Synopsis of the Thesis

PHYTOCHEMICAL INVESTIGATION AND COMPARATIVE PHARMACOLOGICAL SCREENING (VIZ, HEPATOPROTECTIVE, ANTI ULCER, DIURETIC AND ANTIOXIDANT ACTIVITY) OF ETHANOL EXTRACT OF Cucumis sativus Linn.and Trichosanthes cucumerina Linn. with Coriander FORMULATION

> Submitted to THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY, CHENNAI For the degree of DOCTOR OF PHILOSOPHY

> > By

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CERTIFICATE

This is to certify that the work embodied in the synopsis entitled "Phytochemical investigation and Comparative Pharmacological screening (viz, Hepatoprotective, Anti ulcer,Diuretic and Antioxidant Activity) of Ethanol extract of *Cucumis sativus* Linn. *and Trichosanthes cucumerina* Linn. with *Coriander* formulation" submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, was carried out by Mrs. P.Vaijayanthimala (Ref.No. AC-I(2)/29600/2011) in the Department of Pharmaceutical Chemistry, J.K.K. Nataraja College of Pharmacy & Research Centre, Komarapalayam for the award of Doctor of Philosophy in Pharmacy under my direct supervision and guidance.

This work is original and has not been submitted in part or full for any other degree/diploma or academic award of any other university.

Place:

Date :

Dr. S. SureshKumar M.Pharm., Ph.D. Research Guide cum Supervisor

CERTIFICATE

This is to certify that Mrs. P.Vaijayanthimala (Ref.No.AC-I(2)/29600/2011) Carried out her research work on "**Phytochemical Investigation and Comparative Pharmacological screening (viz, Hepatoprotective, Anti ulcer, Diuretic and Antioxidant Activity) of Ethanol extract of** *Cucumis sativus* Linn. *and Trichosanthes cucumerina* Linn. with *Coriander* formulation" for the degree of Doctor of Philosophy (Pharmacy) in The Tamilnadu Dr. M.G.R. Medical University, Chennai, with in the requisite period under the regulation enforce and the synopsis of the thesis is a bonafide record of the work done by her under our supervision and guidance. This research work is original and has not been formed on the basis of the candidate for any other degree/diploma, associate ship, fellowship or other similar title.

We state that the entire synopsis of the thesis represents the independent work of Mrs. P. Vaijayanthimala and all the experimental techniques employed in the work were actually undertaken by the candidate herself under our guidance.

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TABLE OF CONTENT

Chapter No.	Title	Page No.
1.	INTRODUCTION	1-4
2.	LITERATURE REVIEW	5 – 6
3	AIM AND SCOPE OF WORK	7-8
4	PLAN OF WORK	9-10
5.	MATERIALS AND METHODS	11 – 18
6.	OBSERVATION AND INFERENCES	19 – 20
7.	SUMMARY AND CONCLUSION	21
	REFERENCES	22 – 25

1. INTRODUCTION

India has a rich legacy of deep-rooted medicine constituting with its different gears like Ayurveda, unani, homoeopathy siddha and Naturopathy. All the above components based upon the herbs as a medicine. Traditional health care has been successful in this country for many centuries. In India around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional plants use for curing most of the diseases¹.

Herbal constituents are the medicinal products which contain mixture of compounds obtained from plant materials as their pharmacologically active components. These generally consist of complex mixtures of one or more plants and plant materials. The entire plant material contains plant materials viz., leaves, seeds, stems, wood, bark, flowers, fruits, roots, rhizomes, resins, gums and essential oils, etc.²

Plants have provided to mankind a large variety of potent drugs to ease relief from diseases. In spite of amazing advances in synthetic drugs in couple of years, some of the drugs from plant origin have still retained their magnitude. The use of plant based drugs of the world is growing and this is because of the faith that many herbal medicines are free from side effect².

The main objectives of early research in Indian medicinal plants includes

To make India self supporting by allow them to utilize the drugs produced in the country and manufacturing them in suitable form of administration.

To discover medicine from the claim of Tibbi, Ayurvedic and other original sources suitable to be employed by the exponents of western medicine.

To discover the medicine for prevent effecting economy so that these remedies might falls within the means of the great masses in India whose economic condition is very low³.

The increasing demand for herbal medicines unavoidably led to the issue of obtaining and maintaining their quality and purity based on internationally recognized guidelines.

Normal body metabolic behaviors are approved out by liver. After the administration of the drug, the metabolism of the drug is carried out by the liver in the form of first pass metabolism. The proteins and amino acids are metabolized in the liver. The hepato protective drugs are used to protect the liver from toxic substance and also for curing some of the hepatic diseases. Eg: Silymarin⁴.

The liver is the major organ in the body carrying out most of the biochemical synthesis and escritoire functions. Living in a world of poorly prohibited environment, pollution and expanding therapy with potent drugs, when it is always exposed to variety of xe nobiotics and therapeutic agents resulting to structural or functional damage⁵.

The major attention that played to liver disease becoming a global trouble because of increasing alcohol consumption in both developed and developing countries. Anemia, Infection and Malnutrition availability of hepatotoxic drugs over the counter. The usual drugs used in the treatment of liver diseases viz. Corticosteroids and Immunosuppressant agents are sometimes

insufficient and may lead to serious adverse effects. Sometimes, they may themselves cause hepatic damage, as exemplified by cholestatic jaundice with azathioprine and elevation of serum transaminases by interferon and virazole. It is therefore, imperative to search for better drugs to treat liver diseases⁶.

In traditional medicine, the number of plants and herbs has been used to treat gastrointestinal disorders, including gastric ulcers, It is chiefly characterized by the damage of gastric mucosa; it is a disease with a multifactorial etiology. The prompt factors recognized for gastric ulcerogenesis are included bacterial infection, stress, excess intake of alcohol, use of steroids and non-steroidal anti-inflammatory drugs, nutritional lack and trauma ^{7,8}.

Gastric ulcers also occur due to inequality between the destructive injurious, levels of defensive factors and byproducts in the gastric mucosa ^{9,10}. The other factors are oxidative stress, neutrophil accumulation, depletion of antioxidants increase in inflammatory cytokines, reduced blood supply to the gastric mucosa matrix metalloproteinase activity are concerned in the pathophysiology of gastric ulcers¹¹.

Exposure to the ulcerogens results in the excessive manufacture of reactive oxygen species (ROS) which are damaging the gastric mucosa¹², but the mucus layer and endogenous antioxidants helps in the protection against ROS stimulated cytotoxicity ¹³.

The health of the kidneys plays a vital role in maintaining homeostasis and removing waste from the body. They are also responsible for the formation of concentrated urine, regulation of acid-base and ion balance and even regulate the blood pressure. Chronic Kidney Disease (CKD) has an annual death rate of 22% and it can cause secondary complications like hypertension, secondary hyperparathyroidism, anemia, and malnutrition. CKD can result from various preexisting circumstances most commonly diabetes and hypertension. Once kidney

damage take place, hyper filtration in the remaining viable nephrons in an attempt to maintain the glomerular filtration rate (GFR) causes the disease to spread¹⁴.

Oxidative stress depicts the existence of products called free radicals, and reactive oxygen species (ROS), which are formed under normal physiological conditions, but become deleterious when not being eliminated by the endogenous systems¹⁵. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems¹⁶. ROS are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders¹⁷.

From the above revision we came to know that the new drug from herbal is necessary task for the upcoming world. So it designed to carry out the phytochemical investigation and pharmacological screening of the selected plants.

2. LITERATURE REVIEW

The systemic literature survey is the major route for the enlargement of any scientific work and due to the same reasons here the review of literature regarding the *Trichosanthes cucumerina*

L.,*Cucumis sativus* **L.** *and Coriandrum sativum* **L.** have done under various heading like pharmacological review and ethanomedical information.

- Arawwawala et al. (2010) have evolved Gastroprotective activity of *Trichosanthes cucumerina* L. in rats¹⁸.
- Kongtun et al. (2009) have carried out Cytotoxic properties of root extract and fruit juice of *Trichosanthes cucumerina* L.¹⁹
- Raama Murthy et al. (2012) have investigated phytochemical, diuretic activity and anthelmintic Activity of Trichosanthes cucumerina L.²⁰
 - Dong et al. (2014) have analysis and reported Endogenous salicylic acid accumulation is required for chilling tolerance in cucumber (*Cucumis sativus* L.) seedlings²¹.

➢ Gopalakrishnan et al. (2013) have reported the hepatoprotective Activity of the Fruits of Cucumis Sativus L.²²

Subarayan Bothi Gopalakrishnan & Thangaraj Kalaiarasi (2014) have reported the comparative phytochemical screening of the fruits of Cucumis trigonus L . and Cucumis sativus L.²³

➤ Kanthimathi et al. (2013) have reported Antioxidant activity of *Coriandrum sativum* L. and protection against DNA damage and cancer cell migration²⁴.

Samojlik et al. (2013) have reported Antioxidant and hepatoprotective potential of essential oils of Coriander (*Coriandrum sativum* L.) and Caraway (*Carum carvi* L.) (*Apiaceae*) ²⁵.

- > Patel KK et al. (2011) have carried out Pharmacological screening of Coriandrum sativum
- L. for hepatoprotective $activit^{26}$.

3. AIM AND OBJECTIVE OF THE WORK

The growth of fast and precise analytical methodology and the preparation of purified analytical and experimental standards for the analysis of plant phytochemical is a key part of the new research effort aimed at unlocking the mode of action of phytochemical in both plant and animal systems. Often, these compounds are found very low concentration in the plants. They may be accumulated only under certain condition, such as the growth of reproductive structures or under specific types of stress. Accurate analytical methodology, in collaboration with accurate biological studies is required, to determine the effect of these compounds on health. So, there is emerging to have a research and development in the field of medicinal plants has acquired a considerable importance.

- Due to increasing in the scope and demand of herbal drugs, especially in disease like liver, cancer, diabetes, ulcer, diarrhea, arthritis and skin disease etc. Hence It's planned here to study the plants like *Trichosanthes cucumerina* L. (EETC), *Cucumis sativus* L. (EECS) and *Coriandrum sativum* L. (EECRS) for phytochemical investigation and develop a poly herbal formulation and their pharmacological studies..
- 2. The selected plants for the present study were based on its easy availability, degree of research work which is not done in a particular area.
- 3. The literature survey revealed that very less amount of hepato productive and anti ulcer studies has been carried out to the leaves of *Trichosanthes cucumerina* L. and *Cucumis sativus* L.
- 4. Therefore, it was thought valuable to carry out preliminary phytochemical screening (By chemical test), isolation of some compounds and characterization of isolated compounds (by IR, NMR and Mass analysis) the comparative pharmacological screening ring of ethanol extracts of

leaves of *Trichosanthes cucumerina* L., *Cucumis sativus* L. and their formulation with *Coriandrum sativum* L. fruit viz. hepatoprotective, diuretic, antiulcer and antioxidant activity. The plant extracts shows considerable report in pharmacological screening hence it planned to formulate herbal tablet formulation. The extracts of the three plants shows hepatoprotective, diuretic and antioxidant activity. Except Coriander other two plants showed antiulcer activity. Hence for hepatoprotective and diuretic activity all the three plants are used to prepare tablet and named as polyherbal formulation (PHF), for Antiulcer and Antioxidant activity only EETC and EECS are used and named as herbal formulation (HF).

5. The main focal point of this study was hepatoproductive and antiulcer activity with new herbal formulation and in isolated compounds.

4. PLAN OF WORK

The work entitled "Phytochemical investigation and Comparative Pharmacological screening (viz, Hepatoprotective, Anti ulcer, Diuretic and Antioxidant Activity) of an Ethanol extract of *Cucumis sativus* Linn. *and Trichosanthes cucumerina Linn*. with *Coriander* formulation" was planned as follows:

4.1 Phytochemical studies

- > Collection and authentication of plant materials.
- Parts of plant used Leaves of Cucumis sativus L. and Trichosanthes cucumerina L. and fruits of Coriandrum sativum L.
- > Continuous hot extraction of plant material by using Soxhlet Apparatus.
- Preliminary phytochemical screening for all the three plant extracts for the detection of different plant constituents.
- > Isolation of plant constituent by using column chromatography.
- > Characterization of isolated compounds by using IR, NMR and Mass spectral studies.
- > Development of herbal formulation (tablets) and its preliminary evaluation.

PHARMACOLOGICAL STUDIES

To carry out the following pharmacological screening by using an ethanol extracts of *Trichosanthes cucumerina* L., *Cucumis sativus* L. and their isolated compounds and tablet formulation.

- ➢ In vivo study
 - ✤ Acute toxicity of plant extracts.
 - ✤ Hepatoprotective activity
 - ✤ Diuretic activity.
 - ✤ Antiulcer activity.
- > In vitro study
 - Anti Oxidant

5. MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh leaves of *Trichosanthes cucumerina* L., *Cucumis sativus* L. and fruits of *Coriandrum sativum* L. were collected from field of Komarapalayam and authenticated by Dr.P. Satyanarayana, Scientist D & Head office in charge, Southern Regional Centre, TNAU campus, Coimbatore. Voucher specimen (No: J.K.KNCP/0102/12, 13and 14) has been deposited in the Department of Pharmacognosy, J.K.K Nataraja College of Pharmacy, Komarapalayam, Tamilnadu, India

The fruits of *Corriandrum sativum* L. were dried and then crushed into fine powder by using laboratory Homogenizer then stored for further use.

Extraction

The dried powder material was subjected to defat by using Petroleum ether to remove waxy substances and chlorophyll. The marc, which was defatted with petroleum ether was dried and extracted with using ethanol in a soxhlet extractor for 72 hours. The solvent was then subjected to distillation and the resulting semisolid mass was dried and then stored in a desiccator to get a yield.

Preliminary phytochemical screening

The presence of various phytoconstituents viz. steroids and terpenoids, alkaloids, tannins and phenolics, flavonoids, Sugars, amino acids, etc. are detected by usual methods prescribed in standard texts for all the three plant extracts^{27,28}.

Steps necessary for isolating herbal drugs Phytochemistry or natural product chemistry research is the backbone of herbal industry and directly or indirectly responsible for both failure and success of herbal drugs. For promoting the use of herbals in modern medicine, phytochemistry should be investigated for:

- 1. Isolation, purification and characterization of new phytoconstituents.
- 2. Use of newly isolated phytoconstituent as "lead" compound for the synthetic design of analogues with either improved therapeutic activity or reduced toxicity.
- 3. Conservation of lead phytoconstituents into medicinally important drugs.

Hence it was planned to isolate their active constituents from the leaves of *Trichosanthes cucumerina* L. and *Cucumis sativus* L. The isolated compound was characterized by spectral analysis like IR,NMR and MS.

Herbal formulation

An important step in the development of herbal medicine is its formulation. Herbs and herbal extracts cause typical problems in the development of formulation such as hygroscopic nature to get a proper formulation it is necessary to tackle all these problems.

The plant extracts shows considerable report in pharmacological screening hence it planned to formulate herbal tablet formulation. The extracts of the three plants shows hepatoprotective, diuretic and antioxidant activity. Except *Coriandrum sativum* L. Hence it was planned to prepare tablet formulation PHF and HF. By using EETC 50 mg,EECS 50 mg and EECRS 50 mg and studied pharmacological screening like hepatoprotective and diuretic activity. HF Tablet formulation was prepared out by using EETC 50 mg, and EECS 50 mg and studied pharmacological screening like antiulcer and antioxidant activity.

Acute toxicity studies

Acute toxicity was performed as per OECD guidelines 423 and find out effective oral dose for the following studies. Animals aren't shown signs of toxicity including mortality, nature, severity, and duration of effects up to the dose level of 2000 mg/kg of the extract.

Hepatoprotective activity^{29, 30.}

Hepatoprotective drugs are the agent which shields the liver cells from inward bound toxins. Which also allowing it to more effectively processing and also strengthens the liver. The present investigation was carried out to evaluate the hepatoprotective activity of the herbal formulation (PHF) at 150mg/kg prepared from the mixture of dried leaves of ethanol extract of *Trichosanthes cucurmena* L. (EETC) and *Cucumis sativus* L.(EECS) and fruit extract of *Corriandrun sativum* L.(EECRS) against Indomethacine induced ulcer model in Albino rats. Hepatoprotective activity carried out for isolated compound ISO-1 and ISO-2 also.

Albino rats either sex weighing between 175 ± 25 gm was used in this evaluation. The rats were procured from animal house located in J.K.K Nataraja College of Pharmacy, Komarapalayam. They were housed in well ventilated stainless-steel cages at room temperature (24±2°C) in hygienic condition under natural light and dark schedule and were fed on a standard laboratory diet. Food and water were given ad libitum.

The total numbers of 54 Albino rats either sex weighing between 175±25 gm were used in the study. These animals were divided into nine groups of six animals in each.

- Group 1- Normal control rats, which received 0.5% Carboxy Methyl Cellulose (CMC) solution (1ml/kg) One time daily for 7 Days.
- Group 2- Hepatotoxient, administered with paracetamol (3gm/kg) a single dose on day 7.
- Group 3- Standard drug control, receives Silymarin (100 mg/kg) once daily for 7days (Std).
- Group 4- Receives EETC (150mg/kg) once daily for 7 days.
- Group 5 Receives ethanol EECS (150mg/kg) for 7 days.
- Group 6: Receives EECRS (150mg/kg) for 7 days.
- Group 7- Receives formulation PHF for 7 days (150mg/kg).
- Group 8- Receives ISO-1 for 7 days (150mg/kg).
- Group 9- Receives ISO-2 for 7 days (150mg/kg).

Group-3 to Group-9 receives Paracetamol (3gm/kg) as a single dose on 7th day after thirty Minutes administration of drug extract and Silymarin respectively.

In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. After 24 hours of drug treatment, the animals were dissected under ether anaesthesia.from each rat the blood was withdrawn from the carotid artery in the neck and collected in previously labelled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum from blood was separated by centrifugation at 7000 RPM for 10 minutes. The separated serum was used for the estimation of some biochemical parameters like SGPT, SGOT,Total bilirubin, Direct bilirubin and Total protein. Liver section was observed microscopically for histopathological studies.

Antiulcer activity ³¹

The present investigation was carried out to evaluate the gastroprotective activity of the herbal formulation (HF) at 150mg/kg prepared from the mixture of dried leaves of ethanol extract of *Trichosanthes cucurmena* L. (EETC) and *Cucumis sativus* L.(EECS) against Indomethacine induced ulcer model in Albino rats. Antiulcer activity carried out for isolated compound ISO-1 and ISO-2 also.

This activity was compared with Ranitidine as standard in the condition terms of inhibition of release of gastric juice, hydrochloric acid and neutralization activity. The anti ulcer activity was noted after drug administration.

Experimental protocolAlbino rats either sex weighing between 175 ± 25 gm was used in this evaluation. The total numbers of 48 Albino rats either sex weighing between 175 ± 25 gm were used in the study. These animals were divided into eight groups of six animals in each.

Group 1- Normal control rats, which received distilled, water (1ml/kg) orally.

Group 2- Receives Indomethacine (25mg/kg) as a single dose for 3days

Group 3- Receives Rantidine (100mg/kg) as a standard reference drug (Std).

Group 4- Receives EETC (150mg/kg) once daily.

Group 5- Receives EECS (150mg/kg) once daily.

Group 6- Receives HF (150mg/kg) once daily

Group 7- Receives ISO-1 (150mg/kg) once daily

Group 8- Receives ISO-2 (150mg/kg) once daily

Group-2 and Group-8 receives Indomethacine (25mg/kg) as a single dose for 3- days as an ulcerative agent 1 hour before the ulcerogenic procedures.

Following parameters were studied

- 1. Volume of gastric juice secreted: The volume of gastric juice was measured and centrifuged at 1000 rpm for 10 min.
- 2. Determination of total acidity of the gastric juice: From the supernatant, aliquots
- (1 ml of each) were taken for the determination of total acidity and free acidity.
- 3. The ulcer index: gastric mucosa was also examined for ulcers.
- 4. The pH of the gastric secretion was measured by digital pH meter.

Diuretic Activity³²

The present study was carried out to investigate the diuretic activity of ethanol extract of the leaves of *Trichosanthes cucurmena* L. (EETC), *Cucumis sativus* L.(EECS) and fruits of *Corriandrum sativum* L. (EECRS) to make a poly herbal formulation (PHF) and were administered to experimental rats orally at the dose level of of 150mg/kg and compared with standard drug Furosemide (20mg/kg). The diuretic effects of the extracts and PHF were evaluated by measuring the parameters like urine volume, sodium, potassium and chloride contents. The lipschistz method used in rat for the experiment purpose.

Experimental protocol

The method of Lipschitz was employed for the assessment of diuretic activity. Albino rats either sex weighing between 175 ± 25 gm was used in this evaluation. The total numbers of 36 rats were used in this study. These animals are divided into six groups of six animals in each.

Group 1 - Received Normal saline (0.9%) orally at a dose of 10 ml/kg b.wt.

Group 2 – Received standard drug **Furosemide** orally at a dose of 20 mg/kg b.wt.

Group 3- Received EETC at a dose of 150 mg/kg b.wt.

Group 4- Received **EECS** at a dose of 150 mg/kg b.wt.

Group 5- Received EECRS at a dose of 150 mg/kg b.wt.

Group 6 - Received PHF at a dose of 150 mg/kg b.wt.

. The total volume of urine collected and measured for both control and treated groups.

The formulation (PHF) shows significant activity when compared to standard drug. The important parameters like urine volume, sodium, Potassium concentration were determined by flame photometer and chloride concentration was determined by argentometric method.

In Vitro Antioxidant Activity

DPPH assay

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with a hydrogen donor changes to yellow in color. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol 1 and the decrease in absorbance is measured at 490nm.

Procedure ³³

Stock solution of 0.1 mM DPPH in ethanol was diluted using ethanol. 1.0 ml of solvent extract solution of differing concentrations (10–100 mg/ml) was added to 1.0 ml of DPPH and made volume up to 3 ml. The standard for drug Vit. C was also used in this test. The absorbance was measured at 517 nm after 30 min. Inhibition was calculated by using the following equation. Inhibition was calculated by using the following equation.

Percentage inhibition =
$$\frac{OD_{control} - OD_{sample}}{OD_{control}}$$

6. OBSERVATION AND INFERENCES

From the above phytochemical screening the EETC shows the presence of Alkaloids, carbohydrates, saponions, glycosides and phytosterols. EECS shows the presence of proteins and aminoacids, alkaloids, saponins, glycosides and phytosterols. EECRS shows the presence of proteins and aminoacids, saponins, glycosides and phytosterols. These above said constituents present all the three extracts may be responsible for the pharmacological activities.

For hepatoprotective activity animals are divided in to nine groups after seven days treatment with EETC ,EECS ,EECRS, ISO-I and ISO-2 restored the level of serum biochemical parameters(viz SGOT,SGPT,Total biluribin,ALP,Direct biluribin and Total Protein) towards normalization. But the effect of PHF on these parameters was comparable to that of standard Silymarin. Histopathological report also supported that liver section were in normal circumstance.

In antiulcer activity EETC ,EECS ,EECRS, ISO-I and ISO-2, shows a marked effect in gastro protective activity. But HF shows significant effect in reducing ulcer when compared with standard drug Ranitidine.

In diuretic effect the EETC, EECRS and EECS extracts have produced a moderate diuretic activity individually, and in their combination with EECRS in polyherbal tablet formulation to produce significant than the individual extracts. So that it can be used to produce diuresis during edema and also to treat hypertension.

In antioxidant activity EETC, EECS and HF were evaluated for *in vitro* and antioxidant activity which may lead to the finding of most effective agent for the management of diseases

and effective potential source of natural antioxidant that may help in preventing various oxidative stresses. The DPPH method was used for evaluation of antioxidant activity.

7. SUMMARY AND CONCLUSION

Plants from *Cucurbitaceae* family was selected for the investigation because most of research work carried out in this family was focused on seeds and fruits. Hence *Trichosanthes cucumerina* L. and *Cucumis sativus* L. selected from this family and carried out preliminary phytochemical investigation and pharmacological screening like hepatoprotective , antiulcer , diuretic and antioxidant activity.

All the plant extracts shows considerable result in all the above mentioned screening hence it was planned to prepare herbal formulation and isolation of active substance from the plant extract.

As per the serum parameters result the formulation has the significant effect to normalize the serum parameters when compared to standard drug Sylimarin. There is also significant results given by formulation in gastro protective effect. Diuretic and antioxidant activity also shows the same report. Over all it was concluded that the plant extracts of EETC,EECS and EECRS is safe at up to the dose level of 2000mg/kg. It can be used as a natural source of Hepatoprotectant, antiulcer drug,diuretic drug and antioxidants which can be used in the prevention of diseases caused by free radicals. The combination of all these three plant extracts(EETC,EECS and EECRS) showed potent hepatoprotective and diuretic drug. The formulation of other two plant extracts showed potent antiulcer and antioxidant drug. It also need to study to isolate and characterize the some more active compounds that are responsible for the all the above activities

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