shape comprises a cluster of wide circular thin walled xylem elements and a thick band of phloem elements.

EPIDERMIIS IN SURFACE VIEW (Plate-8)

Fairly large squarish thick walled cells. The abaxial epidermal cells are thin, small and elliptical. Paradermal section shows epidermal cells wide with highly wavy anticlinal walls and amoeboid outline. The stomata is dense and diffuse in distribution and are anomocytic type. The guard cells are elliptical and the stomatal pores are slit like.

LAMINA (Plate-9)

The mesophyll is differentialted into upper band of narrow cylindrical palisade cells and lower zone of two or three layers of lobed loosely arranged spongy parenchyma cells. The lateral veins are conspicuous, straight and uniformly thin. The vein islets are distinct having straight vein boundaries. Vein termination are present in the islets some places branched once or twice and spread within the islets.

PLATE-7

T.S OF *C.halicacabum* LEAF MIDRIB(HAND SECTION)

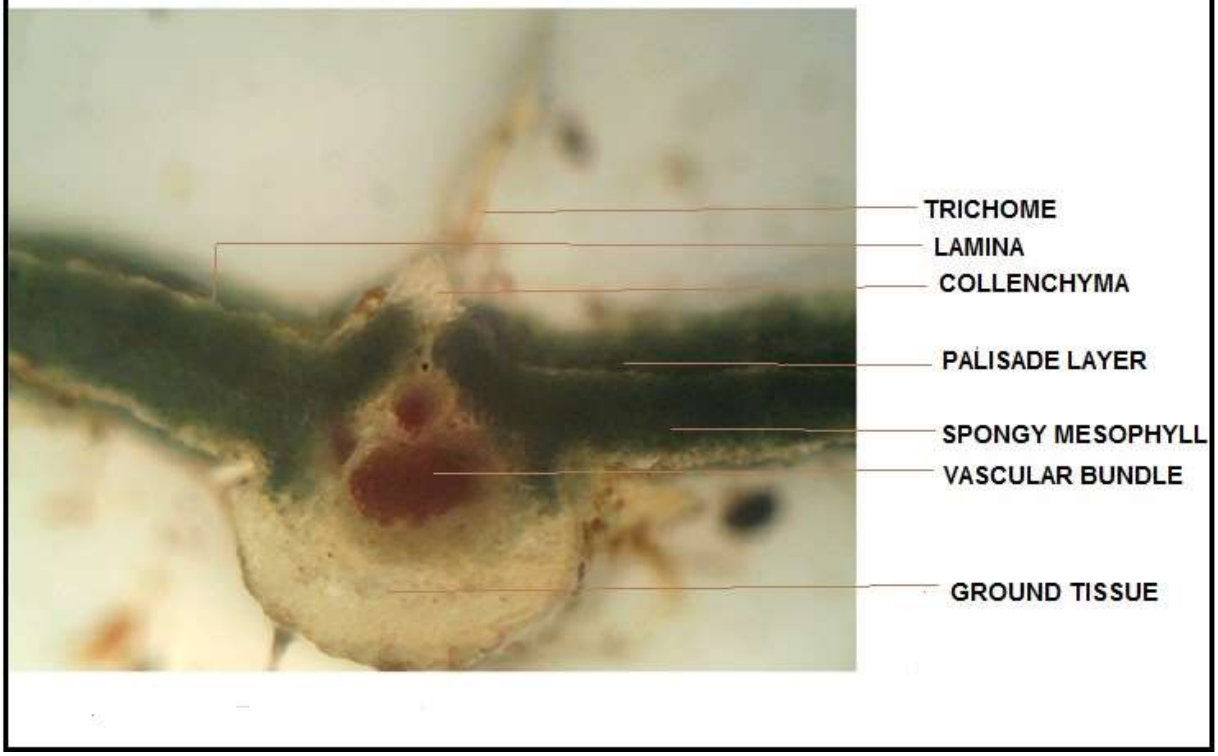
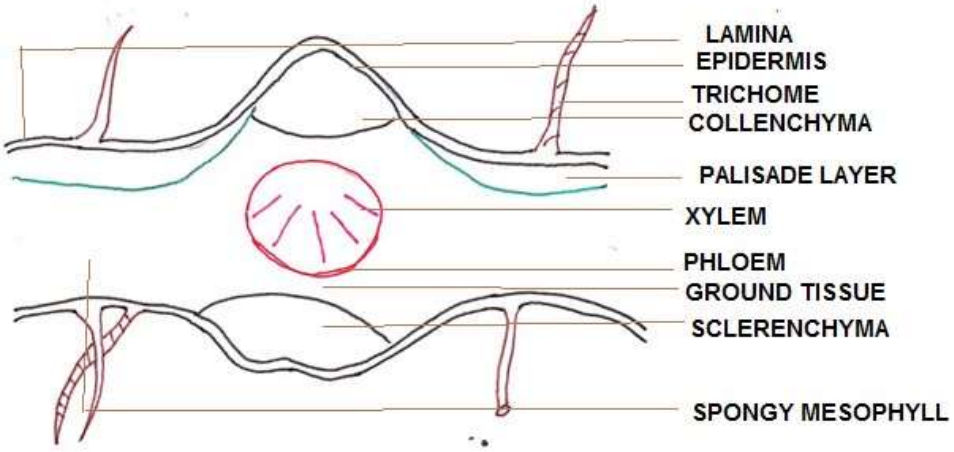


FIGURE-2

T.S OF *C.halicacabum* LEAF MIDRIB

GROUND PLAN



DETAILED DIAGRAM

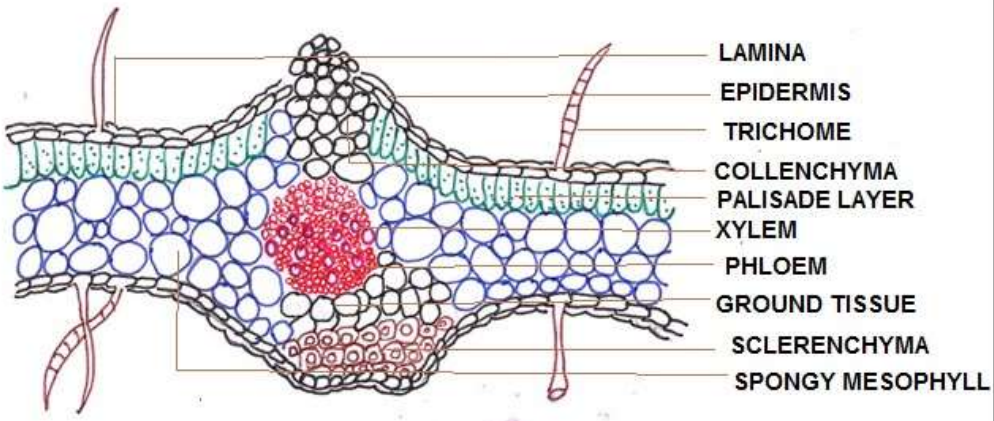
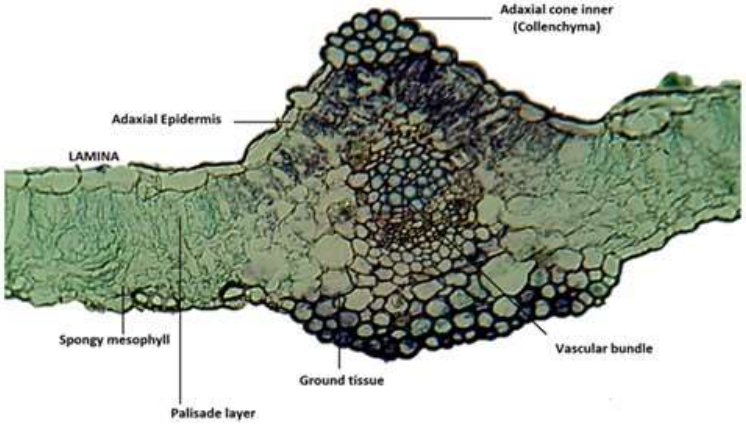


PLATE-7

T.S OF *C.halicacabum* LEAF MIDRIB (MICROTOME SECTION)



ENLARGEMENT OF VASCULAR BUNDLE

(MICROTOME SECTION)

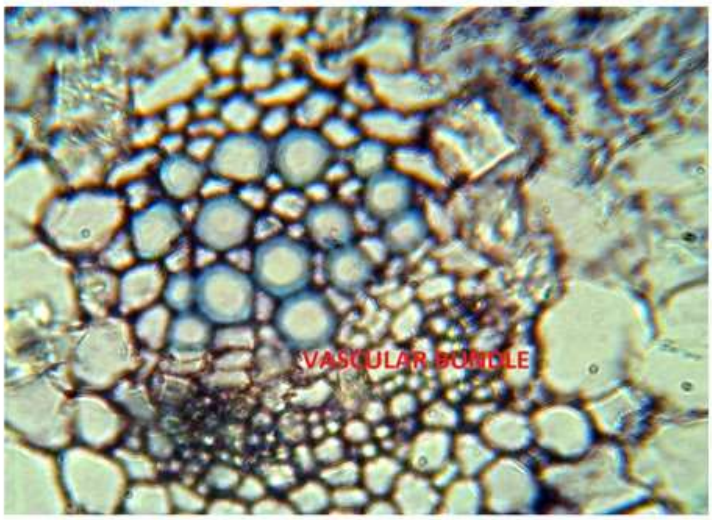


PLATE-7
TRICHOMES OF THE *C.halicacabum* LEAF
(HAND SECTION)

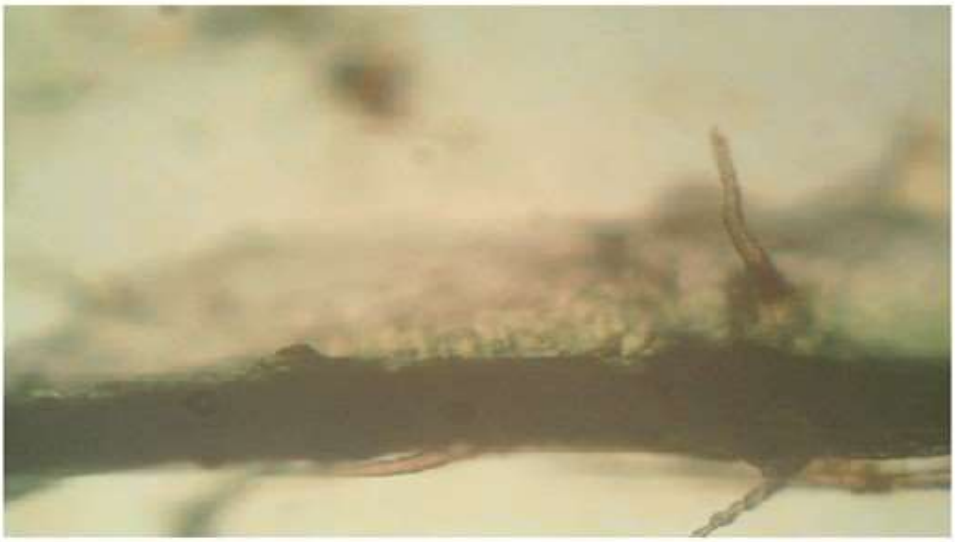


PLATE-8

EPIDERMIS SURFACE VIEW SHOWING

ANAMOCYTIC STOMATA

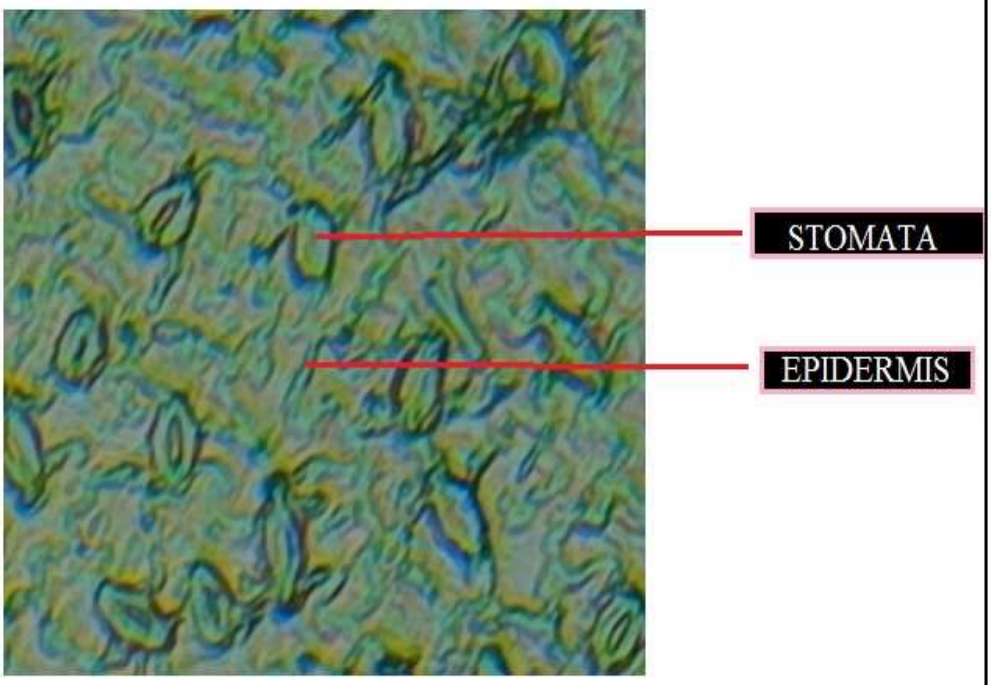
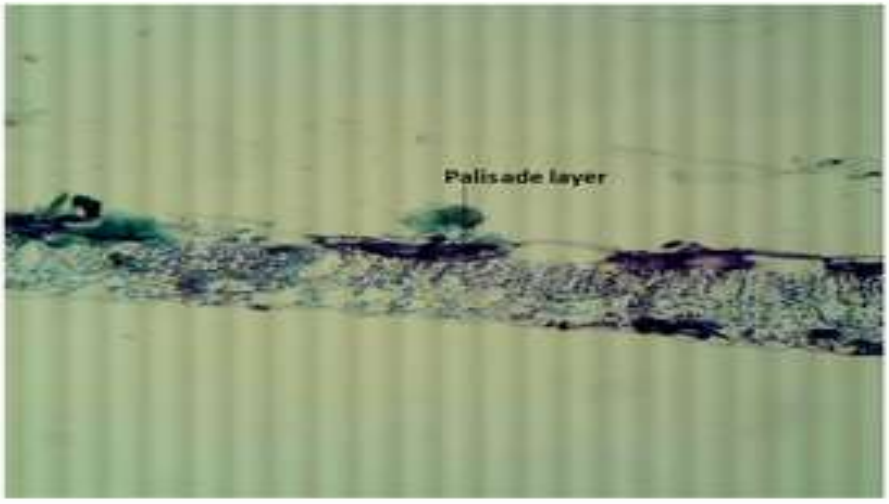
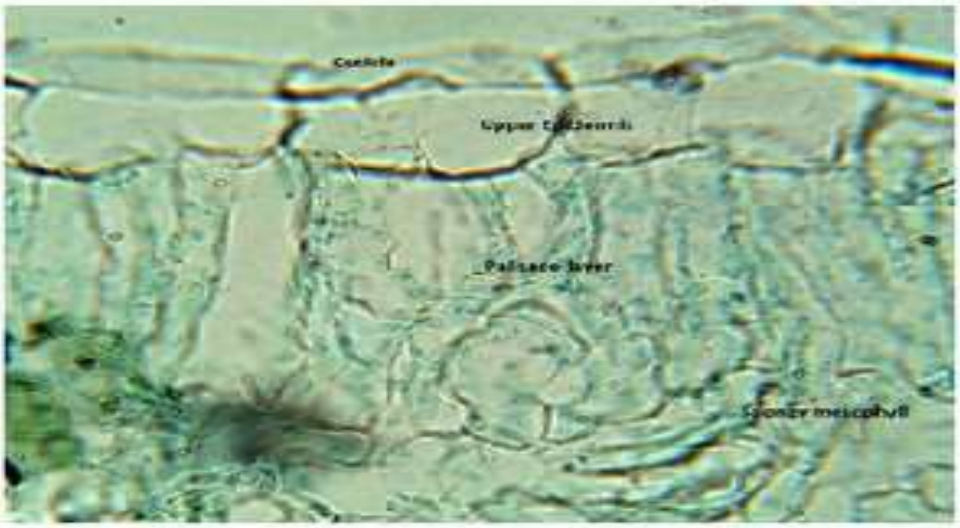


PLATE-9

T.S OF LAMINA



ENLARGEMENT OF LAMINA



T.S OF PETIOLE:(Plate-10,Figure-3)

It shows two prominent grooves towards upper side where as the lower side is round. The epidermis is composed of single layer of cells. Chlorenchymatous hypodermis is present. 4-6 collateral vascular bundles are present in the ground tissue. The xylem is present upper side and phloem towards the lower side. The ground tissue is made up of parenchyma cells.

T.S OF RACHIS:(Plate-11)

Polygonal in outline with two winged projections at the upper side. Single layer of epidermal cells composed of horizontally flattend cells with cuticle. Covering unicellular trichomes are present. Peripheral layer of ground tissue are composed of collenchymatous and chlorenchymatous cells forms the hypodermis. A continuous ring of pericyclic fibres is present in the ground tissue. Vascular bundles are arranged in a ring and are collateral. The rest is parenchymatous.

5.1.3 MICROSCOPICAL STUDY OF LEAF USING SCANNING ELECTRON MICROSCOPE. (Plate-12,13)

SEM microscopy when applied to the surfaces of the leaf revealed the presence of unicellular trichome and ranunculaceous or anomocytic stomata and their distribution.

PLATE-10

T.S OF PETIOLE

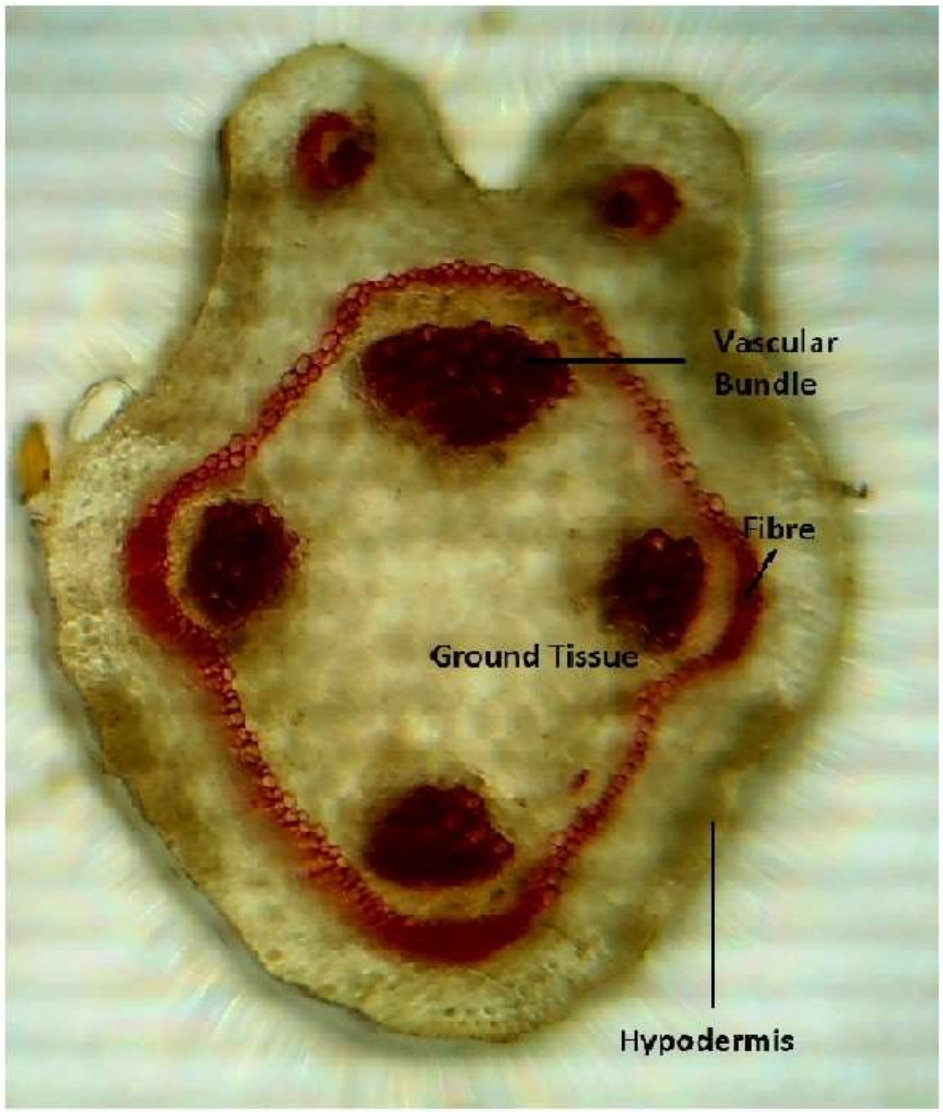
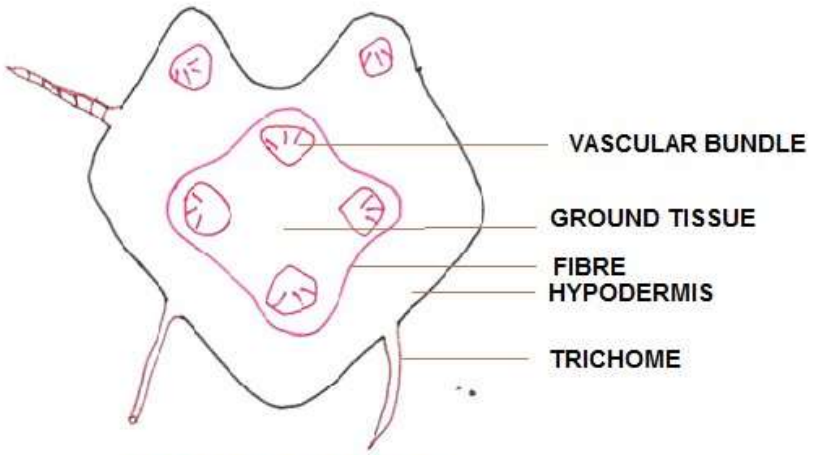


FIGURE-3

T.S OF *C.halicacabum LEAF* PETIOLE

GROUND PLAN



DETAILED DIAGRAM

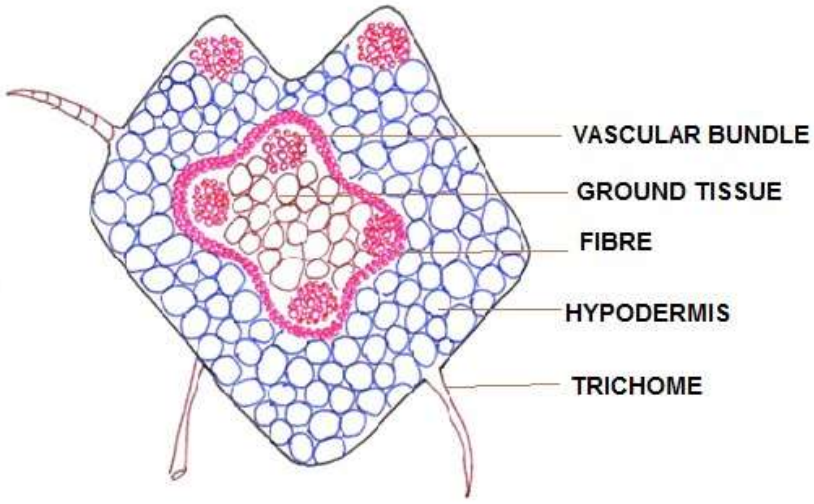
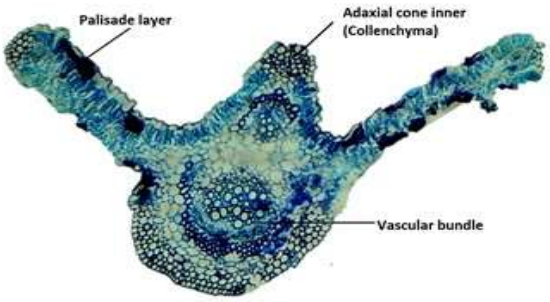


PLATE-11
T.S OF RACHIS



ENLARGEMENT OF RACHIS

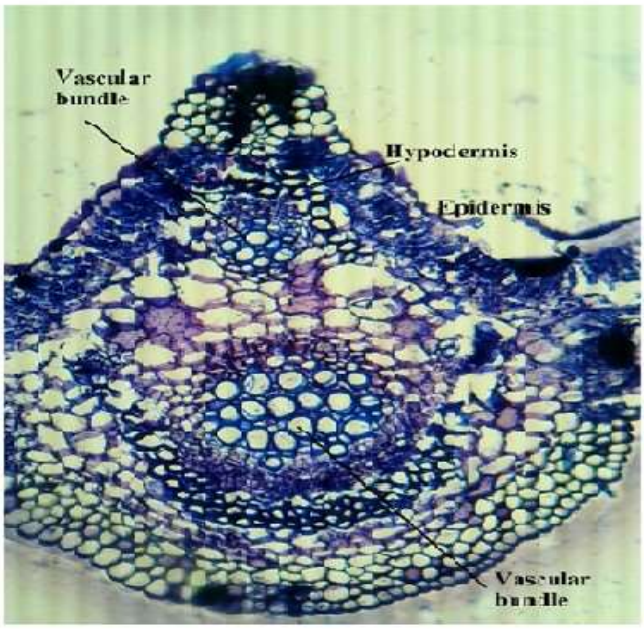
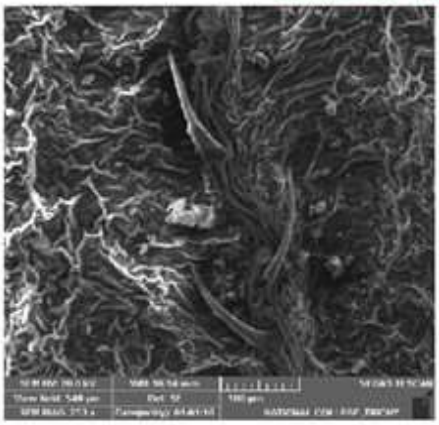
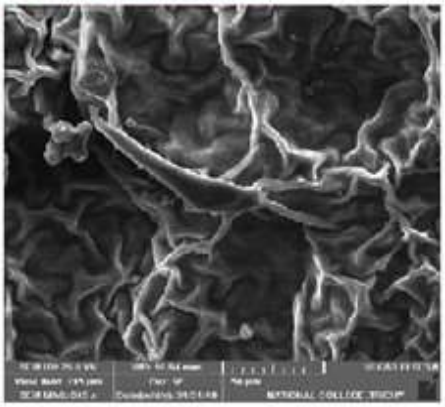


PLATE-12

SEM OF THE LEAF OF *C.halicacabum*





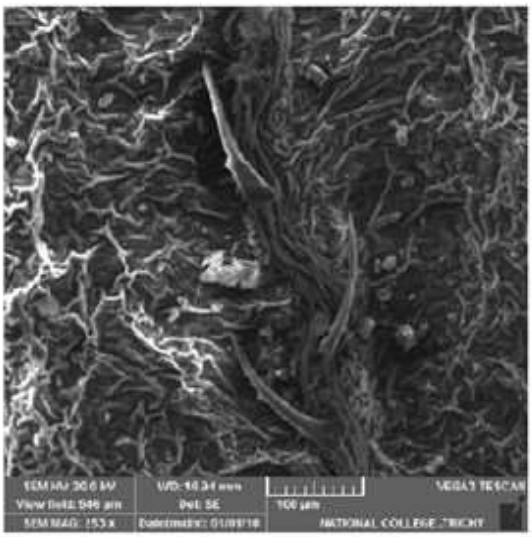
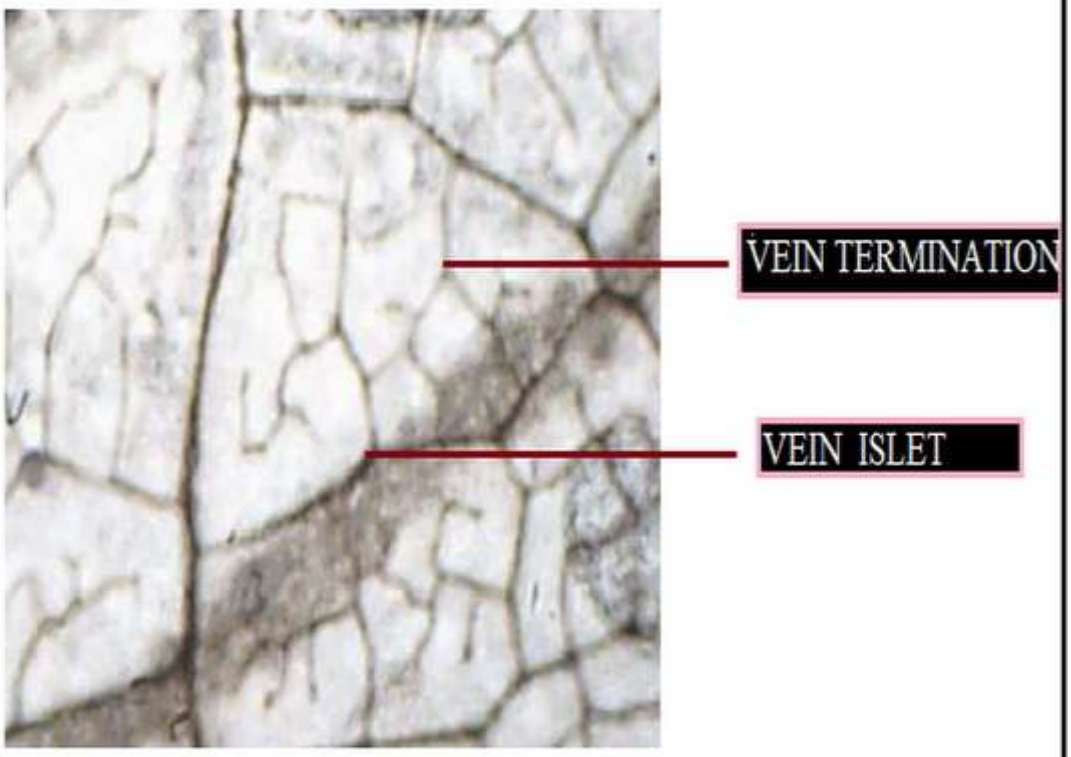


PLATE-13

VENATION PATERN OF *C.halicacabum* LEAF



5.1.4 POWDER MICROSCOPY (Fig-4)

ORGANOLEPTIC CHARACTERS

1.Nature	:	Coarse powder.
2.Color	:	Dark green.
3.Odour	:	Charecterstic odour.
4.Taste	:	Tasteless

5. Pressed in between two filter paper: No oil mark on the paper.

We have observed the following microscopical cell structures,

- Ranunculaceous stomata (Anomocytic),
- Parenchyma,
- Xylem vessels,
- Fibers,
- Collenchyma,
- Uniseriate,multicellular trichomes.

5.1.5 MICROSCOPIC SCHEDULES

As per the methods described in materials and methods, microscopic schedule was carried out and the results were tabulated from the Tables 1-4. The following evaluation were carried out.

FIGURE-4

POWDER MICROSCOPY OF C.halicacabum

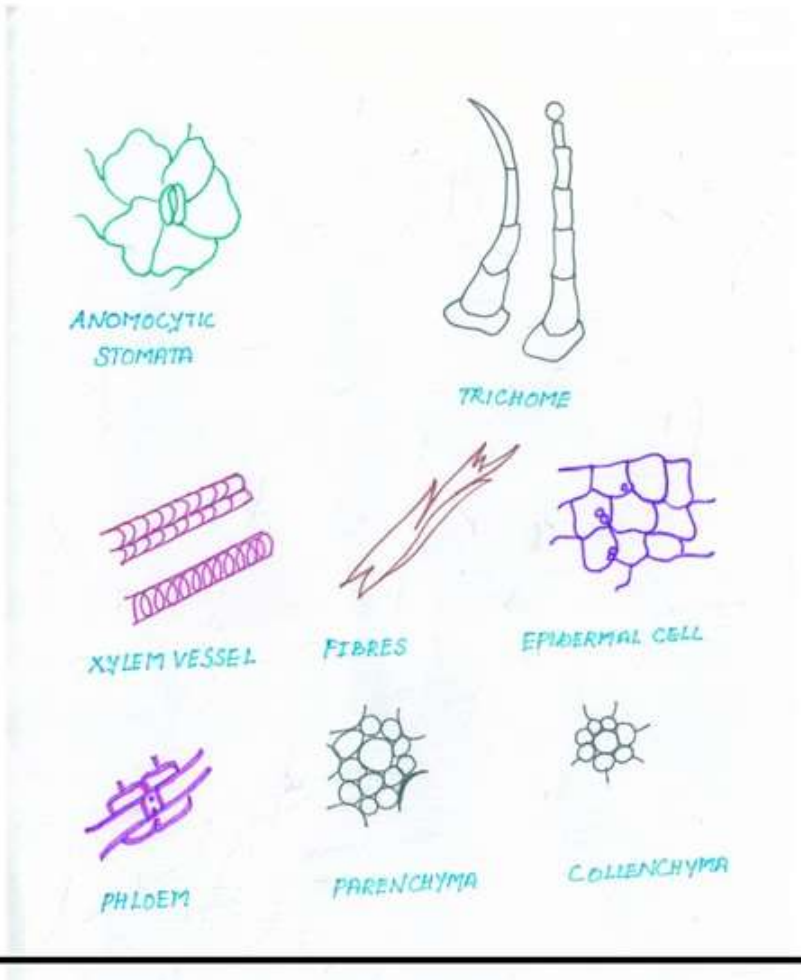


Table – 1

VEIN ISLET AND VEIN TERMINATION NUMBER OF *C.halicacabum* LEAVES

Observation Number	Vein Islet Number	Vein Termination Number
1	13	12
2	11	13
3	15	17
4	14	18
5	15	12
6	10	14
7	11	11
8	12	15
9	17	10
10	22	16

Range	Minimum	Average	Maximum
Vein Islet Number	10	15	22
Vein Termination	10	13	18

Table – 2

STOMATAL NUMBER OF *C.halicacabum*

Observation Number	Lower Epidermis	Upper Epidermis
1	22	25
2	19	23
3	21	24
4	18	23
5	20	26
6	29	27
7	27	28
8	26	21
9	30	20
10	18	22

Range	Minimum	Average	Maximum
Lower Epidermis	18	22	30
Upper Epidermis	20	23	28

Table – 3

STOMATAL INDEX OF *C.halicaca*bum

Observation Number	Lower Epidermis	Upper Epidermis
1	10	11.9
2	10.3	12.1
3	9.9	12.4
4	10.5	12
5	10.7	12.5
6	10.9	12.7
7	11	11.7
8	11.3	13
9	12	13.1
10	11.8	13.5

Range	Minimum	Average	Maximum
Lower Epidermis	9.9	10.7	12
Upper Epedermis	11.7	12.5	13.5

Table – 4

PALISADE RATIO OF *C.halicacabum*

Observation Number	Upper Epidermis
1	6.7
2	7
3	6.8
4	7.7
5	7.5
6	6.7
7	6.9
8	7.6
9	7.8
10	7.9

Range	Minimum	Average	Maximum
Lower Epidermis	6.7	7.5	7.9

5.1.6 PHYSICO CHEMICAL PARAMETERS

As per the methods described in materials and methods, physico chemical parameter was carried out of quantitative microscopy and the results were tabulated from the Tables 5-7. The following evaluation were carried out.

Table – 5

ASH VALUE FOR THE LEAVES OF *C.halicacabum*

Observation Number	Total Ash (%)	Acid Insoluble Ash (%)	Water Soluble Ash (%)
1	5	0.94	-
2	7.6	1	-
3	7.5	0.97	-
4	6.8	1.03	-
5	7.05	1.10	-
6	6.9	-	4.04
7	7	-	4.09
8	7.1	-	4.08
9	7.3	-	4.07
10	7.02	-	4.12

Minimum	5	0.94	4.04
Average	6.8	1	4.08
Maximum	7.6	1.10	4.12

Table – 6

PERCENTAGE OF LOSS ON DRYING FOR THE LEAVES OF *C.halicacabum*

Observation Number		Loss on Drying (%) W/W	
		Leaf Powder	
1		6.08	
2		6.53	
3		6.72	
4		7.01	
5		6.63	
Material	**Minimum**	**Average**	**Maximum**
Leaves powder	6.08	6.72	7.01

Table – 7

EXTRACTIVE VALUES (INDIVIDUAL SOLVENTS)

Solvents	Extractive value (%)w/v
Ethanol	13.5
Water	10.3

5.2. PHYTOCHEMICAL SCREENING

5.2.1. Preliminary Photochemical Screening

Preliminary phytochemical screening of the powdered mature leaves were carried out and the results are as follows (Table 9-10)

TEST FOR ALKALOIDS

Mayer's Test	:	Cream precipitate shows the **Presence** of alkaloids.
Dragendorff's Test	:	Reddish brown precipitate shows the **Presence** of alkaloids.
Hager's Test	:	Yellow precipitate shows the **Presence** of alkaloids.
Wagner's Test	:	Reddish brown precipitate shows the **Presence** of alkaloids.

TEST FOR CARBOHYDRATES

Molish's Test	:	Appearance of purple color shows the **Presence** Of carbohydrate
Fehling's Test	:	Formation of reddish brown precipitate shows the **Presence** of free reducing sugars.
Benedict's Test	:	Formation of reddish brown precipitate shows the **Presence** of free reducing sugars.

TEST FOR GLYCOSIDES

Test A : No red color precipitate shows the **Absence** of glycosides.

Test B : Appearance of red color precipitate less than test A shows the **Absence** of glycosides

Anthraquinone glycosides

Borntrager test : No pink color in ammonical layer shows the **Absence** of anthraquinone glycosides.

Modified borntrager's test : No pink color in ammonical layer shows the **Absence** of anthraquinone glycosides.

Murexide test : No appearance of purple color shows the **Absence** of purine alkaloids

Keller killiani's test : No reddish brown color ring at the junction shows the **Absence** of cardiac glycosides.

Raymond's test : No violet color shows the **Absence** of cardiac glycosides.

Legal's test : No blood red color shows the **Absence** of cardiac glycosides.

TEST FOR CARDIAC GLYCOSIDES:

TEST FOR PHYTO STEROLS

Salkowski's test : Apprearance of red colour in lower layer shows the **Presence** of sterol.

Libermann-Burchard's test : Brown ring at the junction of two layers and the appearance of green colour in the upper layer shows the **Presence** of sterols.

TEST FOR SAPONINS : Froathing occurs indicates the **Presence** of saponins.

TEST FOR TANNINS

Ferric chloride test : Appearance of bluish black color shows the **Presence** of tannins.

Gold beater's skin : Appearance of brown color shows the **Presence** of tannins.

TEST FOR PROTEINS AND FREE AMINO ACIDS

Millon's test : Appearance of red color on heating shows the **Presence** of proteins.

Biuret test : Appearance of violet color shows the **Presence** of proteins.

Ninhydrin test : Formation of violet color shows the **Presence** of amino acids.

TEST FOR MUCILAGE : Appearance of reddish pink color shows the **Absence** of mucilage.

TEST FOR TERPENOIDS : Appearance of pink color shows the **Presence** of terpenoids.

TEST FOR FLAVONOIDS

Shinoda test : Appearance of purple color shows the **Presence** of flavonoids

Alkaline reagent test : Appearance of yellow orange color shows the **Presence** of flavonoids

Acid test : Appearance of yellow orange color shows the **Presence** of flavonoids.

Zinc HCL test : Appearance of red color shows the **Presence** of flavonoids

TEST FOR VOLATILE OIL : Volatile oil not obtained shows the absence of volatile oil.

TEST FOR FIXED OIL : No translucent greasy spot shows the absence of fixed oil.

RESULTS FOR THE PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAVES OF C.halicacabum.

S.NO	TEST	OBSERVATION
I.	**ALKALOIDS**	
	Mayer's reagent	+
	Dragendroff's reagent	+
	Hager's reagent	+
	Wagner's reagent	+
	Murexide Test	-
II.	**CARBOHYDRATES**	
	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
III.	**GLYCOSIDES**	
	Anthroquinone glycosides	-
	Borntrager's test	-
	Modified Borndrager's test	-
	Cardiac glycosides	
	Keller Killiani test	-
	Raymond test	-
	Legal test	-
IV.	**STEROLS**	
	Salkowski test	+
	Lieberman Burchard's test	+
V.	**SAPONINS**	+
VI.	**TANNINS**	
	Ferric chloride	+
	Gold Beater's skin test	+
VII.	**PROTEINS AND FREE AMINO ACIDS**	
	Millon's test	+
	Biuret test	+
	Ninhydrin test	+
VIII.	**MUCILAGE**	-
IX.	**TERPENOIDS**	+
X.	**FLAVONOIDS**	
	Shinoda test	+
	Alkali test	+
	Acid test	+
	Zn/Hcl test	+
XI.	**VOLATILE OIL**	-
XII.	**FIXED OIL**	-

5.2.2 FLUORESCENCE ANALYSIS OF POWDERED LEAF

The fluorescence analysis of the leaf powder of *C.halicacabum* was studied.

The results were as follows (Table 9)

TABLE – 9

FLUORESCENCE ANALYSIS

No	Treatment	Visible light	UV 254 nm	UV 366 nm
1	Powder	Light green	Green	Brownish green
2	Powder + 1N NaoH	Green	Black	Greenish black
3	Powder +Picric acid	Green+ yellow	Black Green	Black
4	Powder + Acetic acid	Green brown	Black green	Green or grey
5	Powder + 1N HCL	Brownish green	Black green	Black
6	Powder + 1N HNO3	Green brown	Black Green	Black
7	Powder + 5 Iodine	Brown Green	Dark brown	Black

PREPARATION OF EXTRACT EECHL

Dark green viscous residue obtained 13.5%w/v.

5.2.3 ESTIMATION FO FLAVONOID CONTENT

Flavonoid content of EECHL was found to be 1.06 mg/g.

5.2.4 ESTIMATION OF TOTAL PHENOLIC CONTENT

Total phenolic content of EECHL was found to be 38.05 mg/g.

5.2.5 DETERMINATION OF TRACE ELEMENTS IN THE LEAF OF *C.halicacabum BY* ENERGY DISPERSIVE X-RAY SPECTROMETER (EDS).

Estimation of the elements like C, O, Mg, P, Cl, K, Cl, Ca showed the following mg weight percentage and atomic percentage.

FIG: 5
ENERGY DISPERSIVE X-RAY SPECTRUM FOR *C.halicacabum* LEAVES

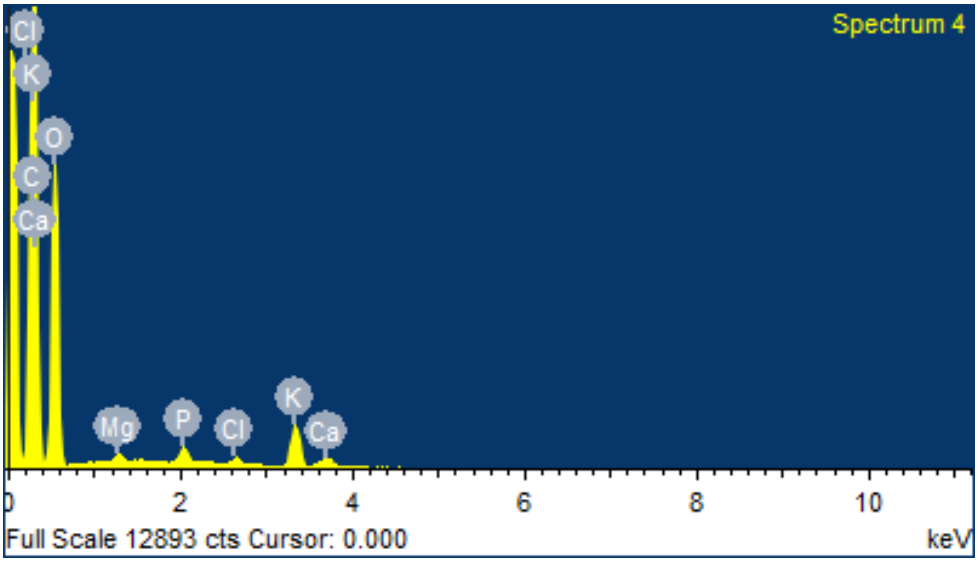


TABLE 10

C.halicacabum LEAVES ELEMENTS WEIGHT PERCENTAGE

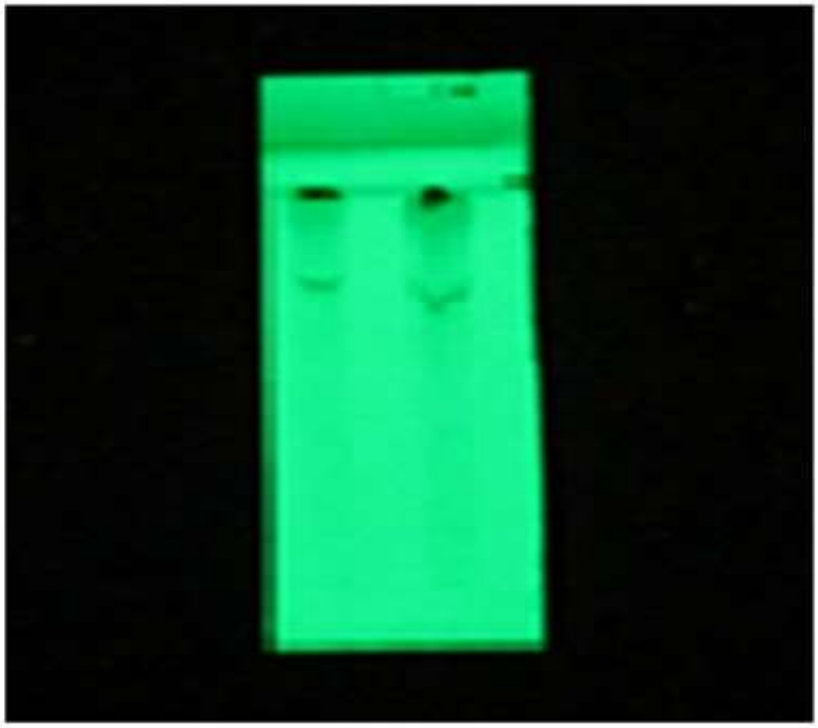
S.NO	ELEMENTS	WEIGHT (%)
1	CK	53.67
2	OK	44.05
3	MgK	0.18
4	P K	0.36
5	Cl K	0.18
6	K K	1.27
7	Ca K	0.28

5.2.6 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY OF THE EECHL

About 5 µl of standard Pinitol, 6µl of EECHL was applied as a band using CAMAG Linomat sample applicator on aluminium sheets pre-coated with silica gel 60 GF 254 HPTLC plates used as a stationary phase. The plates were developed in the mobile phase Chloroform:methanol:water (6:3.5:0.5) to a distance of 16.0mm in CAMAG trough glass chamber. The tracks were scanned using WIN CATS 1.43 software at 254nm. The fingerprint profiles were recorded and presented. The plot showed 9 spots after development with the mobile phase. The Rf value of standard Pinitol 0.68 and Rf value of standard Pinitol 0.69 of EECHL.(0.142mg/100mg)

PLATE-14
TLC OF EECHL

PLATE-15
TLC OF EECHL UNDER UV



winCATSPlanar Chromatography Manager

>6	90.0 mm	20.0 µl	3	CARDIOSPERMUM HALICACABUM	Yes

Development - Glass tank

Chamber type	Twin Trough Chamber 20x10cm
Executed by	KMCHCOP Tuesday, January 02, 2002 12:36:57 AM
Comment	
Pre-conditioning	
Mobile phase	CHLOROFORM:METHANOL:WATER (6:3.5:0.5)
Solvent front position	80.0 mm
Volume	10.0 ml
Drying device	Oven
Temperature	60 °C
Time	5 Minutes
Notes	

Detection - CAMAG TLC Scanner 3

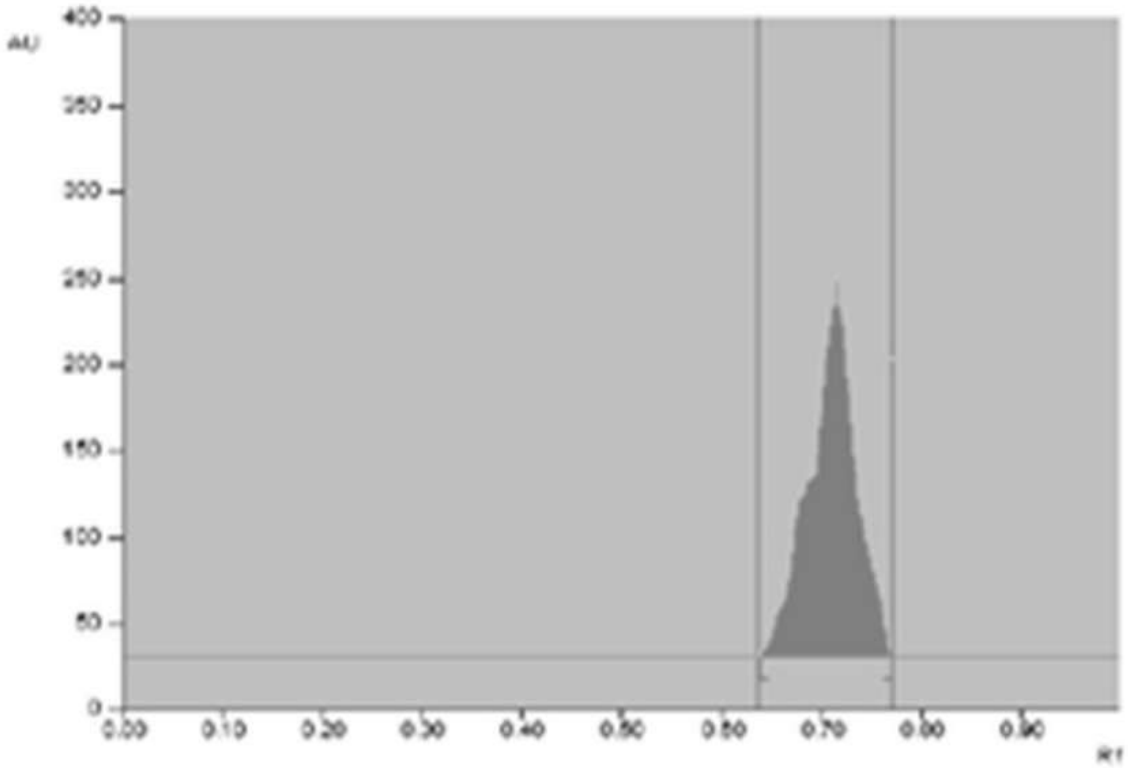
Information	
Application position	10.0 mm
Solvent front position	80.0 mm
Instrument	CAMAG TLC Scanner 3 "Scanner3_170310" S/N 170310 (1.14.28)
Executed by	KMCHCOP Tuesday, January 01, 2002 12:42:46 AM
Position of first track X	10.0 mm
Distance between tracks	16.0 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	75.0 mm
Slit dimensions	6.00 x 0.45 mm, Micro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step
Measurement Table	
Wavelength	254
Lamp	D2 & W
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	281 V
Detector properties	
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (38)

Integration

Properties	
Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	5.0 mm
Track end position	75.0 mm
Display scaling	Automatic

FIG-6

STANDARD PINITOL

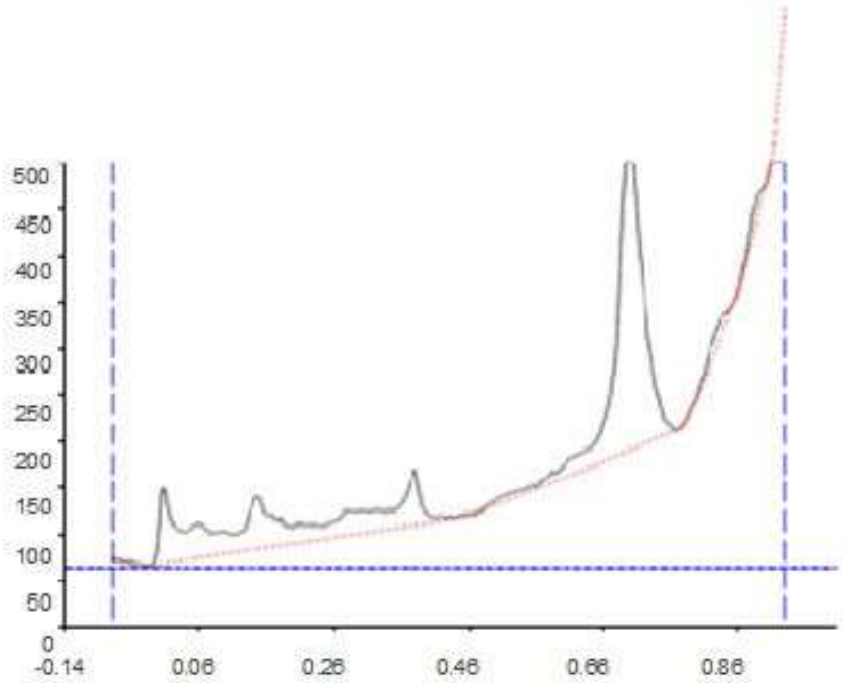


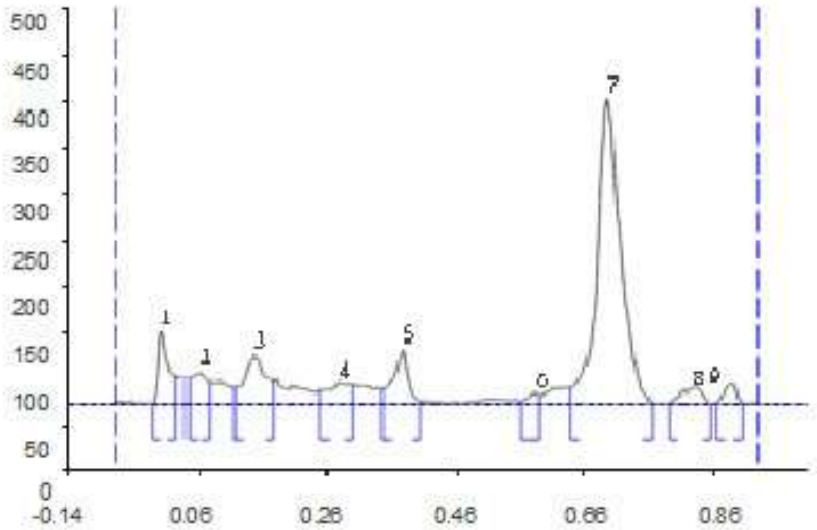
winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	0.2	0.00	80.1	12.33	0.03	33.2	1234.8	7.35	unknown *
2	0.04	31.2	0.06	37.6	5.79	0.07	24.2	817.8	4.87	unknown *
3	0.11	18.8	0.14	55.5	8.55	0.17	25.8	1581.2	9.41	unknown *
4	0.25	16.2	0.28	25.2	3.88	0.30	22.3	742.3	4.42	unknown *
5	0.34	17.3	0.38	61.7	9.50	0.41	2.5	1202.0	7.15	unknown *
6	0.56	2.5	0.58	13.4	2.07	0.59	8.8	164.5	0.98	unknown *
7	0.64	19.2	0.69	332.4	51.19	0.76	0.2	10239.8	60.92	pinitol
8	0.79	0.0	0.82	19.6	3.03	0.85	0.0	465.0	2.77	unknown *
9	0.86	0.6	0.89	23.9	3.67	0.90	0.6	361.0	2.15	unknown *

FIG-7

HPTLC profile of *C.halicacabum*





5.3 PHARMACOLOGICAL STUDIES

5.3.1 WHOLE EMBRYO CULTURE TOXICITY STUDY

Effect of EECHL on the developmental stages of zf embryo was carried out. The eggs were cultured in the embryonic medium. The maximal acceptable toxicant concentration (MATC) was calculated by scoring the malformation 1% DMSO and podophyllotoxin (0.010µg/ml) were used as control and standard toxin. No malformations and incidence of mortality was observed up to the 0.5-1.5 µg/ml concentration level (Score 0). But medium to strong edema, eye malformation, bent tail, weak undulated notochord and twisted notochord were observed from 2 to 4µg/ml concentration up to 80hpf. No mortality was observed in this concentration level. Total mortality was observed in the standard podophyllotoxin at 0.01µg/ml concentration (score 40).

TABLE 11

SCORES FOR THE WHOLE EMBRYO TOXICITY

Conc. µg/ml	Hours	Score
0.5-0.7	**All**	**Nil**
1	12	1
	36	1
	60	2
	80	2
2	12	5
	36	5
	60	10
	80	13

5.3.2 ZEBRAFISH LARVAL TOXICITY STUDY (Fig-8)

Larval toxicity study was carried out on zf larvae of 5dpf cultured in E3 embryonic medium. Five larvae per group was taken and treated with 0.5, 0.75, 1 and 2 µg was taken as a test trail and mortality percentage was calculated.

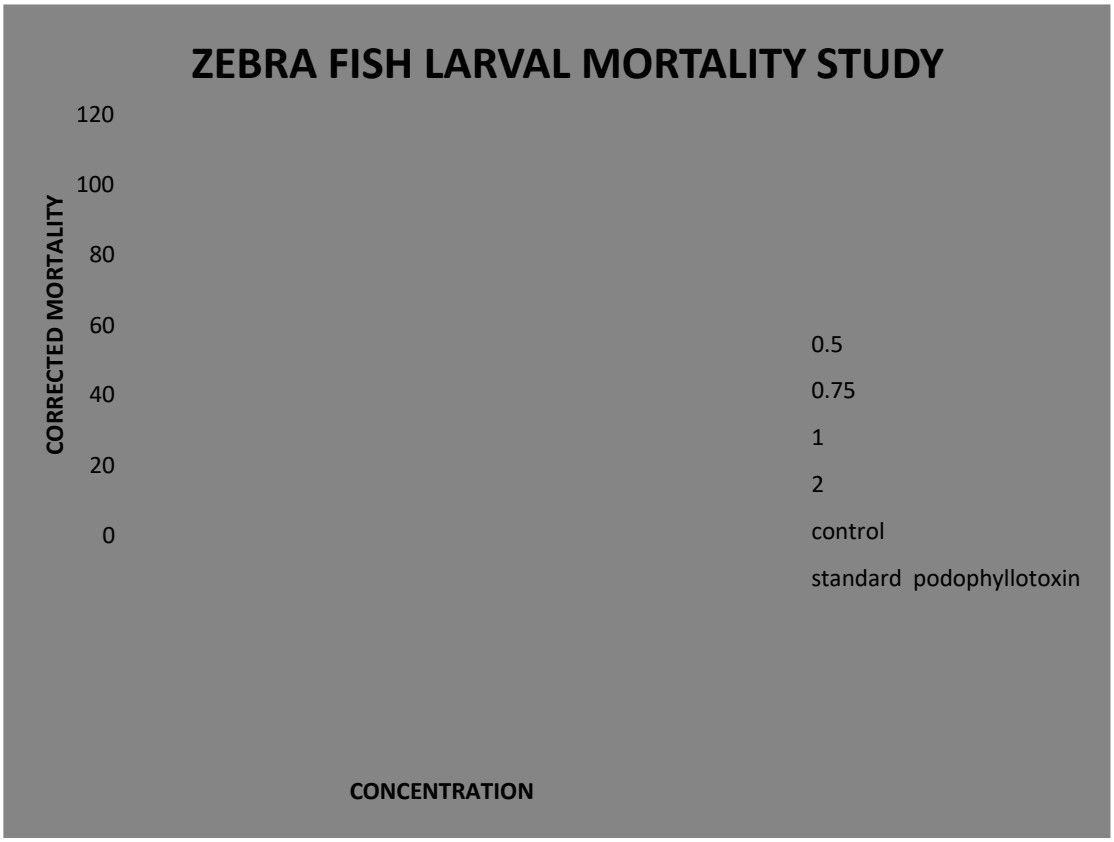
From the experiment it was observed that there was no mortality in 0.5 and 0.75 µg/ml concentrations. But 5% and 10% mortality was observed at 1 and 2 µg/ml concentration respectively. No mortality was observed in both the control viz DMSO and embryonic medium. 100% mortality was observed in the standard podopyllotoxin at 0.5 µg/ml concentration.

TABLE-12

ZEBRAFISH LARVAL TOXICITY STUDY

CONCENTRATION (g/ml)	Number of Larvae	After 4 hrs	Mortality	Corrected Mortality
0.5	20	Nil	-	Nil
	20			
	20			
0.75	20	Nil	-	Nil
	20			
	20			
1	20	Nil	6	6
	20	1		
	20			
2	20	1	11	10.5
	20	1	10	
	20	0	10	
Control	20	Nil	Nil	-
	20			
	20			
Standard Podophylotoxin	20	20	100	
	20	20	100	100
	20	20	100	

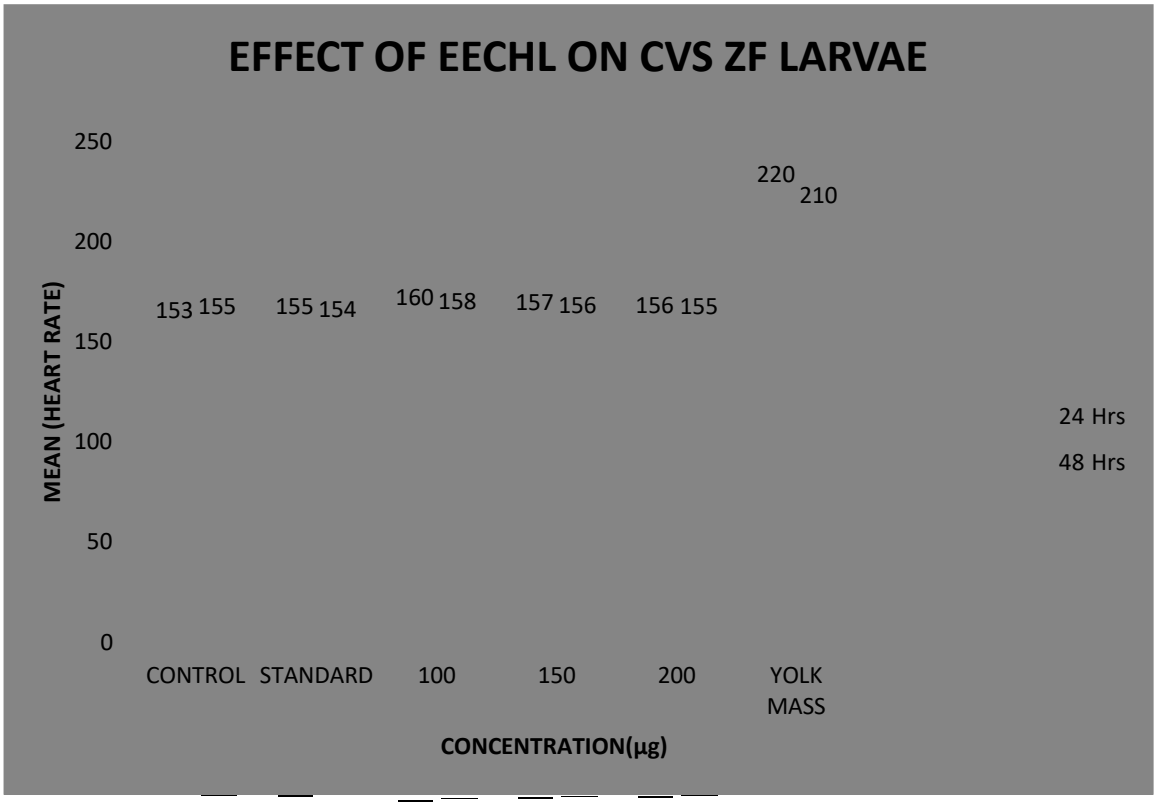
FIGURE-8


ZEBRA FISH LARVAL MORTALITY STUDY
CORRECTED MORTALITY
120
100
80
60
40
20
0
0.5
0.75
1
2
control
standard podophyllotoxin
CONCENTRATION

5.3.3 EFFECT OF EECHL ON THE CVS (HEART RATE, CARDIAC MORPHOLOGY AND BLOOD CIRCULATION) Zf LARVA.(Fig-9)

5dpf larvae were fed with lipid rich diet containing 100, 150, 200 µg/g of EECHL larvae fed with normal diet (paramecia) was also observed. From the result it was observed that there was increase in heart rate after a lipid rich diet in the zf larvae (220 ±2.1) compared with the normal diet fed larvae (153 ±1.7). Moderately elevated heart rate (174±0.96) was observed in the zf treated with 100 µg/g EECHL in 0.15 or 1% yolk mass. But no elevated heart rate were observed in the 150, 200 µg/g of EECHL in yolk mass 160± 1.16, 155± 1.9 respectively.

FIGURE-9


EFFECT OF EECHL ON CVS ZF LARVAE
MEAN (HEART RATE)
250
200
150
100
50
0
153
155
155
154
160
158
157
156
156
155
220
210
CONTROL
STANDARD
100
150
200
YOLK MASS
CONCENTRATION(µg)
24 Hrs
48 Hrs

5.3.4 ASSESMENT OF LIPID LOWERING EFFECT OF EECHL ON HYPERLIPIDEMIC ZEBRAFISH LARVAL MODEL:(PLATE-18,Fig-10).

The developing embryonic and larval zebrafish are small and translucent, enabling non intrusive visualization of organs and biological processes in *vivo* with a high resolution. Therefore we used a high fat diet egg yolk to feed zebrafish larvae and develop a zebrafish hyperlipidemia model and lipids in zf were quantified using ORO staining. Feeding zf with egg yolk for 24,48 hrs we found that the lipids of gut and blood vessel increased with feeding time and blood lipids engorge uniformly at 48h of feeding. Further we observed the clearance time of stainable lipids in zf and found, after removing egg yolk, that lipids of gut and blood vessel were still abundant at 24h and 48 h, lipids were plentiful in gut but poor in vasculature at 72h and gut and vessel lipids were almost gone at 96h.Thus we fed zf with 0.1% egg yolk for 48hrs to establish hyperlipidemia model for assessing lipid lowering efficacy of test drug in this model after drug treatment for 24 and 48h based on quantitative analysis of ORO in zf vena caudalis

To determine whether the zf response to the drugs we treated zf with the EECHL extract in three different concentration (100,150 and 200µg/g) , using ezetimibe 1µM/ml as standard drug were added tp the treatment media at 7dpf and were assessed at 9 dpf. After 24 , 48h treatment test drug and std drug statistically significant positive effect i.e percentage reduced hyperlipidemia zf lipid levels in the blood 24±0.9, 31± 1.2, 40±1.3 and 43± 0.8 and 43±1.1, 63 ± 1, 90 ±1.2 and 95 ±1.3 for test drugs and std drug respectively. Hyperlipidemic control showed 100% elevation of lipid levels. ($p<0.001$)

PLATE-18

EFFECT OF EECHL IN ZEFRA FISH LARVA

YOLK MASS IN INTESTINE OF ZF LARVA

LIPID DEPOSIT IN ZF LARVAE BLOOD VESSEL

LIPID LOWERING IN EECHL TREATED ZF LARVA

LIPID LOWERING IN EZITIMIBE TREATED ZF LARVA

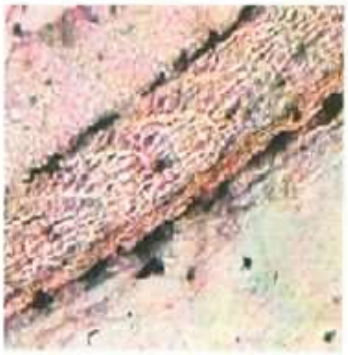
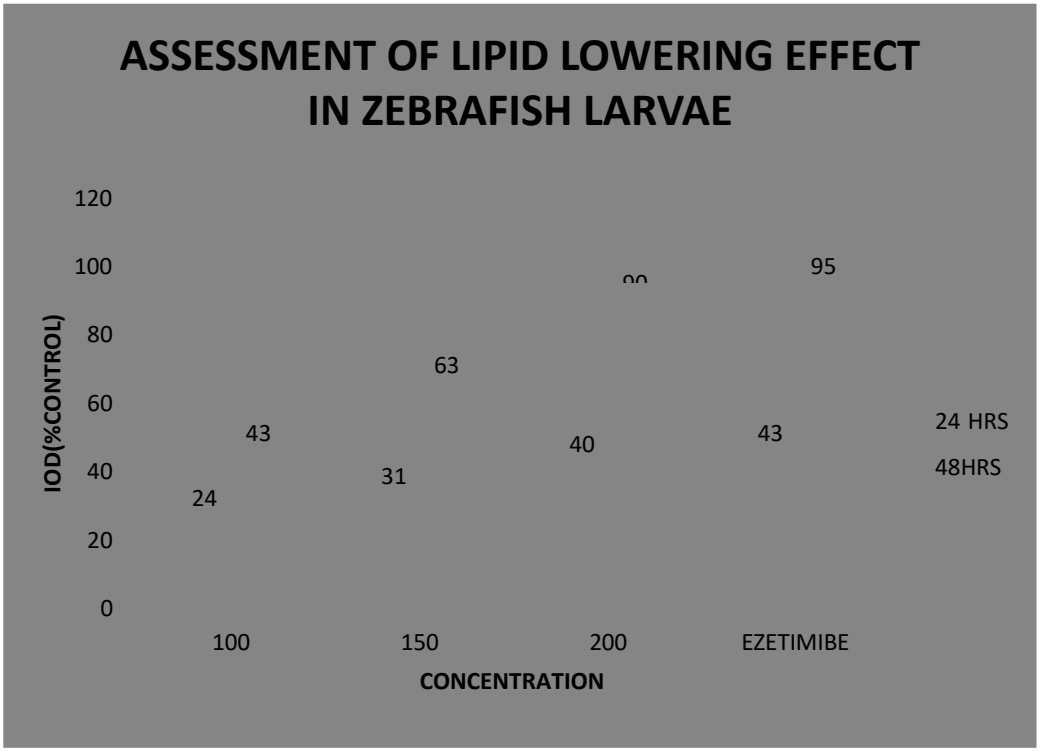
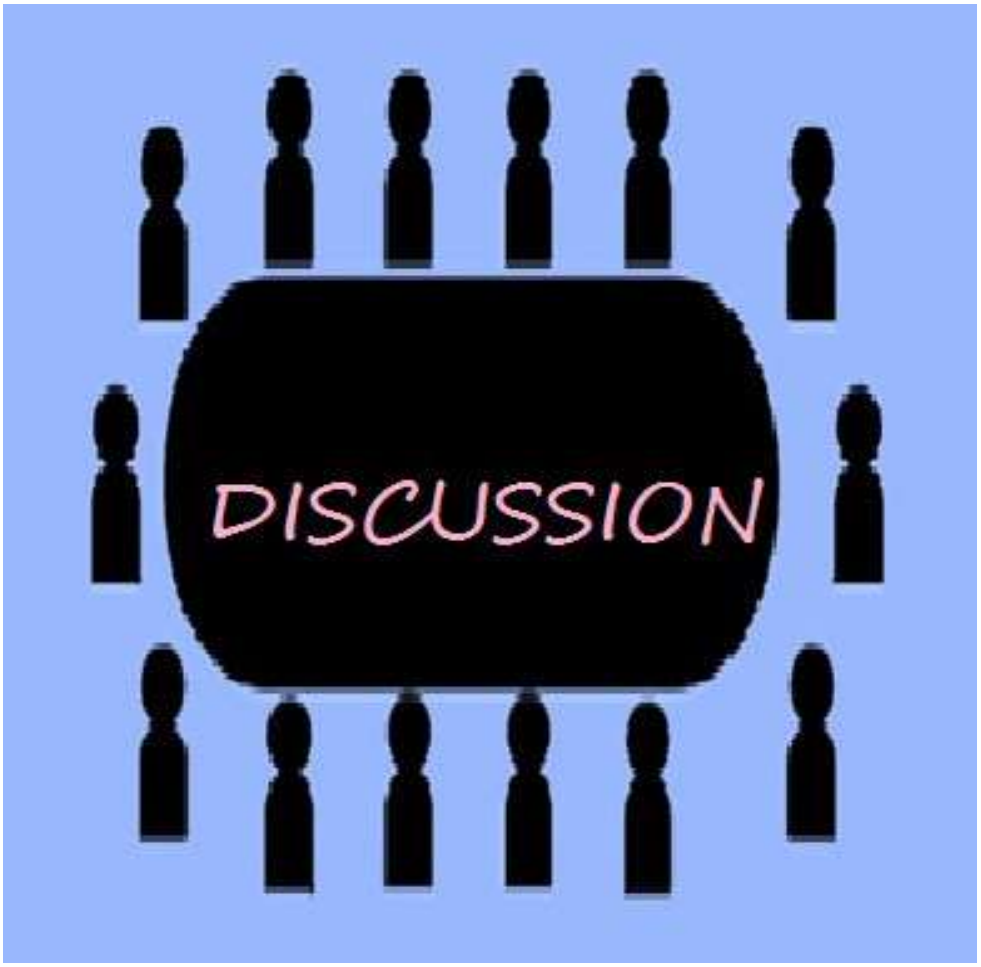


FIGURE-10


ASSESSMENT OF LIPID LOWERING EFFECT IN ZEBRAFISH LARVAE
IOD(%CONTROL)
120
100
80
60
40
20
0
24
43
31
63
40
90
43
95
24 HRS
48HRS
100
150
200
EZETIMIBE
CONCENTRATION


DISCUSSION

CHAPTER VI

DISCUSSION

The dissertation covers a study on the widely available a member of the family Sapinadaceae is known botanically as *Cardiospermum halicacabum* L. called as balloon vine. *Cardiospermum halicacabum* is a cheap natural and easily available plant. It is traditionally known to be useful for the treatment of wide panel of disease like rheumatism,lumbago,stiffness of limbs, snake bites, allergy relief, ear ache, back ache,fever,stomatchic,rubefacient,demulcent inorchitis and dropsy,haemorrhoids. *C.halicacabum* leaf juice cures the ear ache and for Hemorrhoids .Leaves and stalks of the plant are used as diarrhoea and dysentery. .(Patil *et al*.,2011).(G.Ponmari *et al*.,2011).

Leaf reported to possess anxiolytic activity ,memory enhancing activity, hypoglycaemic activity, fever with cough , antihyperglycemic, antioxidant and antihyperlipidemi activity, central nervous system depression , Antirheumatic activity , Anti-inflammatory activity . *(*Malaviya S et al.,2009) .Seed is used as a fever and as a diaphoretic and oral pain relievers. Seed oil have anticancer activity.(Dhayabaran D *et al*.,2012,Krishna murthy naik.V,*et al*.,2014**).**

Root used as a nervous disease and it also used as emetic, laxative.

The plant is much more popular in India than any other country of the world since antiquity. This could be evidence from the numerous citations laid down in the ancient literatures, particularly the Indian scriptures. In India leaves used as food and is rich source of nutrition.

The economics of this crop evidently proved that as commercial crop. In fact the revenue generated by this crop can be further magnified by man folds, if its medicinal applications are scientifically explored well. Therefore research on development of herbal products from the leaves of *Cardiospermum halicacabm* is required to be initiated immediately for exploring the unique potential of this crop which would also minimize the menacing wastage of this leaves. It may be further envisaged that is merely transportation and marketing facilities including the export channels were developed adequately then the revenue generated by this leafy crop would easily exceed that generated by any major crop of the country even with a present level of traditional agreoconomic practices. Therefore as well coordinated effort by the farmers, traders, scientist, technologists, extension workers, physician, administrators and policy makers is required to be initiated to boost up the national economy as well as the proper exploitation of this for proper therapeutic purpose.

PHARMAOGNOSTICAL STUDIES

Morphological and micro morphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacological studies. There was no detailed pharmacognostical work has been carried out including botanical identity based on micro morphology in this leaves of this plant.

The application of morphological studies in drug analysis is pertinent in the field of crude drug authentication. It was studied for the leaf. Interpretation of the morphological characteristic based on different parameters, for the plant organs give a guideline for the diagnosis of the original plant and its adulterants.

Color, size, shape, margin, texture, arrangement were observed and compared with previous data. Leaves is pubescent or nearly glabrous annual or perennial with slender branches climbing by means of tendrillar.Ternately bicompound, biternate essentially 3 folliate with each part divided again into 3 leaflets.

Microscopic techniques help to magnify the fine structure on minute objects and there by confirm the structural details of the plant drug. Though the microscopical evaluation cannot provide complete profile, still it can offer supporting evidences which when combined with other analytical parameters can be used to obtain full evidence for standardization and evaluation of herbal drugs. Consideration must therefore, be given to the types of cells and cell inclusions and the manner in which they are distributed in different organ of the plants.

The leaf is dorsiventral and prominent .T.S of midrib projecting both abaxial and adaxially. The inner part of the adxial cone includes a cluster of angular collenchyma cells.Where as inner side of abaxial are consist of schlerenchyma cells.The palisade is extended up to the shoulders of the cone. The vascular strand is fairly prominent and is triangular in shape comprises a cluster of wide circular thin walled xylem elements and a thick band of phloem elements.

Epidermis is fairly large squarish thick walled cells. The abaxial epidermal cells are thin, small and elliptical. Para dermal section shows epidermal cells wide with highly wavy anticlinal walls and amoeboid outline. The stomata are dense and diffuse in distribution and are anomocytic type. The guard cells are elliptical and the stomatal pores are slit like.

Lamina shows The mesophyll is differentiated into upper band of narrow cylindrical palisade cells and lower zone of two or three layers of lobed loosely arranged spongy parenchyma cells. The lateral veins are conspicuous, straight and uniformly thin. The vein islets are distinct having straight vein boundaries. Vein termination are present in the islets some places branched once or twice and spread within the islets.

Transverse section of petiole shows two prominent grooves towards upper side where as the lower side is round. The epidermis is composed of single layer of cells. Chlorenchymatous hypodermis is present. 4-6 collateral vascular bundles are present in the ground tissue. The xylem is present upper side and phloem towards the lower side. The ground tissue is made up of parenchyma cells

T.S of rachis shows Polygonal in outline with two winged projections at the upper side. Single layer of epidermal cells composed of horizontally flattend cells with cuticle. Covering uniseriate multicellulor trichomes are present. Peripheral layer of ground tissue are composed of collenchymatous and chlorenchymatous cells forms the hypodermis. A continuous ring of pericyclic fibres is present in the ground tissue. Vascular bundles are arranged in a ring and are collateral. The rest is parenchymatous.

Scanning Electron Microscope (SEM) study of the leaf surface showed the presence of unicellular trichome and ranunculaceous or anomocytic stomata and their distribution.

The plant drugs are generally used in the powdered form where the macro morphology is generally destroyed, so the diagnosis of the plant through the

microscopical character is essential. The powdered crude drugs can be identified based on the presence or absence of different cell types. In the powdered microscopy, we have observed xylem vessels, ranunculaceous stomata, unseriate multicellular trichomes, collenchyma, phloem cells, fibers.

Quantitative microscopy includes certain measurements to distinguish some closely related species which are not easily differentiated by general microscopy. The **stomatal number** is the oldest technique but a simple method of diagnosis of fragmentary leaf parts. The **stomatal index** is the percentage in relation to the epidermal cells. Both are very specific criteria for the identification and characterization of leafy drugs. **Vein islet and vein termination number** are another simple technique for distinguishing fragmentary specimens at specific levels. It is used as the distinguishing character for the leaf of the same species or different one.

Palisade ratio is another criteria for identification and evaluation of herbal drugs. This value remains constant within a range for a given plant species and is of diagnostic value in differentiating the species. This value does not alter based on geographical variation and differs from species to species and that is why it is a very useful diagnostic feature for characterization and identification of different plant species.

The ash content of the crude drug is generally taken to be the residue remaining after incineration. If usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also involve the inorganic matter added for the purpose of adulteration. There is a considerable difference within narrow limits in the case of individual drug. Hence ash determination furnishes a

basis for judging the identify and cleanliness of a drug and gives information related to its adulteration with inorganic matter. The ash or residue yielded by an organic chemical compound is a rule to measure the amount of inorganic matter, which is present as impurity. In most cases the inorganic matter is present in small amounts which are difficult to remove in the purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drug in especially in powdered from. The **acid insoluble ash** is of more value to detect the earthy matter adhering to the drug. In this way one can obtain evidence of the presence of foreign matter, which likely to occur with root, rhizomes and also in pubescent leaves. The **water soluble ash** is used to detect the presence of matter exhausted by water. Insufficient drying favours spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles

Extractive values of crude drugs determine the amount of active constituents in a given amount of medicinal plant material when exhausted with solvents. It is employed for that material for which no chemical or biological assay method exist. As mentioned in different official books (IP 1996 and BP 1980, BHP 1990, etc.,) the determination of water soluble and alcohol soluble extractive, is used as means of evaluating crude drugs which are not readily estimated by other means. The extractions of any crude with a particular solvent yield a solution containing different phytoconstituents. The composition of these phytoconstituents in the particular solvent can be the means of providing preliminary information on the quality of a particular drug sample. The **water soluble extract** values play an

important role for the evaluation of crude drugs. It can be used to indicate poor quality, adulteration with any unwanted material or incorrect processing of the crude drug during the drying, storage etc. The **alcohol soluble extractive** is also indicative for the same purpose as water soluble extractive values.

Loss on drying at 105°C is determined as the presence of excess moisture is conductive to the promotion of mold and bacterial growth, and subsequently to deterioration and spoilage of the drug.

THE PRELIMINARY PHYTOCHEMICAL STUDIES

The preliminary phytochemical screening reveals the presence of carbohydrates, proteins and amino acids, flavonoids, terpenoids, alkaloids, saponins, tannins and phytosterols. Glycosides, volatile oils, fixed oil , mucilage were found to be absent.

Ethanolic extract of the *C.halicacabum* leaves (EECHL) was prepared and dark green viscous residue obtained 13.5%w/v.

The reaction of drugs in powdered form in ordinary light and with filtered UV light is importance in several cases by the luminosity in UV light by fluorescent analysis. Many flavonoids showed distinctive colors under UV light: Bright yellow (6-hydroxyl flavonoids and flavones and some chalcones), dark brown most flavanol glycosides, dark may be (isoflavones and flavonols). Hence this parameter can also be used as a diagnostic tool for the standardization of herbal drugs for the detection of adulterants in crude drugs. (Harbone, JB 1973).

Total flavonoid content of EECHL was found to be 1.06 mg/g and total phenolic content was found to be 38.05mg/g.

Identification of inorganic minerals of the leaves of *C.halicacabum* by Electron Dispersive x-ray Spectrophotometer (EDS) showed the presence of minerals C-53.67%, O-44.05%, Mg-0.18%, P-0.36%, Cl-0.18%, K-1.27% Ca-0.28%. (Table-10, Fig.).

HPTLC profile of EECHL was performed and the presence of **Pinitol** were identified and quantified, (0.142mg/100mg).

PHARMCOLOGICAL STUDIES

Investigation on the acute toxicity of the ethanolic extract of the leaves showed no mortality and morbidity even under high dose levels (2000mg/k b.w.) indicating high margin of safety of the plant extract. (Vijayakumari K, Senthilkumar S., 2017).

We perform **preliminary toxicological studies of EECHL on the embryo and larvae of zebrafish.** Effects of compounds any possible retardation of development due to the substance be clearly and distinctly observed. Either mortality or incidence of malformation was not observed with 0.5-0.75 mg/ml. Podophyllotoxin was used as standard toxin which showed total mortality at 0.01 µg/ml concentration (Score 40). This observation showed no pronounced retardation in zf embryo development when exposed to normal concentration which showed that EECHL of leaf would pose no hazard to early life stages *Danio rerio*.

We performed larval toxicity assay after hatching using 5dpf, larvae was observed that 0.5, 0.75 mg/ml concentration showed no mortality and 5%, 10% mortality were observed at 0.75 mg/ml concentration. The standard podophyllotoxin showed 100% mortality at 10 mg/ml concentration. This study showed no significant

mortality or malformation in zf larvae at 24 h exposure at normal concentration but showed moderate toxicity at higher concentration so this evaluation showed no embryo toxicity or larval toxicity and toxicity was dose dependent.

When zf larvae 5dpf were fed with 0.1% egg yolk and ORO for imaging at 24 and 48 h of egg yolk feeding followed by quantitative image analysis through the vena caudalis of individual larvae we found that the lipids in zf gut and vasculature increased with time and indicated that a time dependent increase in whole larval triacyl glycerol content was correlated with the increased level of staining. At 48h of feeding blood lipid engorge uniformly it showed that feeding for48h to be selected for subsequent experiment after removing egg yolk for 24h and 48h lipids of gut and blood vessels were abundant; the lipids were plenty full in the guts and were poor in vasculature at 72h after removing egg yolk and the lipid of gut and blood vessels were not found at 96h after removing egg yolk. Hence, drug treatment for 24h and 48h were selected for quantify efficacy. When compounds and food are provided simultaneously, a major confounding variable is the impact of a drug on feeding behavior such that treatment group. This study design has the advantage that it does not require such assays since feeding is performed prior to drug treatment.

The major previously reported components of EECHL are alkaloids, terpenoids, flavonoids, saponins, pinitol. Apigenin and rutin are the predominant form of flavonoids and many of the beneficial effects may be attributed with them. Pinitol is a cyclitol, a cyclic polyol. It is a known antidiabetic agent isolated from *Sutherlandia frutescens* leaves and *Bougainvillea*. D-pinitol (3-O-methyl-chiroinositol), an active principle of the traditional antidiabetic plant *Bougainvillea spectabilis*, is claimed to

exert insulin-like effects. Pinitol is a completely non-toxic and common constituent of legume plants and is a major component of soybean (*Glycine max* L Merr). Earlier isolated from pine tree the hypoglycemic action and **anti-hyperlipidemic effect of pinitol have been well established**. Clinical trials on the effect of pinitol on glycemic control and lowering cardiovascular risk factors in patients with type II diabetes mellitus have been proved. Pinitol is reported safe and nontoxic as an anti-diabetic agent even at high levels (Pavithra R *et al.,* 2017). A stabilized HPTLC method for identifying and quantifying pinitol was adopted for this study.

This study was under taken with the objective of developing an ideal model for hyperlipidemia that would closely reflect the natural history and metabolic chacteristic of human hyperlipidemia and respond to the pharmacological treatments. The developing larval zf are small and translucent, enabling non-intrusive visualization of organs and biological processes in *vivo* with a high resolution. We used a high-fat diet egg yolk to feed larvae and develop a zf larval hyperlipidemia model and lipids were quantify using ORO staining. **In our model EECHL significantly decrease lipid levels** for 100, 150, 200µg/g 24±0.9, 31±1.2, 40±1.3 for 24 h, 43±1.1, 63±1.3, 90±1.2% for 48h respectively after treatment ($p < 0.001$). Human lipid lowering drug ezetimibe (the Niemann-Pick C1-like 1 NPC1L1 protein cholesterol transporter blocker) significantly reduce lipid levels 43±0.8 for 24h and 95±1.3% for 48h after treatment. In addition we also found that there was increase in heartbeat of hyperlipidemic zf larvae which became normal dose dependently **showing that there was no cardio vascular complication**.

It was already reported that ethanolic extract of the leaves of ***C.halicacabum*** **possesses antioxidant and hypolipidemic effects in diabetic rats**, which may be due to the presence of flavonoids, such as apigenin and luteolin in the extract.(Veeramani C *et al*.,2010).

In our report also we found significant decrease in lipid levels equivalent to standard drug ezetimibe supporting the previous result. We assume that this effect may be due to the presence of **pinitol.**

We recommend further investigation in animal model and clinical trials to confirm this potential therapeutic effect. These aspects remain to be studied.


conclusion

CHAPTER VII

CONCLUSION

The present investigation highlights the pharmacognostical, phytochemical and potential role of lipid absorption inhibition and cardio protection of the leaves of ***Cardiospermum halicacabum*** belonging to the family Sapinadaceae, a widely, easily available edible plant. Ethnomedical information revealed that it was used in various ailments including, rheumatoid arthritis, GI disease, diuretic, stomachic, rubefacient, ear ache, back ache, fever ,lumbago, nervous disease, demulcent, dropsy etc. The economic potentiality of this cash crop remains neglected by the scientists, technologists, physician, traders, administrators, policymakers, farmers, etc.

The morphological evaluation showed the adherence of general characters to the family.

Detailed microscopical characters of the leaves showed the usual leaf characters, vascular bundle, ranunculaceous stomata, non-glandular, glandular simple, unicellular or multicellular, unbranched uniseriate trichomes on both epidermis, The inner part of the adaxial cone includes a cluster of angular collenchyma cells and inner part of abaxial cone consist of schlerenchyma cells. Scanning Electron Mciroscope (SEM) showed the trichomes and ranunculaceous or anomocytic stomata and their distribution.

Petiole shows two prominent grooves towards upper side where as the lower side. epidermis is composed of single layer of cells. Powder microscopy, microscopic

schedules vein islet and vein termination number, stomatal number and stomatal index, palisade ratio, physicochemical parameters ash values, extractive values, loss on drying were determined and presented.

Preliminary phytochemical screening showed the presence of carbohydrates, proteins, amino acids, alkaloids, saponins, flavonoids, tannins, terpenoids and absence of mucilage, volatile oil, fixed oil.

Identification of inorganic minerals of the leaves of *C.halicacabum by* Electron Dispersive x-ray Spector photometer (EDS) showed the presence of minerals C-53.67%, O-44.05%, Mg-0.18%, P-0.36%, Cl-0.18%, K-1.27% Ca-0.28%.

Total flavonoids and total phenolic contents were determined and presented 1.06 mg/g, 38.05 mg/g respectively.

HPTLC profile of the ethanolic extract of the leaves of *C.halicacabum* was presented. Presence of **Pinitol** was confirmed and quantified as 0.142 mg/100mg.

"The **3R's** (**R**eduction, **R**efinement, **R**eplacement) of ethical principle was implemented that help to minimize harms to animals used in science. In our investigation we used zebra fish larvae which is a emerging novel preclinical *in vivo* model that support rapid decision making in the early phases of drug discovery process. It is amenable to medium to High Throught Screening (HTS) because of numerous advantages. The properties of the genome of zf have established it as an excellent model system that is relevant to studies of human diseases.

We performed preliminary toxicological studies of EECHL on the embryo and larvae of zebrafish and found no pronounced retardation in zf embryo development when exposed to normal concentrations (0.5 to 2µg/ml) which showed that EECHL of

leaf would pose no hazard to early life stages *Danio rerio* but standard toxin podophyllotoxin showed 100% mortality at 0.01µg/ml.

Larval toxicity study was carried out on zf larvae of 5dpf , it was observed that there was no mortality in 0.5 and 0.75 mg/ml concentrations. 5, 10, 100% mortalities were observed in 1,2,mg/ml of EECHL and 0.5µg/ml of podophyllotoxin respectively. Previous Investigation on the acute toxicity of the ethanolic extract of the leaves showed no mortality and morbidity even under high dose levels (2000mg/kg b.w.) indicating high margin of safety of the plant extract.

We used a high-fat diet egg yolk to feed larvae and develop a zf larval hyperlipidemia model. In our model EECHL significantly decrease lipid levels at 100, 150, 200µg/ml dose dependently comparable to the standard drug. ($p< 0.001$). Human lipid lowering drug ezetimibe (the Niemann-Pick C1-like 1 NPC1L1 protein cholesterol transporter blocker) significantly reduce lipid levels after treatment. In addition we also found that there was increase in heartbeat of hyperlipidemic zf larvae which became normal and without any morphological changes (heart rate, cardiac enlargement, blood flow, etc.) dose dependently in EECHL treated showing no CVS complication. This conventional zebrafish hyperlipidemia model is predictive, easily available, economic with a short testing duration and could speed up lipid lowering drug research development. Further investigations are needed to elucidate potential biochemical and molecular mechanisms of this biological effect.

The plant leaf possesses several flavonoids such as apigenin, luteolin and the cyclitol pinitol which are reported as the antilipidemic principles. The hypoglycemic

action and **anti-hyperlipidemic effect of pinitol has been well established. The beneficial antihyperlipidemic activity may be attributed to pinitol.**

These results suggest that ethanolic extract of the leaves of *C.halicacabum* (EECHL) exerts in zebrafish larvae a therapeutic effect and that may be used as advantage in therapeutic approach. Further experiments are required to prove the mechanism and advantage of EECHL over other drugs.

The hypercholesterolemic zebrafish can be used as a low-cost andinformative model for testing new drug candidates and for investigating mechanisms of action.

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CHAPTER VIII

REFERENCE

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THANK YOU