PHARMACOGNOSTICAL, PHYTOCHEMICAL STUDIES AND EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF LEAVES OF *Momordica charantia* Linn.,

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Submitted by

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COLLEGE OF PHARMACY

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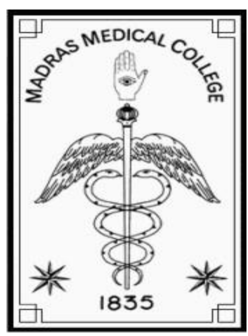

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TAMIL NADU

CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOGNOSTICAL, PHYTOCHEMICAL STUDIES AND EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF LEAVES OF *Momordica charantia* Linn.,"** submitted by **MEGALA.S, Reg. No: 261720652** to The Tamil Nadu Dr. M.G.R. Medical University, examination is evaluated.

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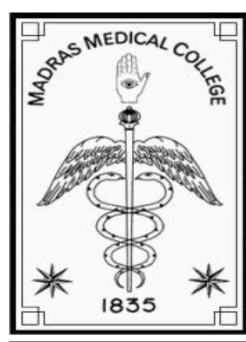

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1. INTRODUCTION

HERBAL MEDICINE[1]

The WHO has recently defined traditional medicine(including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Traditional medicine is the syntheses of therapeutic experience of generations of practicing physicians of comprise medicinal plants, minerals and organic matter etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in India, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include Rigveda, Atharvaveda, CharakSamhita and SushrutaSamhita. The herbal medicines / traditional medicaments have therefore been derived from rich traditions of ancient civilization and scientific heritage.

STATUS OF HERBAL MEDICINE IN INDIA[3]

India has a rich tradition of herbal medicine as evident from Ayurveda, which could not have flourished for two thousand years without any scientific basis. Ayurveda which literally means knowledge (Veda) of life (Ayur) had its beginning in Atharvaveda. CharakSamhita and SushrutaSamhita are the two most famous treatises of Ayurveda several other were compiled over the centuries such as BelaSamhita, KashyapSamhita, AgniveshTantra, Vagbhata'sAshtanghridaya, MadhavaNidan. Vegetable product dominated Indian MeteriaMedica which extensive use of bark, leaves, flowers, fruits, roots, tubers and juices. The theory of rasa, vipaka, virya and prabhava formed the basis of Ayurveda Pharmacology, which made no clear distinction between diet and drug, as both were vital component of treatment.

Based on clinical effects 50 categories of drug have been described such as appetizers, digestive stimulant, laxatives, anti-diarrhea, anti-haemorrhoid, anti-emetic, anti-pyretic, anti-inflammatory, anti-pruritic, anti-asthmatic, anti-epileptic, anti-helminthic, haemoptietic, haemostatic, analgesic, sedative, promoter of life, promoter of strength, complexion, voice, semen and sperm, breast milk secretion, fracture and wound healing, destroyer of kidney stones etc.

ARTHRITIS[2]

Arthritis is an autoimmune inflammatory disorder affecting almost 1-3% of the world population. It is the result of a malfunctioning immune system, although its cause is still unknown. Arthritis means joint inflammation, but the term is used to describe around 200 conditions that affect joints, the tissues that surround the joint, and other connective tissue. It is a rheumatic condition.The most common form of arthritis is osteoarthritis. Other common rheumatic conditions related to arthritis include gout, fibromyalgia, and rheumatoid arthritis (RA).

Rheumatic conditions tend to involve pain, aching, stiffness, and swelling in and around one or more joints. The symptoms can develop gradually or suddenly. Certain rheumatic conditions can also involve the immune system and various internal organs of the body.Some forms of arthritis, such as rheumatoid arthritis and lupus (SLE), can affect multiple organs and cause widespread symptoms.

According to the Centers for Disease Control and Prevention (CDC), 54.4 million adults in the United States have received a diagnosis of some form of arthritis. Of these, 23.7 million people have their activity curtailed in some way by their condition.Arthritis is more common among adults aged 65 years or older, but it can affect people of all ages, including children.

PATHOGENESIS OF ARTHRITIS[4]

The synovium is a thin delicate lining that serves several important functions. Synovial cells synthesize joint lubricants such as hyaluronic acid, as well as collagens that constitute the structural framework of the synovium. Synovial lining or intimal layer is only 1-3 cells thick. In rheumatoid arthritis, this lining is increased to 8-10 cells thick. The initial triggers of arthritis are unclear; hormones, genetics and environmental factors may also play a role.

Once the initial immune response is triggered , cells of the immune system produce autoantibodies and inflammatory cytokines, creating a cascade of inflammation resulting in the formation of pannus, the pannus invades and destroys cartilage and bone. Additional joint damage and systemic complications ensue, resulting from a complex process of inflammatory mediators being released in the affected joints.

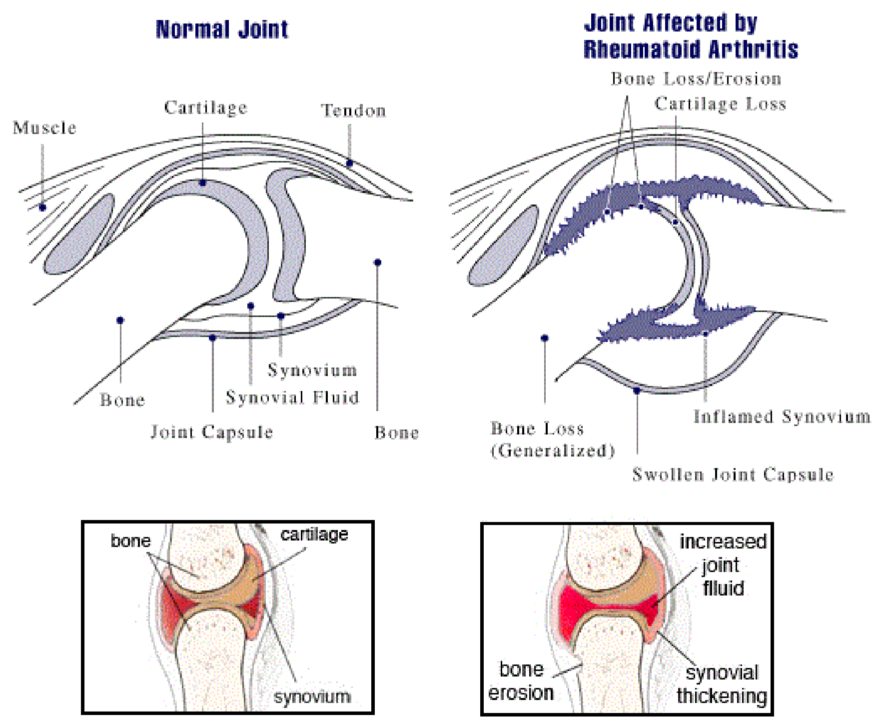


FIG NO. 1 DIAGRAMMATIC REPRESENTATION OF THE NORMAL JOINT AND RHEUMATOID ARTHRITIS AFFECTED JOINT

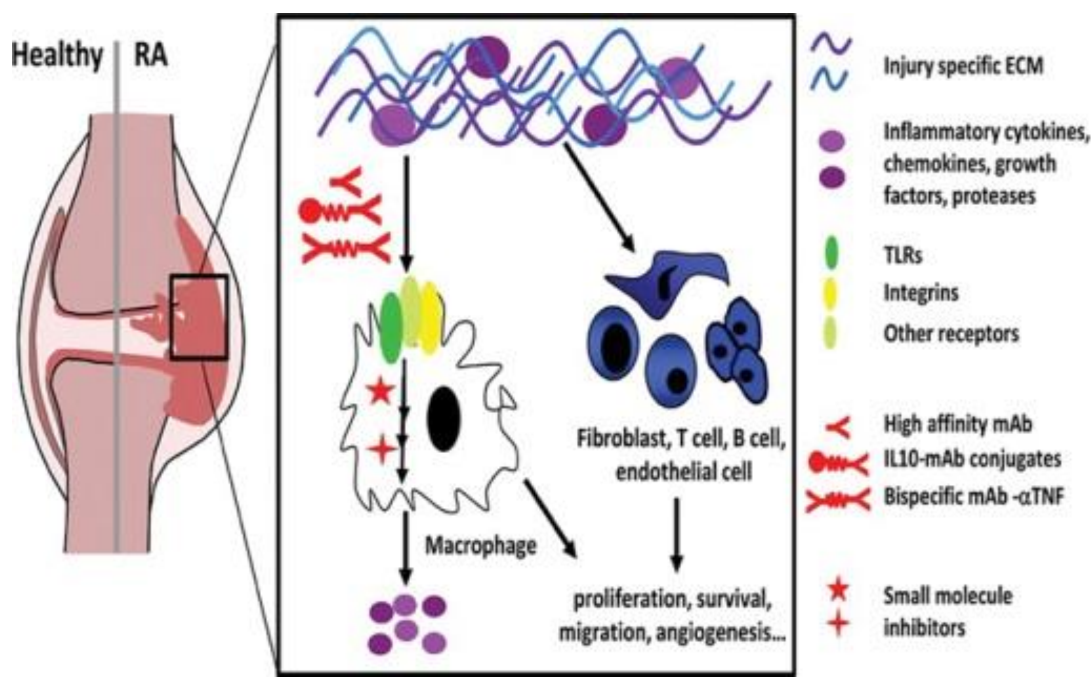


FIGURE NO. 2 PATHOPHYSIOLOGY OF RHEUMATOID ARTHRITIS

Many factors impact the risk of developing arthritis. The risk of developing arthiritis doubles with a first degree relative who has arthritis. There is also a hormonal relationship. Rheumatoid arthritis is more commonly in females, and there are high rate of disease onset associated with pregnancy.

CAUSES

There is no single cause of all types of arthritis. The cause or causes vary according to the type or form of arthritis.

- Most types of arthritis are linked to a combination of factors, but some have no obvious cause and appear to be unpredictable in their emergence.
- Some people may be genetically more likely to develop certain arthritic conditions. Additional factors, such as previous injury, infection and physically demanding occupations, can interact with genes to further increase the risk of arthritis.
- The impact of environmental stressors, especially smoking and chemical exposure, on genes is thought to drive the processes thst induce autoimmune reactions leading up to the inflammation seen in arthiritis.
- Diet and nutrition can play a role in managing arthritis and the risk of arthritis, although specific foods, food sensitivities or intolerances are not known to cause arthritis.
- Gout is one type of arthritis that is closely linked to diet, as it is caused by elevated levels of uric acid which can be a result of a diet high in purines.

SIGN AND SYMPTOMS[2]

- Joint pain, stiffness and swelling are the most common symptoms of arthritis
- Redness and warm joint which is lasts for 6 weeks
- Sudden high fever
- Rheumatoid factor present and red blood cell decreased in blood testing
- Many people with arthritis notice their symptoms are worse in the morning
- Severe rheumatoid arthritis can cause joint deformity if left untreated.

TYPES

There are around 200 types of arthritis, or musculoskeletal conditions

- Inflammatory arthritis
- Degenerative or mechanical arthritis
- Back pain
- Connective tissue disease
- Infectious arthritis
- Metabolic arthritis.

Examples of inflammatory arthritis include:

- Rheumatoid arthritis (RA)
- Osteoarthritis
- Arthritis associated with colitis or psoriasis

TREATMENT

The doctor will likely recommend a course of physical therapies, which help to manage some of the symptoms of arthritis.Treatment for arthritis aims to control pain, minimize joint damage, and improve or maintain function and quality of life. Some people find heating pads and ice packs to be soothing.

Treatment might involve:

- Medications
- Non-pharmacologic therapies
- Physical or occupational therapy
- Splints or joint assistive aids
- Patient education and support
- Weight loss
- Surgery, including joint replacement

Medications will depend on the type of arthritis. Commonly used drugs include:

- Analgesics**,**
  - Hydrocodone
  - Acetaminophen
- Non-steroidal anti-inflammatory drugs (NSAIDs),
  - Ibuprofen
  - Diclofenac
  - Aspirin
- Counterirritants**,**
- Disease-modifying antirheumatic drugs (DMARDs),
  - Sulfasalazine
  - Methotrexate
- Biologics**,**
- Corticosteroids

Herbal treatment for Arthritis

Alternative medicines are still curing arthritis instead of treating symptoms. In an effort to gain relief and take a natural approach, more arthritis patients are seeking herbal remedies than ever before. Certain herbs may have anti-inflammatory properties that can help with rheumatoid arthritis, as well as the ability to reduce pain in all forms of the disease.

Herbs used in the treatment of Arthritis[79]

TABLE NO. 1 HERBS FOR TREATING ARTHRITIS

S.NO	NAME OF THE HERB	PLANT PART USED
1	*Alpinia galangal* Linn	Rhizomes
2	*Cardiospermumhalicacabum*Linn	Roots
3	*Cassia fistula*	Fruits
4	*Commiphoramyrrha*Nees	Gum
5	*Coriandrumsativum*	Fruits
6	*Euphorbia nerifolia L*	Leaf
7	*Gossypiumherbaceum*Linn	Leaves
8	*Heliotropiumindicum*Linn	Whole plant
9	*Hyoscyamusniger*Linn	Leaves and Seed
10	*Ipomoea cairica*Linn	Seeds
11	*Jasminumlanceolarium*Roxb	Leaves and Flowers
12	*Kaempferiagalangal*Linn	Rhizomes and Leaves
13	*Lawsoniainermis*Linn	Leaves
14	*Mangiferaindica*Linn	Roots and Barks
15	*Ocimumbasilicum*Linn	Whole plant
16	*Piper nigrum*	Fruits
17	*Ricinuscommunis*Linn	Leaves
18	*Solanumnigrum*Linn	Whole plant
19	*Tribulusterrestris*Linn,	Whole plant
20	*Vitexnehundo*Linn,	Roots

2. REVIEW LITERATURE

Aim of the current review is to search literature for the pharmacognostic studies, phytochemical investigation, toxicity studies and pharmacological properties of *Momordica charantia* Linn., Literature review of *Momordica charantia* Linn., was carried out to find out the research work on this plant. The review of literature showed that many studies were done on different part of this plant. This review is made to confirm that anti-arthritic activity on the leaves of this plant was not yet proven scientifically.

PHARMACOGNOSTICAL REVIEW:

I. **Rafaela Damasceno et al., (2018)** performed an anatomical study and characterizing the metabolites in leaves of *Momordica charantia*. They revealed information about the type of trichomes, cuticle, vascular bundles, arrangement of the tissues that determine the botanical identity of this species. The histochemistry allowed determining the location of metabolites and along with chemical microanalyses, to identify the type of crystal in the leaf blade.[5]

II. **Poonam sethi (2012**) studied the pharmacognostical characters of leaves of *Momordica charantia* and also evaluated the trace elements and the mineral contents of the leaves. This pharmacognostical and phytochemical constituents help in proving authencity of the drug[9]

PHYTOCHEMICAL REVIEW:

I. **Javed Ahamad, Saima Amin and ShowkatR.Mr., (2017)** presented the information related to chemical composition of *Momordica charantia* Linn., which is used as traditional functional food and medicine in many Asian countries. The earliest report of the chemical screening of *Momordica charantia* contains cucurbitacins, sterols, alkaloids, proteins and triterpenoids.[18]

II. **Wen Li et al., (2015**) studied the chemical constituents of the leaves of *Momordica charantia* and established the method for determination of their total saponin content.[24]

III. **Prarthna Daniel et al.,(2014)** studied the phytochemical analysis of *Momordica charantia.* In *Momordica charantia* primary metabolites are common sugars, protein and chlorophyll while secondary metabolites are alkaloids, flavonoids, tannins, saponins, diosgenin, calcium, copper etc. Secondary metabolites are responsible for medicinal activity of *Momordica charantia.*[28]

IV. **J Kubola et al., (2008)** studied the phenolic contents of *Momordica charantia* leaves.[36]

V. **Yoshio Takeda et al., (2003)**isolated the three new cucurbitane triterbenoids, 1,3 and 6 from the leaves of *Momordica charantia* along with two other known compounds, momordicine I and II. The structures of the new metabolites were determined by interpretation of spectral data.[39]

PHARMACOLOGICAL REVIEW:

I. **Saahithya Rajamohan, R. Sridhar, S. Hemalatha, P. Sriram., (2018)**investigated the effect of*Momordica charantia* L. on serum biochemical parameters in experimentally induced atypical acinar cell tumors. They observed significant alteration in ALT, ALP, triglyceride, cholesterol, lipase and amylase level. These alterations were compared with the control group and which revealed the high level of protective effect of aqueous extract of *Momordica charantia*, on the serum biochemical alteration.[40]

II. **Xueli Cao et al., (2018)** reported Antiaging of Cucurbitane Glycosides from Fruits of *Momordica charantia* L.[41]

III. **LekhniSoni, et al., (2016)**evaluated the anti-arthritic activity of the ethanolic extract of root of *Momordica charantia* (EEMR)by using Freund's adjuvant induced arthritis model. This study showed that ethanolic extract of the plant in dose of 400mg/kg produced more significant reduction in arthritic score, joint diameter and paw volume. On the basis of result, they concluded that EEMR showed significant anti-arthritic activity.[52]

IV. **R.NuralHusna et al., (2013)** stuided the acute toxicity studies on leaves of *Momordica charantia*.[61]

V. **MS Akhtar et al., (2007)** attempted to screen the alleged activity, blood glucose levels of normal and alloxan diabetic male albino rabbits treated orally with various doses of dried *Momordica charantia* fruits. They concluded that the plant possessed a significant and consistent hypoglycaemic effect. It is suggested that probably *Momordica charantia* contains more than one type of hypoglycaemic principles.[72]

VI. **Nafisa PC Fernandes et al., (2007)**reported the hypoglycemic and antilipidemic properties of *Momordica charantia* fruit extract.They suggested that plant extract enhance insulin secretion by islets of Langerhans, reduce glycogenesis in liver tissue, enhance peripheral glucose utilization and increase serum protein levels.[71]

VII. **J.K.Grover et al., (2004)** studied the pharmacological action and potential uses of *Momordica charantia.*[77]

3. PLANT PROFILE

Plant name : *Momordica charantia* Linn.

Common name : Bitter melon

Family : Cucurbitaceace

VERNACULAR NAMES[14]

Tamil : Pavakkai

Kannada : Haggalakai

Hindi : Karela

Bengali : Karola

Telugu : Kakarakai

Malayalam : Kaypa

Marathi : Karli

Gujarathi : Karela

Sanskrit : Karavelli

TAXONOMY[14]

Kingdom : Plantae

Subkingdom : Viridiplantae

Division : Tracheophyta

Subdivision : Spermatophytina

Class : Mangnoliopsida

Suborder : Rosanae

Order : Cucurbitales

Family : Cucurbitaceae

Genus : Momordica

Species : *Momordica charantia* Linn.

GEOGRAPHICAL DISTRIBUTION[11]

It was possibly domesticated in India and southern China and is now found naturalized in almost all tropical and subtropical regions. It appears to be native to the African and Australian continents, but its actual origin has been obscured by its spread as a food crop.

Fig No. 3 Plant of *Momordica charantia*

Fig No. 4 Leaf of *Momordica charantia*

HABITAT[11,13]

This climbing plant is cultivated in gardens everywhere in India for its fruit. It is the major weed in tropical pastures and vegetable crops. It grows sea level to over 1300m and in area with annual rainfall as low as 480mm. Minimum average annual temperatures may be as low as 12.5^{o}C, and grows in soil with pH ranginf from 4.3 to 8.7. It also occurs as a ruderal in disturbed, uncultivated habitats such as roadsides, gardens, fencelines and around houses and farm buildings.

VARIETIES[11]

There are two varieties, one which a small roundish or ovoid fruit (uchche) and the other longer and more cucumber-like (Kerula in Bengali).

PHYTOCONSTITUENTS[13]

Fruits and leaves contains charantin, a steroidal saponin which shows blood sugar lowering activity. Karela fruit also contain a cathartic principle called

momordicin. The drug also contains carbohydrates (10%), mineral (1.5%) and ascorbic acid (88-188mg/100gm). Additionally, alkaloids, glucoside, saponins and mucilage are the other contents of karela.

PLANT DESCRIPTIONS[11,13,14]

TABLE NO. 2 PLANT DESCRIPTIONS

S. NO	PARTS	IMAGES	DESCRIPTION	USES
1	Leaves		Simple, alternate, 3-12 cm wide, suborbicular shape, base is widely cordate	Used in the treatment of menstrual troubles, burning sensation, fever, constipation. Traditional medicine as an antiviral for measles[56]
2	Stem		Slender, slightly pubescent, grooved and light green. It branches at the base	Used as cosmetic ingredient in skin conditioning. Used for diabetes[43] and cancer[40]. Bitter tonic
3	Flowers		Sepals are lanceolate, 4-6mm long and 2mm wide, glabrous. The petals are yellow, obovate. 10-20mm long and 7-15mm wide.	Used to treat hepatitis and asthma.
4	Fruits		Fleshy, broadly ovoid oblong to fusiform, 4-20cm long and 2.5-4cm wide, 3 valved at the apex when mature,	Used in asthma, cough, diabetes, helminthiasis, ulcer and inflammation[54]. Used as blood purifier, beneficial in treating

			surface rough	and preventing the liver damage.
5	Seeds		Oval elliptic, almost toothed at the tops, 10-16mm long and 7-9mm wide, 2-3mm thick. Seeds are covered by red mucilage	Used in the treatment of diabetes, high cholesterol, intestinal paeasites, heal wounds.
6	Roots		Taproot which can be become rhizomatous.	Used in the treatment of syphilis, rheumatism[52], septic swelling, opthelmia. Root juice helps to reduce the problem of Pyorrhea. To abort pregnancy.

4. RATIONALE FOR THE SELECTION OF THE PLANT

- Arthritis is the autoimmune disorder affecting almost 1-3% of the world population. The research work is decided to study the herbal plant drug which cures arthritis.
- The plant *Momordica charantia* Linn., belonging to the family Cucurbitaceae was selected for the present work.
- Traditionally, *Momordica charantia* Linn., is a popular herb used as supplementary agents to treat diabetic melitus and it having anti-inflammatory, abortifacient, anthelmintics, antiviral acticities.
- Anti-arthritic activity was evaluated in roots[52] and fruits[47] of *Momordica charantia* and the leaves of *Momordica charantia* Linn., has a claim to treat arthritis which is not proven scientifically.
- Hence the present work taken to establish the effect of *Momordica charantia* Linn., leaves in the treatment of arthritis.

5. AIM AND OBJECTIVE

AIM

- To evaluate the Pharmacognostical, Phytochemical and Anti-Arthritic Activity of the leaves of *Momordica charantia* Linn.,

OBJECTIVE

Pharmacognostical studies:

- Establishing Pharmacognostical profile of the leaves of *Momordica charantia* Linn.,

Phytochemical studies:

- Phytochemical screening and determination of phytoconstituents.

Pharmacological studies:

- To select the active extract by *in-vitro* anti-arthritic studies.
- To evaluate *in-vivo* anti-arthritic activity on the leaves of *Momordica charantia* Linn.,.

6. PLAN OF WORK

- **COLLECTION OF PLANT MATERIALS**
- **AUTHENTICATION**
- **PHARMACOGNOSTICAL STUDIES**
  - **Macroscopy**
  - **Microscopy**
    - **Powder microscopy**
    - **Histochemical studies**
    - **Quantitative microscopy - Linear measurement**
  - **Physiochemical analysis**
  - **Qualitative and Quantitative analysis**
    - **Heavy metals**
    - **Inorganic elements**
- **PHYTOCHEMICAL STUDIES**
  - **Preparation of extract**
  - **Preliminary phytochemical screening**
  - **Fluorescence analysis**
  - **Quantitative estimation of phytoconstituents**
  - **Chromatography**
    - **Thin layer chromatography**
    - **High performance thin layer chromatography**

- **PHARMACOLOGICAL STUDIES**
  - ***Invitro* evaluation**
    - **Protein denaturation method**
    - **Membrane stabilization method**
  - **Acute toxicity study**
  - ***Invivo* evaluation**
    - **Adjuvant induced arthritis**
    - **Paw volume measurement**
    - **Body weight change**
    - **Hematological parameters**
    - **Histopathological studies**

7. MATERIAL AND METHODS

7.1 PHARMACOGNOSTICAL STUDIES

COLLECTION OF PLANT MATERIAL

The leaves of *Momordica charantia* Linn., was collected from Erode, Tamilnadu in September 2018.

AUTHENTICATION

The plant material was identified as **leaf of *Momordica charantia* Linn., (Code: M12091801C)** and authenticated by **Dr.K.N.Sunilkumar, R.O and HOD Pharmacognosy**, Siddha Central Research Institute, Arumbakkam, Chennai-106.

7.1.1 MACROSCOPY[5,7,8,14]

The leaves of *Momordica charantia* Linn., were categorized to sensory characteristics. Organoleptic evaluation provides the simplest and quickest means to establish the identity, purity and quality of a particular sample. Hence this observation is of primary important before any further testing can be carried out.

7.1.2 MICROSCOPY[8,9]

Sectioning method[5,7]

Fixation of plant organ

The leaves cut fixed in FAA solution (Formalin 5 + Acetic acid 5ml + 70% Ethyl alcohol 90ml). The specimen was dehydrate after 24 hours of fixing. The leaves were graded with series of tertiary butyl alcohol, as per the standard.

Infiltration of the specimen

It was carried out by gradual addition of (Melting point-58 to 60˚C) paraffin wax until Tertiary butyl alcohol (TBA) solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the section is 10-12µg. Dewaxing of the sections were customary procedures. The sections were stained with Toludine blue. Since toludine blue is a polychromatic stain. The staining results were remarkably good. The dye

rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to mucilage and blue to the protein bodies. Whenever, necessary sections were also stained with safranin, fast green and iodine for starch.

7.1.2.1 POWDER MICROSCOPY[7]

The shade dried, powdered plant material was used for powder microscopic analysis. The organoleptic characters were observed and to identify the different characteristic features various staining reagents are used. Powder was stained with 1% phloroglucinol in 90% ethanol, concentrated hydrochloric acids and observed through microscope. All the lignified cells stained with pink colour.

7.1.2.2 QUANTITATIVE MICROSCOPY[10,12]

Determination of leaf constants and linear measurement of crude drugs helps in the identification, characterization and standardization of leaves.

Linear measurement

The powder analysis of the leaves of *Momordica charantia* Linn.,showed the presence of trichomes, so we proceed for measuring the dimensions of those powder characters.

Leaf constants

The important histological features on the epidermal surfaces of leaves were the stomata, trichomes. The measurements like Stomatal index, Veinislet number and Veinlet termination number were considered to be important parameters in the microscopical evaluation.

Stomatal number[12]

Stomatal number is an average number of stomata present per square millimeter of epidermis of leaf.

Procedure

Clear the piece of the leaf (middle part) by boiling with potassium hydroxide solution or alternatively with chlorinated soda. Peel out upper and lower epidermis separately by means of forceps. Keep it on slide and mount in glycerin water. Arrange

a camera lucida and drawing board for making the drawing to scale. Draw a square of 1mm by means of stage micrometer. Place the slide with cleared leaf (epidermis) on the stage. Trace the epidermis cell and stomata. Count the number of stomata present in the area 1 sq. mm. Include the cell if at least half of its area lies within the squares. Record the results for each of the ten fields and calculate the average number of stomata per sq.mm.

Stomatal index[12]

The stomatal index is the percentage in which the number of stomata form to the total number of epidermal cells; each stomata being counted as one cell.

It is calculated by using the following equation,

$$\text{Stomatal number (S.I)} = S / (E+S) \times 100$$

Where,

S - Stomata per unit area

E - Number of epidermal cells per unit area

Procedure

Clear the piece of the leaf (middle part) by boiling with potassium hydroxide solution or alternatively with chlorinated soda. Peel out upper and lower epidermis separately by means of forceps. Keep it on slide and mount in glycerin water. Arrange a camera lucida and drawing board for making the drawing to scale. Draw a square of 1mm by means of stage micrometer. Place the slide with cleared leaf (epidermis) on the stage. Trace the epidermis cell and stomata. Count the number of stomata, also the number of epidermal cells in each field. Calculate the stomatal Index using the above formula. Determine the values for upper and lower surface (epidermis) separately.

Vein islet number and Vein termination number[10]

Vein islet number

Small vascular bundle surrounded by many conducting tissues is called vein islet. The Vein islet number is defined as the number of vein-islet per sq.mm of the leaf surface midway between the midrib and the margin.

Procedure

Clear the piece of the leaf (middle part) by boiling with potassium hydroxide solution for about 30 mins. Arrange camera lucida and drawing board for making drawings to scale. Place stage micrometer on the microscope and using 16mm objectives, draw a line equivalent to 1mm as seen through the microscope. Construct a square on this line. Move the paper so that the square is seen in the eye piece, in the centre of the field.

Place the slide with the cleared leaf (epidermis on the stage). Trace off the veins which are included within the square, completing the outlines of those islets which overlap two adjacent sides of the square. Count the number of vein islets in the square millimeter, where the islets are intersected by the sides of the square, include those on two adjacent sides and exclude those islets on the other sides. (To obtain critical results for a leaf, 4sq.mm. should be used, preferably in one large area of 4 sq.mm.)

Find the average number of vein islets from the four adjoining squares, to get the values for one sq.mm.

Vein termination number

Veinlet termination number is defined as the number of veinlet termination per sq.mm of the leaf surface midway between the midrib and margin.

Procedure

Clear the piece of the leaf (middle part) by boiling with potassium hydroxide solution for about 30 mins. Arrange camera lucida and drawing board for making drawings to scale. Place stage micrometer on the microscope and using 16mm objectives, draw a line equivalent to 1mm as seen through the microscope. Construct a square on this line. Move the paper so that the square was seen in the eye piece, in the center of the field.

Place the slide with the cleared leaf (epidermis on the stage). Trace off the veins which are included within the square, completing the outlines of those islets which overlap two adjacent sides of the square. Count the number of vein

terminations present within the square millimeter. Find the average number of vein termination number from the four adjoining squares, to get the values for one sq.mm.

7.1.2.3 HISTOCHEMICAL STUDIES

The sections of leaves of *Momordica charandia* Linn.,were stained by using specific reagents such as N/50 Iodine, dilute Ferric chloride, Phloroglucinol and Conc. Hydrochloric acid, picric acid and Dragendroff reagent to observe and locate lignin, starch, alkaloids, protein and tannins respectively as per the protocols.

7.1.3 PHYSIOCHEMICAL ANALYSIS[17]

Shade dried powdered plant material of leaves of *Momordica charantia* Linn.,used for the determination of physiochemical parameters in accordance with WHO guidelines.

DETERMINATION OF ASH VALUES

Ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration.

Total ash

About 2–4g of ground air-dried material was accurately weighed, in a previously ignited and tarred crucible (usually of platinum or silica). The material was spread in an even layer and ignite by gradually increasing the heat to 500–600 °C until it will be in white,indicating the absence of carbon. Cooled in a desiccator and weighed. The residue isallowed to cool in a suitable desiccator for 30 minutes and weighed without delay. Thecontent of total ash was calculate in mg per g of air-dried material.

Water-soluble ash

To the crucible containing the total ash, 25 ml of water was add and boil for 5 minutes. The insoluble matter was collect in a sintered-glass crucible or on an ashless filter- paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450 °C. The weight of this residue in mg is subtract from

the weight of total ash. The content of water-soluble ash was calculate as mg per g of air-dried material.

Acid-insoluble ash

To the crucible containing the total ash, 25 ml of hydrochloric acid (~70 g/l) was added and covered with a watch-glass and boiled gently for 5 minutes. Filtered and the insoluble matter was collected on an ashless filter-paper and washed with hot water until the filtrate was neutral. Filter-paper containing the insoluble matter was transferred to the original crucible, dried on a hotplate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes and weighed without delay. The content of acid insoluble ash was calculated in mg per g of air-dried material.

Sulphated ash

Silica crucible was heated to redness for 10 minutes, allowed to cool in the desiccator and weigh. 2g of sample were accurately weighed, ignited gently then thoroughly charred. Cool, moistened the residue with 1ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800±25°C until all black particles have disappeared. Crucible was allowed to cool, add few drops of sulphuric acid and heat. Ignite as before, allow cooling and weighing. This process was repeated until two successive weighing differ by more than 0.5mg.

DETERMINATION OF EXTRACTIVE VALUES

Water soluble extractive

4.0 g of coarsely powdered air-dried material was accurately weigh, in a glass stoppered conical flask. 100 ml of water was added and weighed to obtain the total weight including the flask. Shaken well and allow to stand for 1 hour. Reflux condenser was attached to the flask and boil gently for 1 hour, cool and weigh. Readjust, with the solvent to the original total weight specified in the test procedure. Shaken well and filtered rapidly through a dry filter. 25 ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporate to dryness on a water-bath. Dried at 105 °C for 6 hours and cool in a desiccator for 30 minutes, then weigh without delay. The content of extractable matter was calculated as mg per g of air-dried material.

Alcohol soluble extractive

4.0 g of coarsely powdered air-dried material was accurately weigh, in a glass stoppered conical flask and macerate with 100 ml of the ethanol specified for the plant material select for 6 hours, shaken frequently, allow and kept for 18 hours. Filter rapidly, taking care not to lose any solvent, 25 ml of the filtrate was transfered to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105 °C for 6 hours, and cool in a desiccator for 30 minutes. The content of extractable matter was calculated in mg per g of air-dried material.

Non-volatile ether soluble extractive value

A suitable weighed quantity of the sample was transferred to an extraction thimble and extracted was solvent petroleum ether in a soxhlet for 6 hours. The extract was filtered into a tarred evaporating dish, evaporated and dried at 105°C to constant weight. The percentage of non volatile ether soluble extractive value with reference to the air dried was calculated.

LOSS ON DRYING

5 g of the prepared air-dried material, or the quantity specified in the test procedure for the herbal material concerned is accurately weighed, in a previously dried and tarred flat weighing bottle. The sample was kept in an oven at 100-105°C; in a desiccator over phosphorus pentoxide under atmospheric pressure. The loss of weight was calculated in mg per g of air-dried material.

FOAMING INDEX

1 g of the herbal material was reduced to a coarse powder (sieve size no. 1250), weigh accurately and transferred to a 500ml conical flask containing 100ml of boiling water by maintaining at moderate boiling for 30minutes. Cool and filtered into a 100-ml volumetric flask and sufficient water was added through the filter to dilute to volume. The decoction was poured into 10 stoppered test-tubes (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml, 3ml, etc. up to 10ml and the volume of the liquid in each tube is adjust with water to 10ml. The tubes were sealed and shaken in a lengthwise motion for 15 seconds (two shakes per second). Allowed to stand for 15 minutes and the height of the foam are measure. The results were assessed as follows: If the height of the foam in every tube is less than 1cm, the foaming index is less than 100. If the height of foam of 1cm is measured in any tube, the volume of the herbal material decoction in this tube (*a*) is used to determine the

index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain more precise result. If the height of the foam is more than 1cm in every tube, the foaming index is over 1000. In this case, repeat the determination using a new series of dilution of the decoction in order to obtain results.

Foaming index calculated using the following formula

Foaming Index = 1000/a

Where,

a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

SWELLING INDEX

The swelling index was the volume in ml occupied by the swelling of 1gm of plant material under specified conditions. A specified quantity of the plant materials were previously reduced to the required fineness is accurately weigh and transfer into a 25ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125mm, marked in 0.2ml divisions from 0 to 25ml in upward direction. Unless otherwise indicated in the test procedure, add 25ml of water and make the mixture thoroughly every 10min for 1hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material was measured including any sticky mucilage. Calculate the mean value of the individual determination, related to 1gm of plant material.

7.1.4 QUALITATIVE ANALYSIS AND QUANTITATIVE ESTIMATION OF HEAVY METALS AND INORGANIC ELEMENTS

Plant minerals play a vital role in metabolism. Presence of elements vary with the soil, climate conditions etc. There are essential and non-essential elements which may beneficial or harmful to living things. Non-essential elements like lead, arsenic, cyanide, chromium, cadmium, aluminium and silver bring about toxic effects resulting in intoxification. Hence, qualitative and quantitative estimation of inorganic elements in the plant *Momordica charantia* are carried out.

7.1.4.1 QUALITATIVE ESTIMATION OF INORGANIC ELEMENTS[23,25,35]

To the ash of the drug material 50% v/v hydrochloric acid was added and kept for 1 hours. It was filtered and the filtrate was used for the following tests.

Aluminium: White gelatinous precipitate of aluminium hydroxide was formed on addition of ammonia solution. It was slightly soluble in excess of the reagent. The

precipitate dissolves readily in strong acid and base, but after boiling it becomes insoluble.

Arsenic: Arsenious salts in neutral solution react with solution of copper sulphate to form green precipitate (Scheele's green) which on boiling gives red precipitate of cupric oxide.

Borate: The mixture obtained by the addition of sulphuric acid and alcohol (95%) to a borate when ignited, burns with flame tinged with green.

Calcium: Solution of calcium salts when treated with ammonium carbonate solution, yield a white precipitate after boiling and cooling the mixture (it is insoluble in ammonium chloride solution).

Carbonate: Carbonate, when treated with dilute acid effervescence, liberating carbon dioxide which is colorless and produces a white precipitate in calcium hydroxide solution.

Chlorides: Chlorides, when treated with silver nitrate solution, yield a white crude precipitate which is insoluble in nitric acid, but soluble after being well washed with water, in diluted ammonia, from which it was re-precipitated by the addition of nitric acid.

Copper: An excess of ammonia, added to a solution of cupric salt, produces first a bluish precipitate and then a deep blue coloured solution.

Iron: Solution of ferric salts, when treated with potassium ferrocyanide solution, yields an intense blue precipitate which is insoluble in dilute Hcl.

Lead: Strong solution lead salts, when treated with Hcl, yields a white precipitate. Which soluble in boiling water and is re-deposited as crystals when the solution was cooled.

Magnesium: Solution of magnesium salts, when treated with ammonium carbonate solution and boiled, yield a white precipitate, but yield no precipitate in the presence of ammonium chloride solution.

Mercury: Solution of mercury salts, when treated with sodium hydroxide solution, yields a yellow precipitate.

Nitrate: With solution of ferrous sulphate no brown colour was observed but if sulphuric acid was added (slow from the sides of test tube) a brown colour was produced at the junction of two liquids, indicating the presence of nitrates.

Phosphate: Solution of phosphate when treated with silver nitrate with dilute ammonia solution and in dilute nitric acid yield yellow precipitate of normal silver ortho phosphate (distinction from meta and pyrophosphate) solution.

Potassium: Moderately strong potassium salts, which have been previously ignited to remove ammonium salts, when treated with perchloric acid (60%) yield a white crystalline precipitate.

Silver: Solution of silver salts, when treated with potassium iodide solution yield a cream coloured precipitate which was insoluble in dilute ammonia solution and in nitric acid.

Sulphates: Solution of sulphates, when treated with lead acetate solution yields a white precipitate which was insoluble in ammonium and in sodium hydroxide.

7.1.4.2 QUANTITATIVE HEAVY METALS ESTIMATION BY ICP-OES METHOD[19,26,29]

INSTRUMENTATION PARAMETERS

Instrument Name: Inductively Coupled Plasma Optical Emission Spectrometry.

Instrumental Model: PE Optima 5300 DV ICP-OES

Optical system: Dual View- axial or radial.

Detector System: Charge coupled detector (UV-VISIBLE detector which is maintaining at 40˚C) to detect the intensity of the emission line wavelength range from 165 to 782 nm.

Torch (Light source): Positional horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a simple software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Standard alumina injector: 2mm inner diameter.

Spray chamber: Scott type

Nebulizer: Cross flow Gem tip.

Preparatiom of samples by acid digestion method

Weighed 50gm of powdered drug, treated with acid mixture of sulphuric acid: water in the ratio of 4:1 in the kjeldahl flask and heated continuosly till the solution is colorless. The sample mixture was then transfer in a 25ml volumetric flask and made upto the volume with distilled water. Blank solution was prepared as above without sample. The standards of Lead(Pb), Arsenic(Ar), Mercury(Hg), Cadmium(Cd) and Iron(Fe) were prepared as per the protocol and the calibration curve was developed for each of them.

Detection

Samples were analyzed for the detection and quantification of the lead, Arsenic, Mercury, Cadmium and Iron by inductively Coupled Plasma Optical Emission Spectrometry.

7.2 PHYTOCHEMICAL STUDIES[18,24,28]

Phytochemical evaluation is used to determine the nature of phyto constituents present in the plant by using suitable chemical tests. It is essential to study the pharmacological activities of the plant. It can be done by confirmation with different chromatographic techniques like TLC and HPTLC. Therefore a complete investigation is required to characterize the phytoconstituents qualitatively and quantitatively.

7.2.1 PREPARATION OF EXTRACTS[21]

Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites into the extracting solvent depends upon its polarity.

Extraction

The dried coarsely powdered leaves of *Momordica charantia* Linn., was first extract with n-Hexane (60-70°c) in soxhlet apparatus and then with solvents of increasing polarity like Chloroform, Ethyl acetate and Ethanol at 60-70°C. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were record and proceeded for further detailed phyto chemical and pharmacological screening.

7.2.2 PRELIMINARY PHYTOCHEMICAL SCREENING[16,24,39]

The different qualitative chemical tests can be performed for establishing profile of *Momordica charantia* leave extract for its chemical composition.

1. Detection of Alkaloids

Dragendroff's reagent: The substance was dissolved in 5mlof distilled water, to this 5ml of 2M HCl was added until an acid reactions occurs, then 1ml of Dragendroff'sreagentias added and examined for an immediate formation of an orange red precipitate.

Mayer's reagent: The substance was mixed with the little amount of dilute hydrochloric acid and Mayer's reagent and examined for the formation of white precipitate.

Wagner's reagent: The test solution was mixed with Wagner's reagent and examined for the formation of reddish brown precipitate.

2. Detection of glycosides

Borntrager's test: The powdered material was boiled with 1ml of sulphuric acid in a test tube for five minutes. Filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour was produced in the ammonical layer.

Modified Borntrager's test: The test was boiled with 2ml of the dilute sulphuric acid. This was treated with 2ml of 5% of ferric chloride solution (freshly prepared) for 5 minutes, and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour was produced in the ammonical layer.

3. Detection of Steroids and Terpenoids

Libermann Burchards test: The powdered drug was treated with few drops of acetic anhydride, boiled and cooled. Conc.sulphuric acid was added from the sides of test tube, brown colour ring was formed at the junction of two layers and upper layer turns green which showed steroids and formation of deep colour indicates presence of tri terpenoids.

Salkowski test: The extract was treated with few drops of concentrated sulphuric acid, red color at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of tri terpenoids.

4. Detection of Flavonoids

Shinoda test: To the solution of extract, few piece of magnesium turnings and concentrated Hcl was added drop wise, pink to crimson red occasionally green to blue colour appears after few minutes indicates the presence of flavonids.

Alkaline reagent test: To the test solution few drops of sodium hydroxide solution was added, intense yellow color was formed which turns to colorless on addition of few drops of dilute acid indicates presence of flavonoids.

5. Detection of carbohydrates

Molisch's Test: To the test solution few drops of alcoholic alpha napthol and few drops of conc. sulphuric acid were added through the sids of test tube, purple to violet color ring appears at junction.

Felhing's Test: The test solution was mixed with Fehling's I and II and heated, examined for the appearance of red coloration for the presence of sugar.

6. Detection of Phenols

Ferric chloride Test: A small quantity of substance were dissolved with 2ml distilled water and a few drops of 10% aqueous ferric chloride solution was added and observed for appearance of blue or green colour.

7. Detection of Tannins

Lead acetate test: The test solution was mixed with basic lead acetate solution and examined for formation of white precipitate.

Ferric chloride test: A few drops of 5% Ferric chloride solution was added to 2ml of an aqueous extract of the drug and examined for the appearance of bluish black colour.

8. Detection of saponins

A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigoursly and left for 3 minutes. Development of any honey comb like froth was examined.

9. Detection of Gum and Mucilage: Small quantities of test substance was dissolved in 5 to 10ml of acetic anhydride by means of heat, cooled and add 0.05ml of

concentrated sulphuric acid it was examined for the formation of bright purplish red color.

10. Detection of fixed oils and fats: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils and fats.

7.2.3 FLUORESCENCE ANALYSIS

Many crude drug show the fluorescence when the samples exposed to UV radiation. Evaluation of crude drugs based on fluorescence in day light was not much used, as it was usually unreliable due to the weakness of the fluorescence effect. Fluorescent lamps are fitted with suitable filter, which eliminate visible radiation from the lamp and transmits UV radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation.

7.2.4 QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Total phenolic compound content[22,31,36]

An aliquots of gallic acid standard and extracts were transferred to 250ml of flask separately, added 50ml of distilled water, the solution was centrifuged at 500rpm about 20 minutes. Then added 2ml of super saturation solution transferred to flask, added 2.5ml of 7.5% of sodium carbonate. After incubation at $40^{o}C$ for 30 minutes, the absorbance of the reaction mixtures was measured at 765nm in a spectrometer.

Total flavonoid content[20,27]

Total flavonoid content was measured by the aluminium chloride colorimetric assay. An aliquots (1ml) ofextract or standard solution of quercetin (20,40,60,80 and 100µg/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To the flask was added 0.30ml of 5% $NaNO_2$, after 5 mins 0.3ml of 10% $AlCl_3$was added. After 5 mins, 2 ml of 1M NaOH was added and the volume was made upto 10ml with distilled water. The solution was mixed and the absorbance is measured against the blank at 510nm. The total flavonoid content was expressed as mg quercetinequivalents(QE).

Total Saponin content[12]

20 g of plant sample was dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55 ºC. The mixture is filter and the residue re-extract with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transfer into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process will repeat. 60 ml of n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven into a constant weight. The saponin content was calculated in percentage.

IR SPECTROSCOPY

IR spectroscopy is an important and popular tool for structural elucidation and compound identification. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. It can be seen in the annotated spectrum. By interpreting the infra spectrum, the chemical bonds in a molecule can be determined.

1. 3540-3300 cm^{-1} N-H Stretching Vibration
2. 3670-3230cm^{-1}H Stretching Vibration
3. 1690-1630cm^{-1}C=N Stretching Vibration
4. 2975-2840 cm^{-1}C-H Aliphatic Stretching Vibration

Instrument **:** ABB MB 3000-PH FT-IR Spectrometer [Horizon software]

Sample technique **:** KBr Pellet technique.

Sample preparation

Ethanolic extract of leaves of *Momordica charantia* Linn., was used for FTIR analysis. 10 mg of the dried extract was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The sample was loaded in FTIR spectroscope, with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

7.2.5 CHROMATOGRAPHY[32]

Chromatography methods were important analytical tool in the separation, identification and estimation of compounds present in the plant.

7.2.5.1 THIN LAYER CHROMATOGRAPHY[38]

Principle

Thin layer chromatography is a technique used for the separation, identification and estimation of single or mixture of components present in the various extracts. It is reliable technique in which solute undergoes distribution between two phases, stationary and mobile phase. The separation is mainly based on the differential migration that occurs when a solvent flows along the thin layer of stationary phase. This may be achieved by partition and adsorption depending on stationary phase used.

TLC Plate preparation

The plates were prepared by using TLC spreader. 40gm of silica gel G was mixed with 85ml of water to prepare homogenous suspension and poured in the spreader. 0.25mm thickness of plates were prepared and dried until the transparency of the layer disappeared, then dried at 110°C for 30 minutes and kept in desiccators.

Selection of mobile phase

Solvent mixture was selected on the basis of phyto constituents present in each extract. Factors such as nature of components, stationary phase, mobile phase, polarity, influence the rate of separation constituents. From the vast analysis, best solvent was select which show good separation with maximum number of components.

The retention Factor (R_f) was calculate by using following formula,

$$R_f = \frac{\text{Distance travelled by solute from the origin}}{\text{Distance travelled by solvent from the origin}}$$

7.2.5.2 HIGH PERFORMACE THIN LAYER CHROMATOGRAPHY- Fingerprint Profile

HPTLC is one of the versatile chromatographic methods which helps in the identification of the compounds and thereby authentication of purity of herbal drugs. The time required in this method for the demonstration of most of the characteristic constituents of a drug is very quick and short. In addition to qualitative detection, HPTLC also provides semi-quantitative information on major active constituents of a drugs, thus enabling an assessment of drug quality.

HPTLC serves as a convenient tool for finding the distribution pattern of phytoconstituents which is unique to each plant. The fingerprint obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extract.

Instrument conditions

Sample used : Ethanol extract
Instrument : CAMAG HPTLC
HPTLC Applicator : CAMAG LINOMAT IV
HPTLC Scanner : CAMAG TLC SCANNER II
Sample dilution : 5mg/ml in methanol Volume of injection : 20µl
Mobile phase : Toluene : Ethyl acetate : Formic acid (6:2.5:0.5)
Wavelength : 254nm
Lamp : Deuterium
Stationary phase : TLC silica gel G 60 F_{254} (Merck)

Equipment

CAMAG HPTLC system equipped with a sample applicator Linomat IV, Twin trough plate development chamber, TLC scanner II.

Chromatographic conditions

The estimation of has been done using the following chromatographic conditions. Chromatography was performed on a 10×10cm pre- activated HPTLC silicagel G 60 F254 plate. Samples were applied to the plate as 6mm wide band with an automatic TLC applicator Linomat IV with nitrogen flow (CAMAG, Switzerland), 8mm from the bottom. Densitometric scanning was performed on CAMAG scanner II. The plates were pre-washed with solvent ethyl acetate.

7.3 PHARMACOLOGICAL STUDIES[50,51,52]

7.3.1 *INVITRO* EVALUATION[44,47,54]

7.3.1.1 PROTEIN DENATURATION METHOD

Principle

Protein denaturation is the process in which the protein lose their secondary and tertiary structure by application of external stress, heat, compounds (acid, base) and mineral salts. Proteins lose their biological function. This protein denaturation is well documented in cause of inflammation. The ability of extract to inhibit protein denaturation will be studied by this method.

Procedure

The reaction mixtures (0.5ml) consist of 0.45ml bovine serum albumin (5% aqueous solution) and 0.05ml of test compound (100, 200, 400, 800 and 1000mg/ml of final volume). pH was adjust at 6.3 using a small amount of 1N HCL. The samples were incubate at 37°C for 3 mins. After cooling the samples 2.5ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660nm. For control tests 0.05ml distilled water was used instead of synthesized product control tests lacked bovine serum albumin.

The percentage inhibition of protein denaturation was calculate as follows,

$$\text{Percentage inhibition} = \frac{(\text{O.D Test compounds} - \text{O.D Control})}{(\text{O.D control})} \times 100$$

7.3.1.2 MEMBRANE STABILIZATION METHOD[55]

Principle

The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since human red blood cells

(HRBC) membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by the extracts.

Procedure

Human blood was collected from healthy volunteers. The collected blood was missed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42% and distilled water 100ml) and centrifuged ithisosaline. To 1ml of HRBC suspension, equal volume of test drug in various concentration () was added. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560nm.

The percentage of protection can be hence calculated from the equation as given below;

$$\text{Percentage of protection} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

Where,

OD of test = Test sample's absorbance

OD of control = Absorbance of negative control

7.3.2 ACUTE TOXICITY STUDY (OECD 423 GUIDELINES)

Acute toxic study for *Momordica charantia* Linn., has been carried out already by using OECD 423 (Acute Toxic Class Method). In this study there was no toxicity/death observed up to dose of 2000mg/kg. The acute toxicity study in rats showed that up to 2000mg/kg dose, the plant is safe for consumption and medicinal uses.

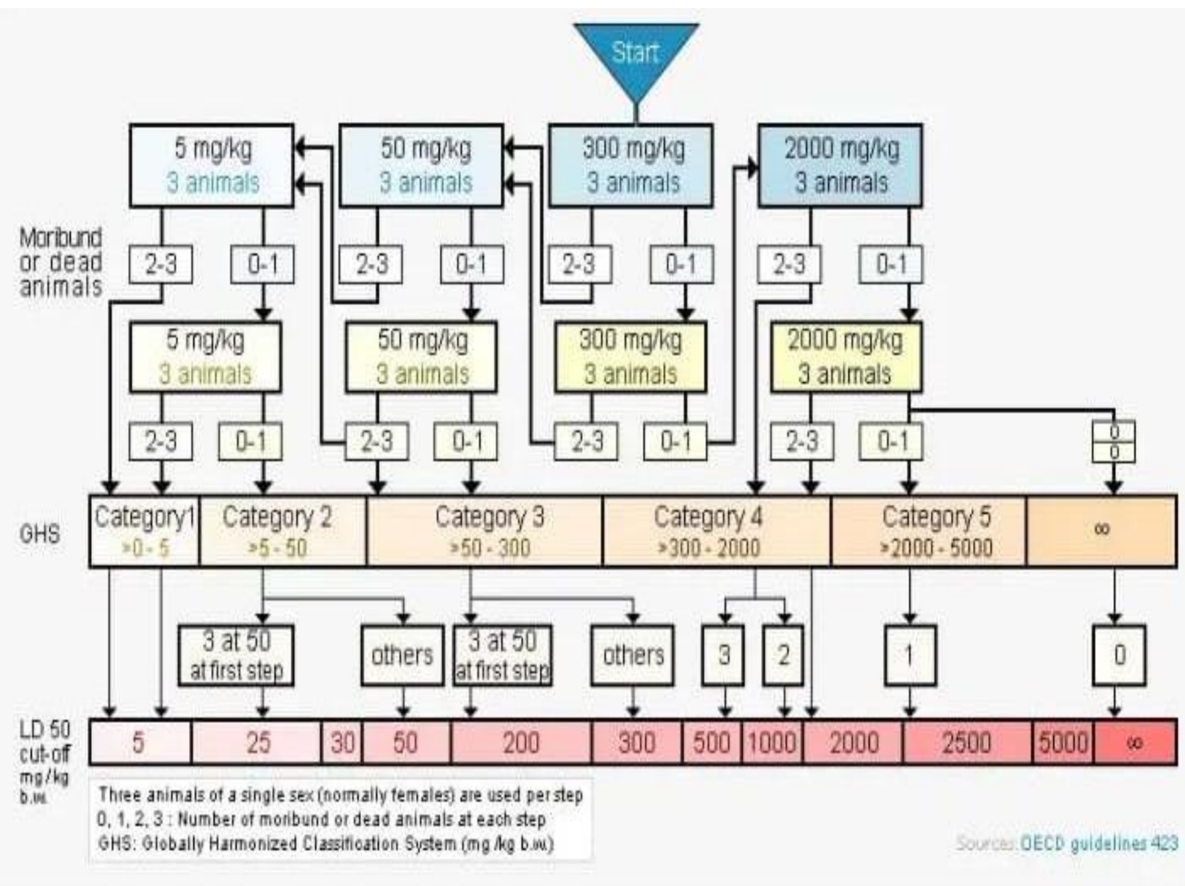


FIGURE NO 5. OECD 423 GUIDELINES FOR ACUTE TOXICITY STUDY

7.3.3 *INVIVO* EVALUATION[46,68]

7.3.3.1 Adjuvant induced Arthritis[62]

This is one of the most commonly used animal models for evaluating anti-arthritic activity. Arthritis was induced in rats by injection with 0.1 ml of Complete Freund‟s Adjuvant in the sub-plantar region of the right hind paw except the vehicle control. The Complete Freund‟s Adjuvant contains 10 mg/ml of heat-killed *Mycobacterium tuberculosis.*

Experimental analysis[43]

A total of 30 adult Wistar albino rats weighing (150-200g) were divided into 5 groups of 6 animals in each group.

TABLE NO: 3 GROUPING OF ANIMALS

GROUP	NAME OF THE GROUP	TREATMENT
I	Positive control	Treatment with vehicle for 21 days
II	Arthritic control	Treated with CFA
III	CFA+ Standard	Treatment with Diclofenac sodium 15mg/kg for 21 days
IV	CFA+Test drug 1 (Low dose)	Treatment with ethanol extract 200mg/kg for 21 days
V	CFA+Test drug 2 (High dose)	Treatment with ethanol extract 400mg/kg for 21 days

7.3.3.2 PARAMETERS STUDIED

Change in body weight

Body weight are observe every week.

Paw volume measurement

Paw volume of all the animal groups are measured by using Plethysmograph at 0,7,14 and 21 days. The percentage inhibition of paw volume can be determined by using the formula,

$$\text{Paw volume} = \frac{(Vc - Vo) - (Vt - Vo)}{(Vc - Vo)} \times 100$$

Where,

Vc - is the paw volume after induction

Vo - is the paw volume before induction

Vt - is the paw volume after treatment

Hematological parameters

At the end of the experimental period (22nd day), the blood sample was collected from animals through retro orbital vein puncture of all groups for the study of biochemical parameters such as Hemoglobin content, Total RBC count, Total WBC count and ESR..

Histopathological studies[63]

The animals were sacrificed by cervical dislocation and the ankle joints of hind limps were removed and placed in 10% buffered formalin. The fixed tissues were then decalcified and the sections were stained with Haematoxylin and Eosin. Slides were reviewed for evaluation of histopathological changes like soft tissue swelling, bony erosion, pannus formation and narrowing of the space between the joints.

RESULTS AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

MACROSCOPY

Organoleptic characters

- Colour : Green
- Taste : Intensely bitter
- Odour : Characteristic

Morphological Features

- Type : Simple and alternate
- Shape : Palmate and loped
- Margin : Crenate, Irregularly toothed
- Size : 8-12cm long, 4-6cm wide
- Apex : Acute
- Base : widely cordate
- Surface : Adaxial, apaxial
- Blade : Smooth and thick
- Simple tendril present in that base and both sides of leaf has few scattered hairs along the nerves on the lower surface.

FIG NO. 6 MACROSCOPY OF*Momordica charantia* Linn.,

MICROSCOPY

- The petiole of *Momordica charantia*, in cross section, has a convex outline with two ribs on the adaxial surface and a triangular outline on the abaxial surface.
- The midrib is distinct. Upper and lower epidermis are in a line the rectangle, ovoid cells. The epidermis is composed of a single layer of cells and covered with a thin cuticle.
- The angular collenchyma is discontinuous, observed below the epidermis and composed by one to three layers of cells.
- The vascular system is constituted by seven bicollateral bundles in the central region of the petiole and two bundles in the ribs.
- It is situated some collenchyma cells under the xylem. Leaves are dorsiventral, parenchyma cells are 1-2 layered, spongy parenchyma cells are 2-3 layered.

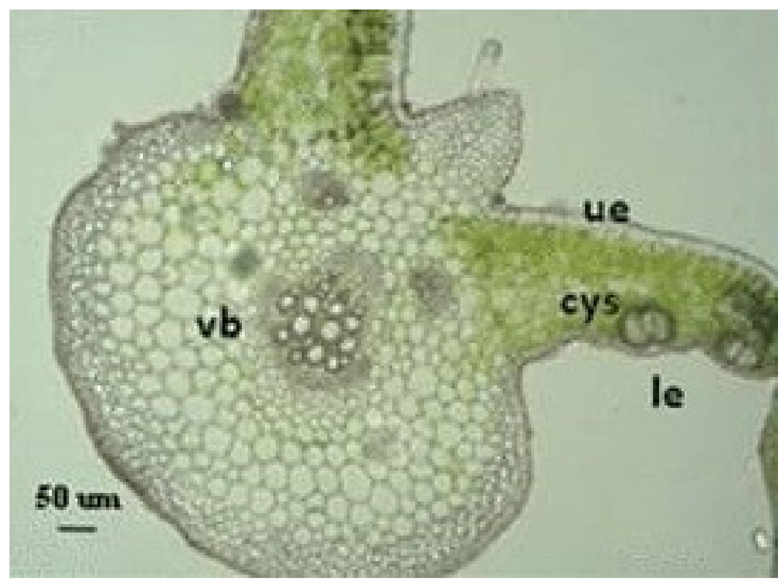


FIGURE NO. 7 T.S OF LEAF OF *Momordica charantia* Linn.,

(vb - vacular bundle, cys - cystolith, le - lower epidermis, ue - upper epidermis)

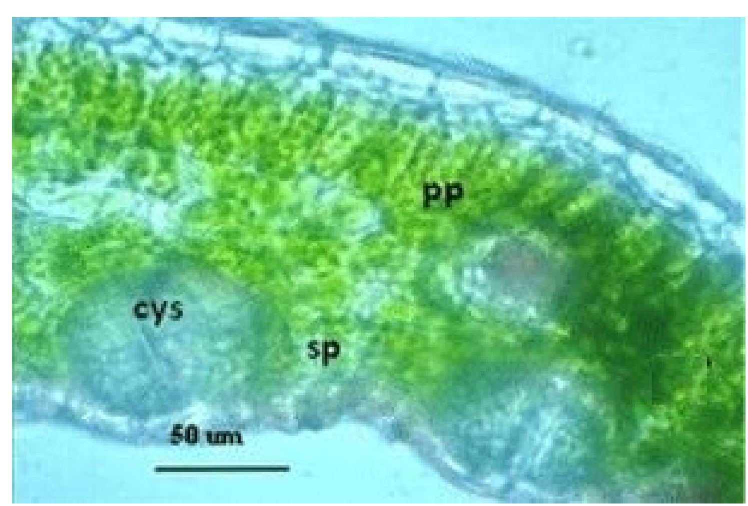


FIGURE NO.8MESOPHYLL

(pp – palisade parenchyma, sp – spongy parenchyma)

POWDER MICROSCOPY

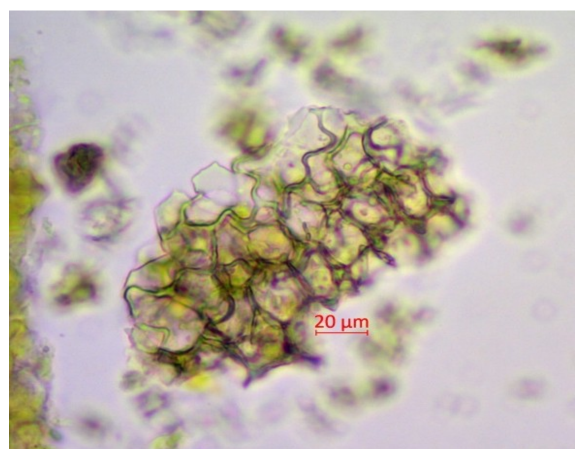


Figure No. 9 Epidermal cells in surface view

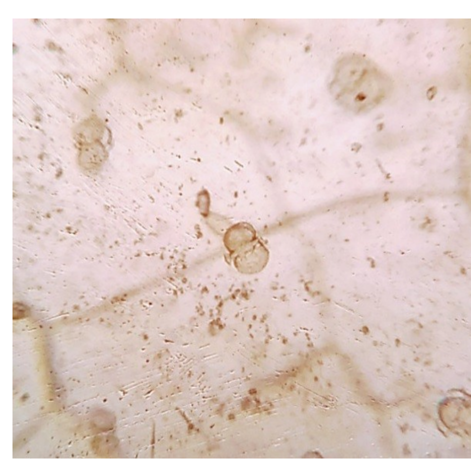


Figure No. 10 Anomocytic stomata

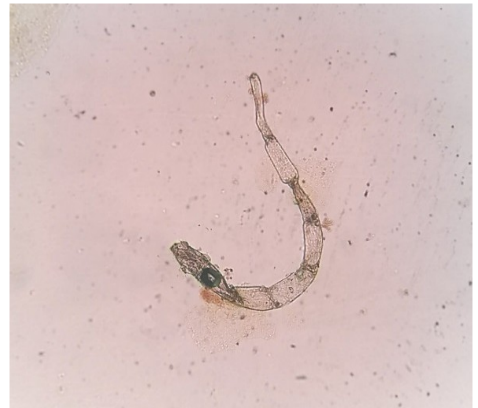
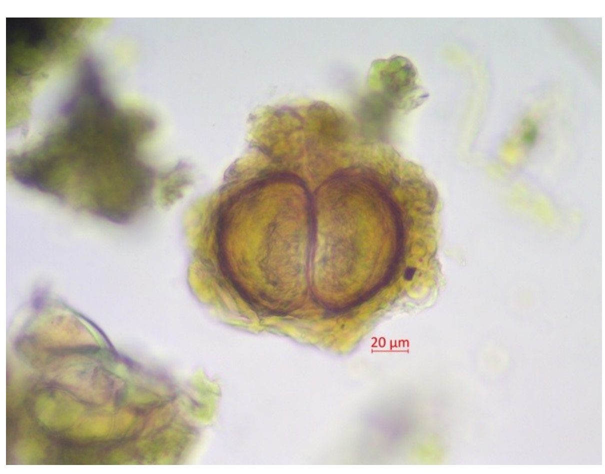


Figure No. 11 Non-glandular trichomes



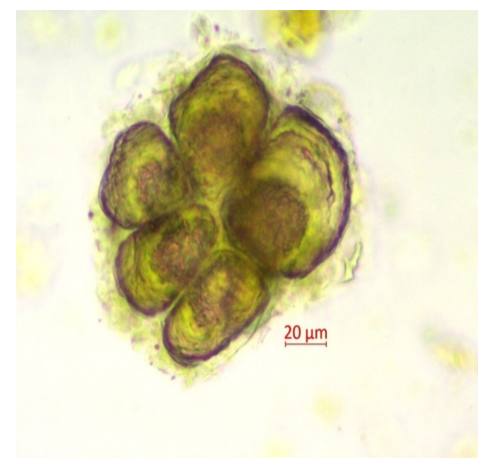


Figure No. 12 Idioblasts

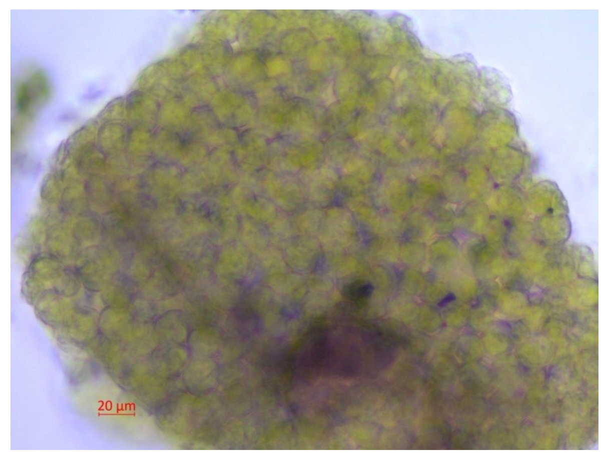


Figure No. 13 Spongy parenchyma

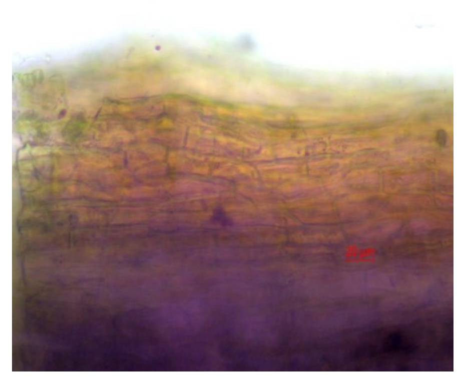


Figure No. 14 Epidermis in sectional view

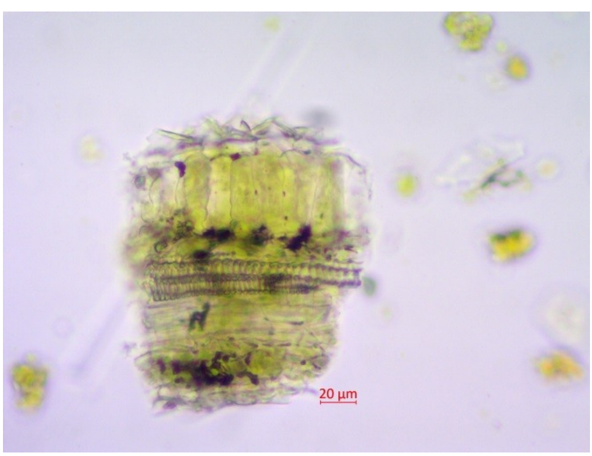


Figure No. 15 Pallisade cells

Figure No. 16 Fibre

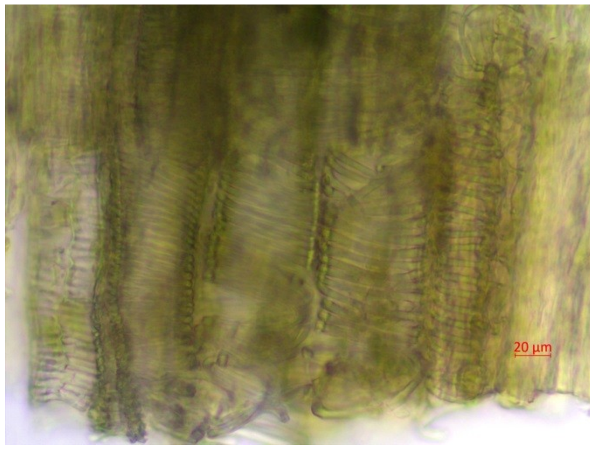


Figure No. 17 Vessel

QUANTITATIVE MICROSCOPY

LINEAR MEASUREMENT OF TRICHOMES

The length and width of the trichomes were measured in the powdered leaves of *Momordica charantia* Linn., and the results were shown in table

TABLENO. 4 LINEAR MEASUREMENT OF TRICHOMES

Dimension	Minimum (μ)	Average (μ)	Maximum(μ)
Length	141.1	221.6	340.3
Width	24.9	31.5	41.5

The length of trichomes were in range 141.1μm - 221.6μm and the width of trichomes were found in the range 24.9μm - 31.5μm.

LEAF CONSTANTS

Stomata

The stomata was found in abaxial surface, it composed of anomocytic type. The stomata surrounded by varying number of subsidiary cells.

Veinislet number and Veinlet termination number

Leaf surface showed the presence of veins and veinislets and vein termination. The primary and secondary veins branched and gave rise to ultimate veinlets. Each vein islets had one or two vein terminations.

TABLE NO. 5 LEAF CONSTANTS OF *Momordica charantia* Linn.,

S.NO	PARAMETERS	VALUES IN SQ mm
1.	Stomatal number (Lower epidermis)	432-459
2.	Stomatal Index (Lower epidermis)	12-14
3.	Vein islet number	7-9
4.	Vein termination number	4-6

HISTOCHEMICAL COLOUR RAECTIONS

Transverse section of leaves of *Momordica charantia* Linn., was treated with various reagents like Dragendroff's reagent, N/10 iodine, Phloroglucinol and Conc.HCL, ferric chloride, picric acid and toluidine blue. The histochemical colour reactions of transverse section of leaves showed different stained cells were observed. These characteristic features were observed and reported in the table 6.

TABLE NO. 6 HISTOCHEMICALCOLOUR REACTIONS OF *Momordicacharantia*Linn.,

S.NO	REAGENTS	TEST FOR	NATURE OF CHANGE	HISTOLOGY	RESULTS
1.	Phloroglucinol + HCL	Lignin	Red	Xylem	++
2.	N/50 Iodine solution	Starch	Blue	Parenchyma in midrib	++
3.	Dil. Ferric chloride	Tannin	Dark blue black	Cuticle	++
4.	Picric acid	Protein	Yellow colour	--	--
5.	Dragendroff's reagent	Alkaloid	Orange colour	Epidermal cell	++
6.	Phloroglucinol + HCL	Calcium oxalate crystals	Dissolved	Idioblast	++

Note : + presence, - absence

PHYSIOCHEMICAL ANALYSIS

TABLE NO. 7 PHYSIOCHEMICAL ANALYSIS OF THE LEAVES OF *Momordica charantia* Linn.,

S.NO	PARAMETERS	VALUES(%w/w)
I.	**ASH VALUE**	
1	Total ash	13.83±0.67
2	Water soluble ash	7.04±0.45
3	Acid insoluble ash	6.58±0.47
4	Sulphated ash	14.37±0.46
II.	**EXTRACTIVE VALUES**	
1	Water soluble extractive	4.2±0.39
2	Alcohol soluble extractive	6.25±0.33
3	Ether soluble extractive	2.95±0.40
III.	**Crude fibre content**	21.49±0.82
IV.	**Loss on drying**	13.73±1.86
V.	**Foaming index**	<1
VI.	**Swelling Index**	Nil

Values are expressed as Mean ± SD, n=6

INORGANIC ELEMENTS AND HEAVY METAL ANALYSIS

TABLE NO. 8 QUALITATIVE ESTIMATION OF INORGANIC ELEMENTS OF *Momordica charantia* Linn.,

S.NO	ELEMENTS	OBSERVATION
1.	Calcium	+
2.	Sodium	+
3.	Copper	-
4.	Magnesium	+
5.	Phosphorus	+
6.	Silver	-
7.	Zinc	+
8.	Iron	+
9.	Potassium	+
10.	Aluminium	-
11.	Borate	-

Note : + Presence, - Absence

TABLE NO. 9 QUANTITATIVE ESTIMATION OF INORGANIC ELEMENTS OF *Momordica charantia* Linn.,

S.NO	INORGANIC ELEMENTS	TOTAL AMOUNT (%W/W)
1.	Calcium	0.031
2.	Sodium	0.021
4.	Magnesium	0.003
5.	Phosphorus	0.055
6.	Zinc	0.001
8.	Potasium	0.069
9.	Manganese	0.001

Quantitative estimation of Heavy metals by ICP OES method

The quantification of the individual heavy metals was analysed for the powdered mixture of *Momordica charantia* Linn., by ICP-OES technique the following metals like were detected.

TABLE NO.10 QUANTITATIVE ESTIMATION OF HEAVY METALS

S.NO	ELEMENTS	RESULTS (ppm)	SPECIFICATION AS PER WHO GUIDELINES
1	Mercury	Not detected	NMT 0.5ppm
2	Arsenic	0.024	NMT 5.0ppm
3	Lead	0.045	NMT10ppm
4	Cadmium	0.008	NMT0.3ppm

The estimation of heavy metals in the sample revealed heavy metals are within the prescribed limits. It is safe and does not cause any harm on consumption.

PHYTOCHEMICAL ANALYSIS

Percentage yield of extracts

TABLE NO. 11 PERCENTAGE YIELD OF SUCCESSIVE EXTRACTS OF LEAVES OF *Momordica charantia* Linn.,

S.NO	EXTRACT	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD (%w/w)
1.	n-Hexane	Continuous Hot percolation method using Soxhlet apparatus	Semisolid	Brown	3.8
2.	Chloroform		Sticky soild	Black	7.75
3.	Ethyl acetate		Sticky	Greenish black	5.8
4.	Ethanol		Oily Semisolid	Brown	6.7
5.	Aqueous	Cold maceration	Solid	Brown	2.2

QUALITATIVE PHYTOCHEMICAL ANALYSIS

TABLE NO. 12 QUALITATIVE PHYTOCHEMICAL ANALYSIS

S. No	Phytoconstituent	Powdered drug	n-Hexane	Chloroform	Ethyl acetate	Ethanol	Aqueous
1.	Glycosides	+	+	-	+	+	+
2.	Steroids	+	+	+	+	+	+
3.	Carbohydrate	+	-	-	+	+	+
4.	Alkaloids	+	+	+	+	+	+
5.	Phenolic compound	+	+	+	+	+	-
6.	Flavonoids	+	+	+	+	+	+
8.	Tannins	-	-	-	+	-	-
9.	Terpenoids	+	+	+	+	+	+
10.	Saponins	+	+	+	+	+	+
11.	Resins	-	-	-	-	-	-

Note: + Presence, - Absence.

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

The *Momordica charantia* Linn., was found to contain various phytochemical constituents. The phytoconstituents like flavonoids, alkaloids, saponins and phenolic constituents were estimated quantitatively.

TABLE NO.13 QUANTITATIVE ESTIMATION OF ALL THE EXTRACTS

S. NO	Phyto Constituents	N-hexane	Chloroform	Ethyl acetate	Ethanol
1	Flavonoids (%w/w)	0.97	4.35	3.96	9.61
2	Phenolic compounds (%w/w)	1.74	10.82	7.43	14.37
3	Saponins (%w/w)	2.53	3.27	5.22	11.48

FLUORESCENCE ANALYSIS

TABLE No. 14 FLUORESENCE CHARACTERS OF POWDERED LEAVES OF *Momordica charantia* Linn.,

S.NO	TREATMENT	DAY LIGHT	SHORT UV (254nm)	LONG UV (366nm)
1.	Powder	Light green	Reddish Brown	Green
2.	Powder + water	Yellowish Green	Brown	Yellowish green
3.	Powder + ethanol	Dark green	Dark brown	Dark brown
4.	Powder + 1N Hcl	Dark Brown	Blackish brown	Brownish black
5.	Powder + H_2SO_4	Yellowish green	Reddish brown	Yellowish green
6.	Powder + KoH	Light green	Dark brown	Green
7.	Powder +NaoH	Greeninsh yellow	Brown	Yellowish green
8.	Powder + Acetic acid	Yellowish brown	Yellowish brown	Greeninsh black
9.	Powder + Nitric acid	Brown	Black	Reddish brown
10.	Powder + Iodine	Yellowish green	reddish	Dark green
11.	Powder + Picric acid	Light green	brown	Dark brown

TABLE NO.15 FLUORESCENECE ANALYSIS OF VARIOUS LEAVES EXTRACTS OF *Momordica charantia* Linn.,

S.NO	TREATMENT	DAY LIGHT	SHORT UV (254nm)	LONG UV (366nm)
1.	n-Hexane	Brown	Yellowish brown	Brown
2.	Chloroform	Brownish black	Brown	Dark brown
3.	Ethyl acetate	Greenish black	Black	Black
4.	Ethanol	Brown	Brown	Brown
5.	Aqueous	Brown	Brown	Brown

There was no characteristic fluorescence were seen with either powdered leaves or the extracts.

IR SPECTROSCOPY

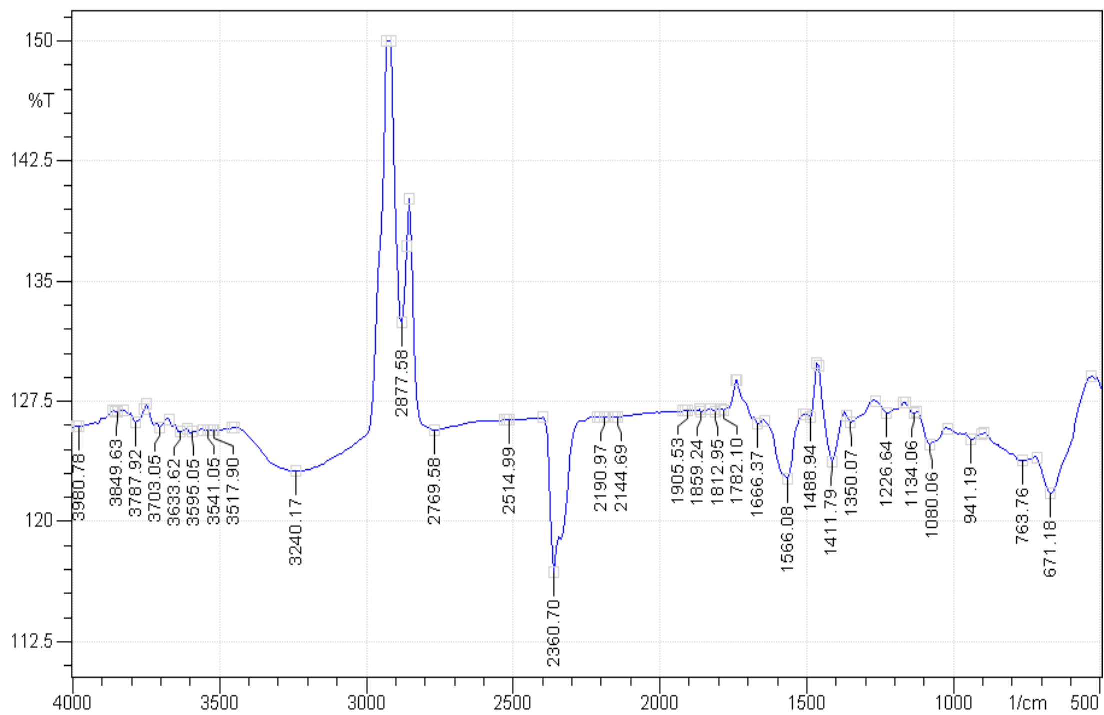


FIGURE NO 18. IR SPECTRUM OF ETHANOLIC EXTRCT OF *Momordica charantia* Linn.,

TABLE NO 16. FTIR PEAKS OF ETHANOLIC EXTRACT OF OF *Momordica charantia* Linn.,

S. No	Wavenumber (cm^{-1})	Assignment	Width (cm^{-1})	Height (%t)	Peak area (Abs. Sum)	Peak area (%)
1.	1134.06	C-O stretching	38.57	-2.76	115.05	0.4
2.	1411.79	C-C stretching	92.58	-5.96	377.24	1.3
3.	1666.37	C=N stretching	92.58	-5.19	454.09	1.6
4.	2877.58	C-H Aliphatic stretching	54	-8.3	348.09	1.3
5.	3517.9	N-H stretching	23.14	-8.68	757.34	0.7
6.	3541.05	N-H stretching	69.43	-9.24	263.68	2.5
7.	3633.62	H streching	61.72	-6.52	421.28	1.4

CHROMATOGRAPHIC STUDIES

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was done with all the five extracts and their R_f values were tabulated in the table 16.

TABLE NO. 17 THIN LAYER CHROMATOGRAPHIC STUDIES OF EXTRACT

S.NO	CHEMICAL CONSTITUENT	SOLVENT SYSTEM	EXTRACTS	NO. OF SPOTS	R_f VALUE
1.	Phenolic content	Toluene : Ethyl acetate (93:7)	n-Hexane	6	0.15, 0.37, 0.42, 0.62, 0.75, 0.90
			Chloroform	9	0.07, 0.13, 0.20, 0.39, 0.46, 0.55, 0.70, 0.77, 0.90
			Ethyl acetate	6	0.10, 0.14, 0.31, 0.49, 0.55, 0.87
			Ethanol	8	0.07, 0.23, 0.27, 0.31, 0.49, 0.55, 0.87
			Aqueous	-	-
2.	Flavonoid	Ethyl acetate: Formic acid : acetic acid : water (100:11:11:26)	n-Hexane	1	0.86
			Chloroform	3	0.64, 0.75, 0.89
			Ethyl acetate	3	0.46, 0.70, 0.89
			Ethanol	4	0.10, 0.15, 0.43, 0.89
			Aqueous	1	0.20

3.	Steroids	Chloroform: Methanol (27:3)	n-Hexane	3	0.48, 0.70, 0.84
			Chloroform	6	0.19, 0.26, 0.34, 0.58, 0.71, 0.82
			Ethyl acetate	7	0.18, 0.29, 0.39, 0.44, 0.58, 0.66, 0.85
			Ethanol	7	0.11, 0.18, 0.40, 0.58, 0.65, 0.74, 0.87
			Aqueous	1	0.15
4.	Alkaloids	Toluene : Ethyl acetate (7:3)	n-Hexane	4	0.53, 0.68, 0.79, 0.86
			Chloroform	7	0.15, 0.18, 0.21, 0.27, 0.45, 0.55, 0.70
			Ethyl acetate	6	0.11, 0.20, 0.32, 0.40, 0.68, 0.76
			Ethanol	5	0.17, 0.23, 0.29, 0.71, 0.82
			Aqueous	4	0.21, 0.73, 0.82, 0.92
5.	Saponins	Chloroform: Methanol:Water (13:7:2)	n-Hexane	2	0.85, 0.95
			Chloroform	2	0.82, 0.87
			Ethyl acetate	3	0.42, 0.69, 0.82
			Ethanol	3	0.69, 0.79, 0.87
			Aqueous	2	0.74, 0.90

FIGURENO. 19 TLC OF VARIOUS EXTRACTS OF LEAVES OF *Momordica charantia* Linn.,

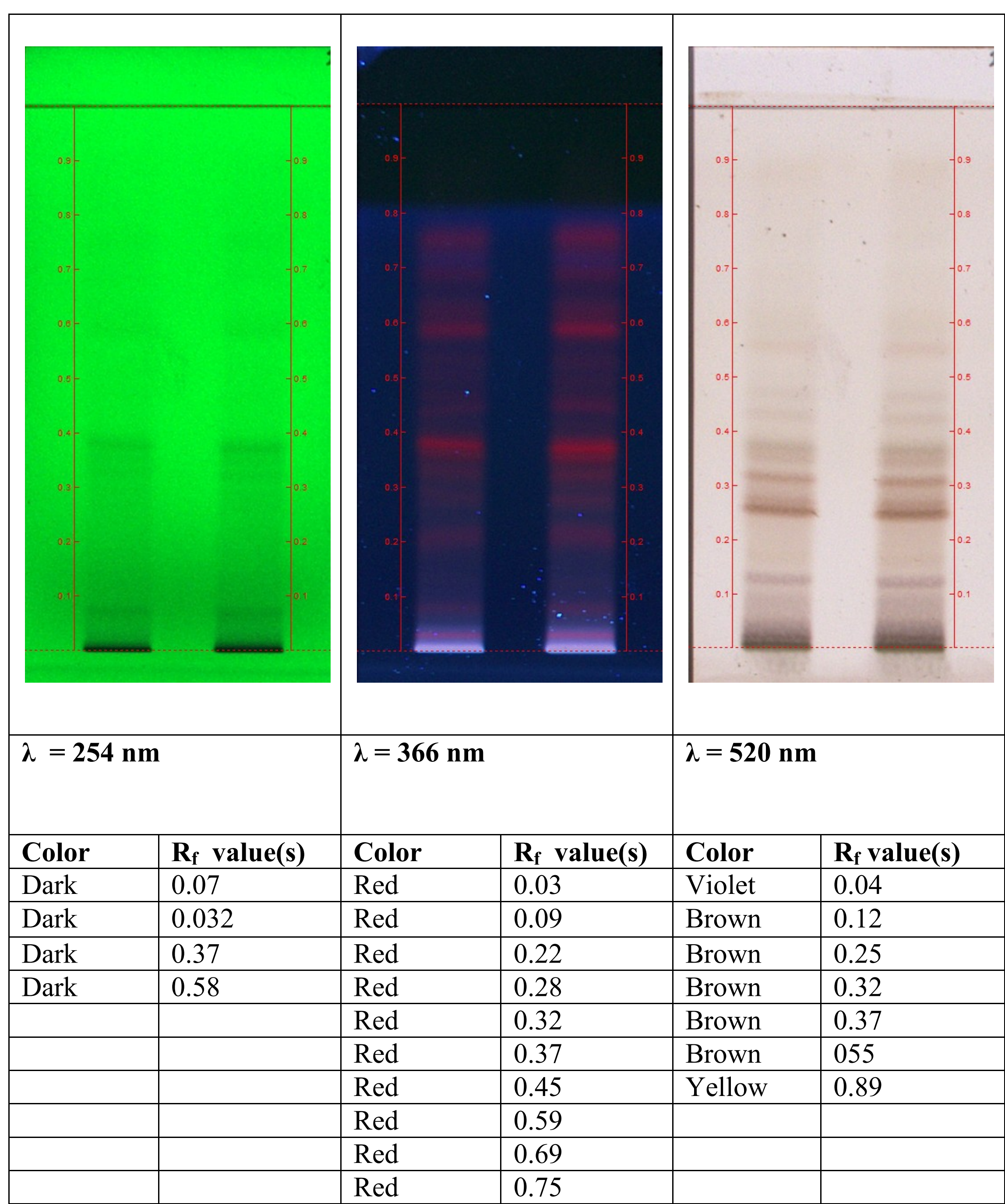
TLC of various extracts		
TLC of Steroids	**TLC of Phenolic compounds**	**TLC of Alkaloids**

HPTLC FINGER PRINT PROFILE

HPTLC Finger print Data of ethanolic extract of *Momordica charantia* Linn.,

Since secondary metabolites are responsible for biological activity, this study would be the leading pathway of information for selection of extract for pharmacological activity.High performance thin layer chromatography finger printing was performed with the ethanol extract of *Momordica charantia* Linn.,

FIGURE NO 20. HPTLC OF ETHANOLIC EXTRACT



λ = 254 nm		**λ = 366 nm**		**λ = 520 nm**	
Color	**R_f value(s)**	**Color**	**R_f value(s)**	**Color**	**R_f value(s)**
Dark	0.07	Red	0.03	Violet	0.04
Dark	0.032	Red	0.09	Brown	0.12
Dark	0.37	Red	0.22	Brown	0.25
Dark	0.58	Red	0.28	Brown	0.32
		Red	0.32	Brown	0.37
		Red	0.37	Brown	055
		Red	0.45	Yellow	0.89
		Red	0.59		
		Red	0.69		
		Red	0.75		

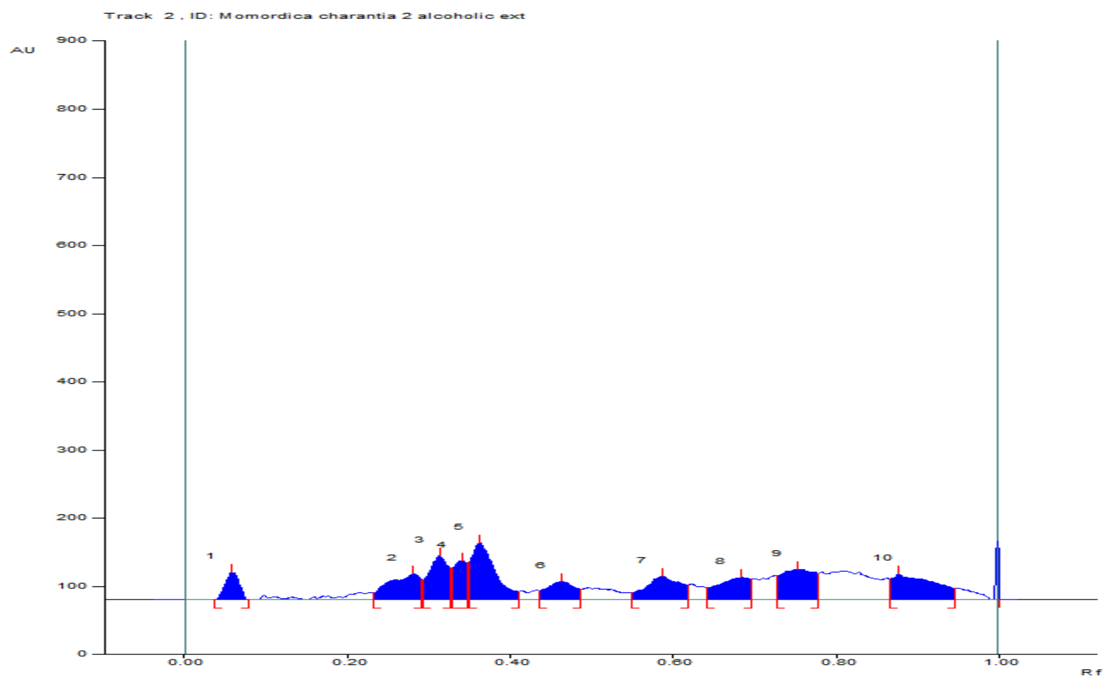


FIGURE NO. 21 HPTLC FINGER PRINT AT 254nm

TABLE NO. 18 HPTLC FINGERPRINT DATA AT 254nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.1 AU	0.06 Rf	40.0 AU	8.75 %	0.08 Rf	0.1 AU	641.8 AU	4.72 %
2	0.23 Rf	9.5 AU	0.28 Rf	38.3 AU	8.36 %	0.29 Rf	29.2 AU	1338.1 AU	9.85 %
3	0.29 Rf	29.3 AU	0.31 Rf	63.9 AU	13.97 %	0.33 Rf	46.2 AU	1423.4 AU	10.48 %
4	0.33 Rf	46.3 AU	0.34 Rf	57.2 AU	12.49 %	0.35 Rf	53.4 AU	906.1 AU	6.67 %
5	0.35 Rf	53.6 AU	0.36 Rf	83.4 AU	18.23 %	0.41 Rf	11.6 AU	2274.6 AU	16.74 %
6	0.44 Rf	13.1 AU	0.46 Rf	26.8 AU	5.85 %	0.49 Rf	15.6 AU	864.3 AU	6.36 %
7	0.55 Rf	10.0 AU	0.59 Rf	34.2 AU	7.46 %	0.62 Rf	21.4 AU	1349.4 AU	9.93 %
8	0.64 Rf	17.4 AU	0.68 Rf	32.1 AU	7.02 %	0.70 Rf	29.5 AU	1193.2 AU	8.78 %
9	0.73 Rf	35.3 AU	0.75 Rf	44.2 AU	9.65 %	0.78 Rf	37.5 AU	1751.3 AU	12.89 %
10	0.86 Rf	30.5 AU	0.88 Rf	37.6 AU	8.22 %	0.95 Rf	15.8 AU	1844.3 AU	13.57 %

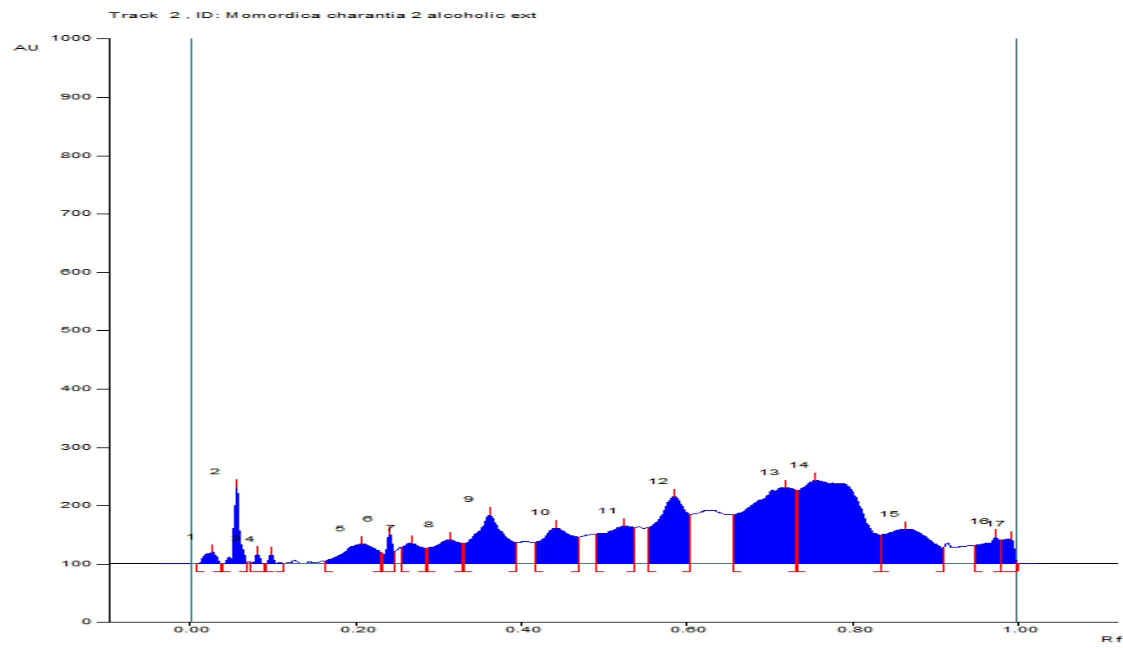


FIGURE NO. 22 HPTLC FINGER PRINT AT 366nm

TABLE NO. 19 HPTLC FINGERPRINT DATA AT 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.03 Rf	20.1 AU	1.85 %	0.04 Rf	0.1 AU	246.4 AU	0.66 %
2	0.04 Rf	0.0 AU	0.06 Rf	131.8 AU	12.12 %	0.07 Rf	2.1 AU	761.3 AU	2.05 %
3	0.07 Rf	2.0 AU	0.08 Rf	17.3 AU	1.59 %	0.09 Rf	0.0 AU	76.1 AU	0.20 %
4	0.09 Rf	0.0 AU	0.10 Rf	14.8 AU	1.36 %	0.11 Rf	0.1 AU	75.8 AU	0.20 %
5	0.16 Rf	4.3 AU	0.21 Rf	33.2 AU	3.06 %	0.23 Rf	18.8 AU	1238.5 AU	3.33 %
6	0.23 Rf	17.6 AU	0.24 Rf	50.3 AU	4.62 %	0.25 Rf	19.7 AU	366.5 AU	0.99 %
7	0.25 Rf	28.1 AU	0.27 Rf	34.8 AU	3.20 %	0.29 Rf	26.3 AU	793.4 AU	2.13 %
8	0.29 Rf	26.8 AU	0.31 Rf	41.0 AU	3.78 %	0.33 Rf	34.5 AU	1215.1 AU	3.27 %
9	0.33 Rf	35.1 AU	0.36 Rf	83.1 AU	7.65 %	0.39 Rf	35.8 AU	2833.2 AU	7.62 %
10	0.42 Rf	36.2 AU	0.44 Rf	60.8 AU	5.59 %	0.47 Rf	44.7 AU	2141.9 AU	5.76 %
11	0.49 Rf	50.5 AU	0.52 Rf	64.8 AU	5.97 %	0.54 Rf	61.0 AU	2311.4 AU	6.22 %
12	0.55 Rf	61.9 AU	0.58 Rf	114.4 AU	10.53 %	0.60 Rf	83.9 AU	3840.5 AU	10.33 %
13	0.65 Rf	83.7 AU	0.72 Rf	130.5 AU	12.01 %	0.73 Rf	25.7 AU	7092.3 AU	19.08 %
14	0.73 Rf	125.8 AU	0.75 Rf	142.6 AU	13.12 %	0.83 Rf	49.3 AU	9615.5 AU	25.87 %
15	0.83 Rf	49.4 AU	0.86 Rf	59.3 AU	5.45 %	0.91 Rf	26.7 AU	3037.3 AU	8.17 %
16	0.95 Rf	30.9 AU	0.97 Rf	45.6 AU	4.20 %	0.98 Rf	38.7 AU	972.1 AU	2.61 %
17	0.98 Rf	39.8 AU	0.99 Rf	42.5 AU	3.91 %	1.00 Rf	2.3 AU	557.8 AU	1.50 %

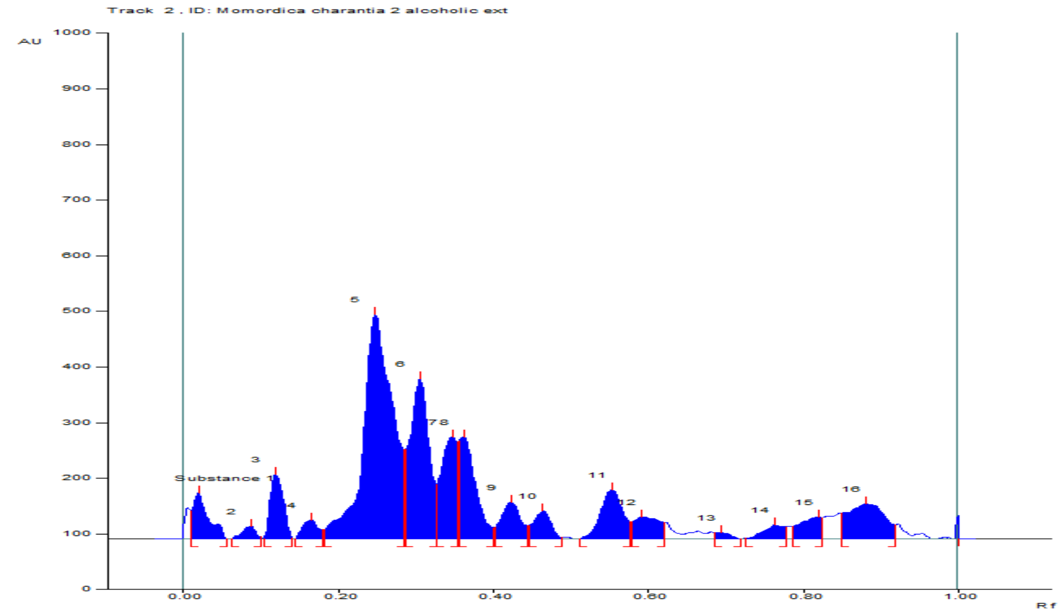


FIGURE NO. 23 HPTLC FINGER PRINT AT 520nm

TABLE NO. 20 HPTLC FINGERPRINT DATA AT 520nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	49.7 AU	0.02 Rf	82.6 AU	4.88 %	0.05 Rf	0.1 AU	1552.6 AU	3.54 %
2	0.06 Rf	0.0 AU	0.09 Rf	22.2 AU	1.31 %	0.10 Rf	3.6 AU	365.4 AU	0.83 %
3	0.10 Rf	0.3 AU	0.12 Rf	116.1 AU	6.86 %	0.14 Rf	0.8 AU	1843.8 AU	4.21 %
4	0.14 Rf	0.2 AU	0.16 Rf	33.6 AU	1.98 %	0.18 Rf	16.5 AU	656.8 AU	1.50 %
5	0.18 Rf	16.7 AU	0.25 Rf	403.3 AU	23.82 %	0.29 Rf	60.6 AU	14477.0 AU	33.05 %
6	0.29 Rf	161.1 AU	0.30 Rf	287.2 AU	16.96 %	0.33 Rf	97.0 AU	6565.0 AU	14.99 %
7	0.33 Rf	97.6 AU	0.35 Rf	183.6 AU	10.85 %	0.35 Rf	75.1 AU	3552.7 AU	8.11 %
8	0.35 Rf	176.3 AU	0.36 Rf	183.4 AU	10.84 %	0.40 Rf	20.2 AU	3746.3 AU	8.55 %
9	0.40 Rf	20.5 AU	0.42 Rf	65.6 AU	3.88 %	0.44 Rf	24.2 AU	1534.1 AU	3.50 %
10	0.45 Rf	24.3 AU	0.46 Rf	50.0 AU	2.95 %	0.49 Rf	3.0 AU	1052.1 AU	2.40 %
11	0.51 Rf	0.2 AU	0.55 Rf	87.8 AU	5.19 %	0.58 Rf	30.9 AU	2374.5 AU	5.42 %
12	0.58 Rf	31.2 AU	0.59 Rf	39.7 AU	2.35 %	0.62 Rf	29.3 AU	1261.5 AU	2.88 %
13	0.69 Rf	11.1 AU	0.69 Rf	11.8 AU	0.70 %	0.72 Rf	0.1 AU	200.9 AU	0.46 %
14	0.73 Rf	1.4 AU	0.76 Rf	24.6 AU	1.45 %	0.78 Rf	21.6 AU	656.1 AU	1.50 %
15	0.79 Rf	22.9 AU	0.82 Rf	38.9 AU	2.30 %	0.82 Rf	38.2 AU	1073.4 AU	2.45 %
16	0.85 Rf	46.3 AU	0.88 Rf	62.4 AU	3.68 %	0.92 Rf	24.4 AU	2897.6 AU	6.61 %

HPTLC was scanned at 366nm with the best solvent to detect the maximum number of components and peak abundance quantitatively. The finger print of ethanolic extract of leaves of *Momordica charantia* Linn., showed 12 peaks with different R_f values.

PHARMACOLOGICAL STUDIES

IN-VITRO ARTHRITIC ACTIVITY

PROTEIN DENATURATION METHOD

TABLE NO. 21PERCENTAGE INHIBITION OF EXTRACTS- PROTEIN DENATURATION METHOD

DRUGS	% INHIBITION AT VARIOUS CONCENTRATIONS				
	100µg/ml	200µg/ml	400µg/ml	800µg/ml	1000µg/ml
Diclofenac sodium	24.15	32.50	41.87	60.23	72.13
n- Hexane	13.54	17.84	24.45	28.27	36.33
Chloroform	4.81	9.50	18.90	26.61	35.18
Ethyl acetate	16.81	21.54	37.73	41.95	48.15
Ethanol	21.25	28.12	42.51	57.17	63.75
Aqueous	2.84	5.97	15.70	23.33	31.35

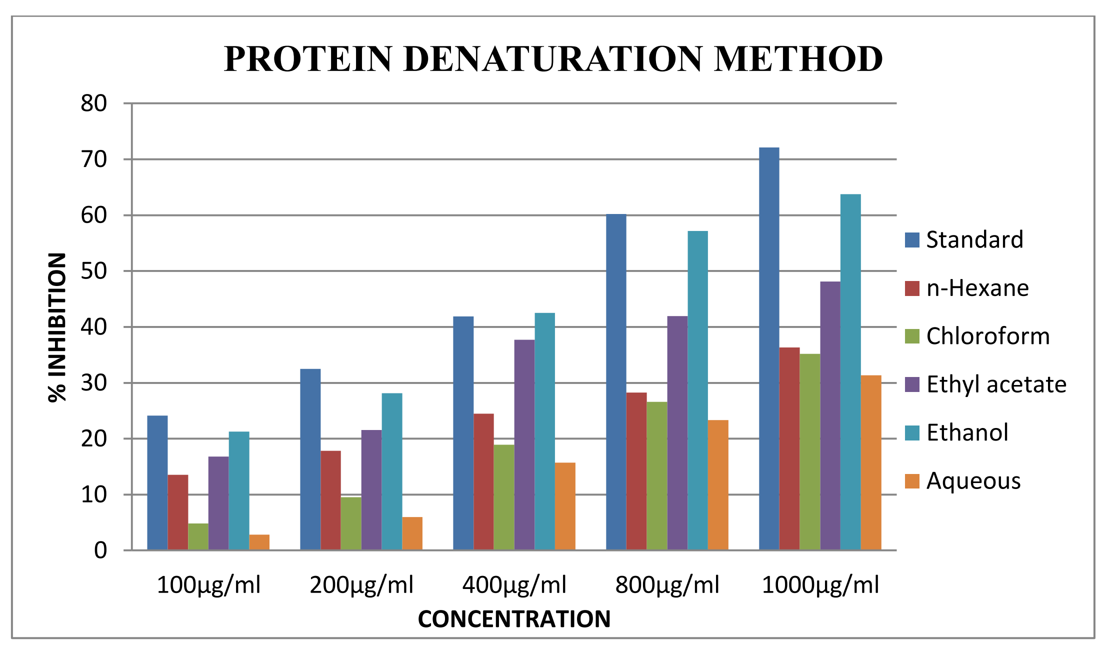


FIGURE NO. 24 GRAPHICAL DATA OF PERCENTAGE INHIBITION

The percentage inhibition of protein denaturation assay by n-Hexane, Chloroform, Ethyl acetate, Ethanol and aqueous extracts was found to be 36.33, 35.18, 48.15, 63.75 and 31.35 respectively at maximum concentration 1000µg/ml. Percentage inhibition of Diclofenac sodium (Standard) was found to be 72.13. The results indicated that ethanolic extract showed the maximum percentage inhibition which is compared with the standard.

MEMBRANE STABILIZATION METHOD

TABLE NO.22 PERCENTAGE MEMBRANE STABILIZATION OF EXTRACTS

DRUGS	% MEMBRANE STABILIZATION				
	25µg/ml	50µg/ml	100µg/ml	200µg/ml	400µg/ml
Diclofenac sodium	65.09	71.63	75.93	83.26	87.51
n- Hexane	19.65	24.53	31.76	37.19	45.02
Chloroform	18.27	23.73	25.64	34.02	38.27
Ethyl acetate	22.43	28.39	37.44	53.93	61.82
Ethanol	38.08	42.18	49.29	67.01	72.65
Aqueous	15.37	19.72	27.49	37.29	39.58

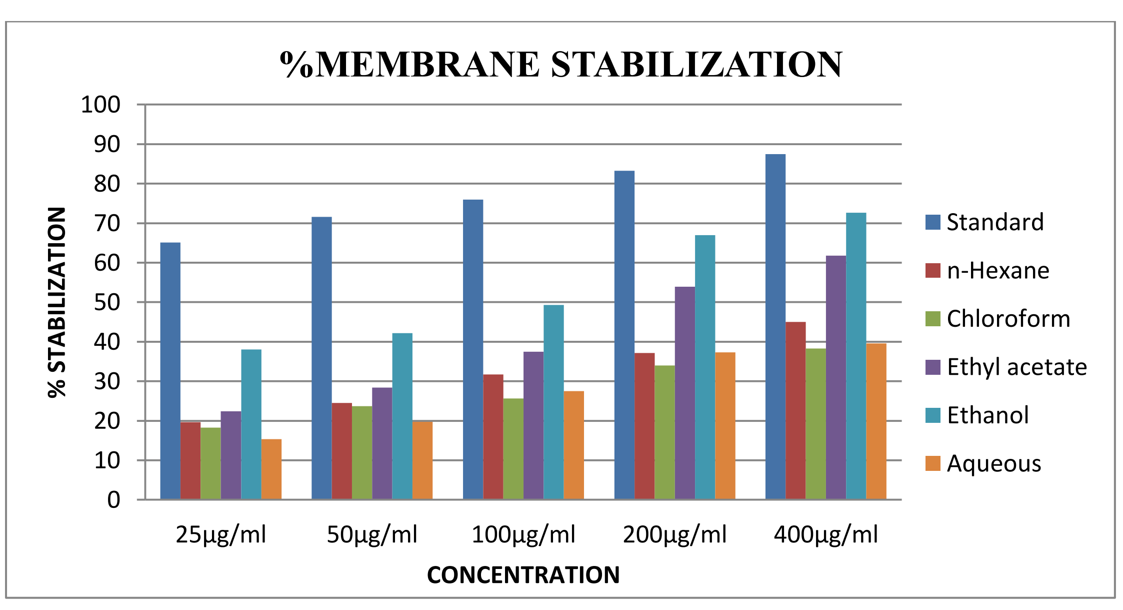


FIGURE NO.25 GRAPHICAL DATA OF MEMBRANE STABILIZATION

The percentage of membrane stabilization by n-Hexane, chloroform, ethyl acetate, ethanol and aqueous extracts was found to be 45.02, 38.27, 61.82, 72.65 and 39.58 respectively at maximum concentration of 400µg/ml. Percentage membrane stabilization of Diclofenac sodium was found to be 87.51. The results indicated that ethanolic extract showed the maximum percentage membrane stabilization which is compared with the standard.

Both inhibition of protein denaturation assay and membrane stabilization method indicated that of all the extracts, the ethanolic extract showed the maximum inhibition and stabilization activity. This correlates with the findings of the phytochemical study where the ethanol extract showed the presence of most of the phytoconstituents.

***IN-VIVO* EVALUATION OF ANTI-ARTHRITIC ACTIVITY BY ADJUVANT INDUCED ARTHRITIS (Complete Freund's Adjuvant)**

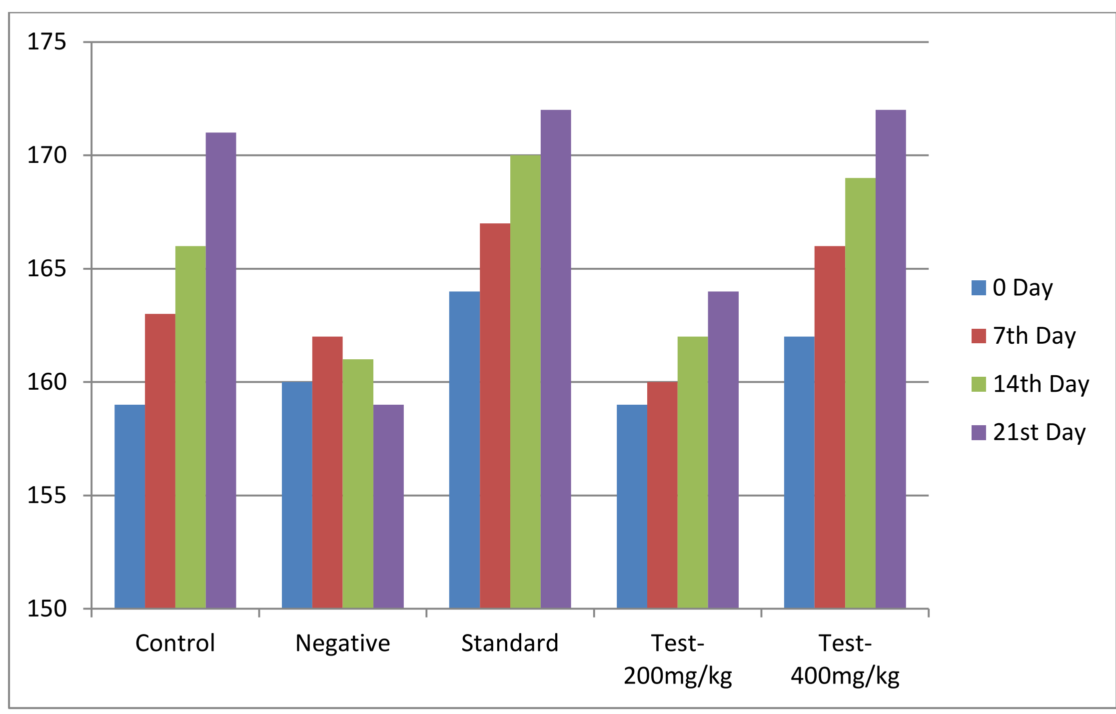
TABLE NO. 23 CHANGES IN THE BODY WEIGHT(GM) IN ADJUVANT-INDUCED ARTHRITIC RATS

TREATMENT	0 DAY	7 DAY	14 DAY	21 DAY
Group I (Normal control)	159±4.57	163±3.97	166±3.89	171±3.16
Group II (Arthritic control)	160±3.16	162±2.66	161±2.61	159±2.61
Group III (Standard)	164±4.07	167±3.58	170±3.49	174±3.44
Group IV (200mg/kg)	159±5.71	160±6.01	162±6.42	164±5.93
Group V (400mg/kg)	162±7.19	166±7.28	168±6.55	170±6.16

Values represented in the result are mean ± SD (n=6)

$p < 0.001$ as compared to the positive and arthritic control. The data was analysed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

FIGURE NO.26 GRAPHICAL DATA OF CHANGE IN BODY WEIGHT – ADJUVANT INDUCED ARTHRITIC RATS



The body weight of the different groups of rats was noted. During the treatment the body weight of the control, standard, test dose I and test dose II animals were increased except arthritis induced rats.

PAW VOLUME MEASUREMENT

TABLE NO. 24 MEASUREMENT OF PAW VOLUME USING PLETHYSMOGRAPH IN ADJUVANT INDUCED ARTHRITIC RATS

TREATMENT	0 DAY	7 DAY	14 DAY	21 DAY
Group I (Normal control)	5.49±0.07	5.70±0.06	5.87±0.10	6.03±0.10
Group II (Arthritic control)	5.84±0.12	22.02±1.01	22.84±1.20	24.40±0.56
Group III (Standard)	5.52±0.34	19.45±1.55	15.52±1.93	8.99±0.51
Group IV (Low dose)	5.54±0.29	20.97±1.69	17.84±0.77	15.32±1.50
Group V (High dose)	5.68±0.35	20.45±1.64	16.30±1.41	12.81±1.22

Values are expressed as mean ± SD; n=6; p<0.001

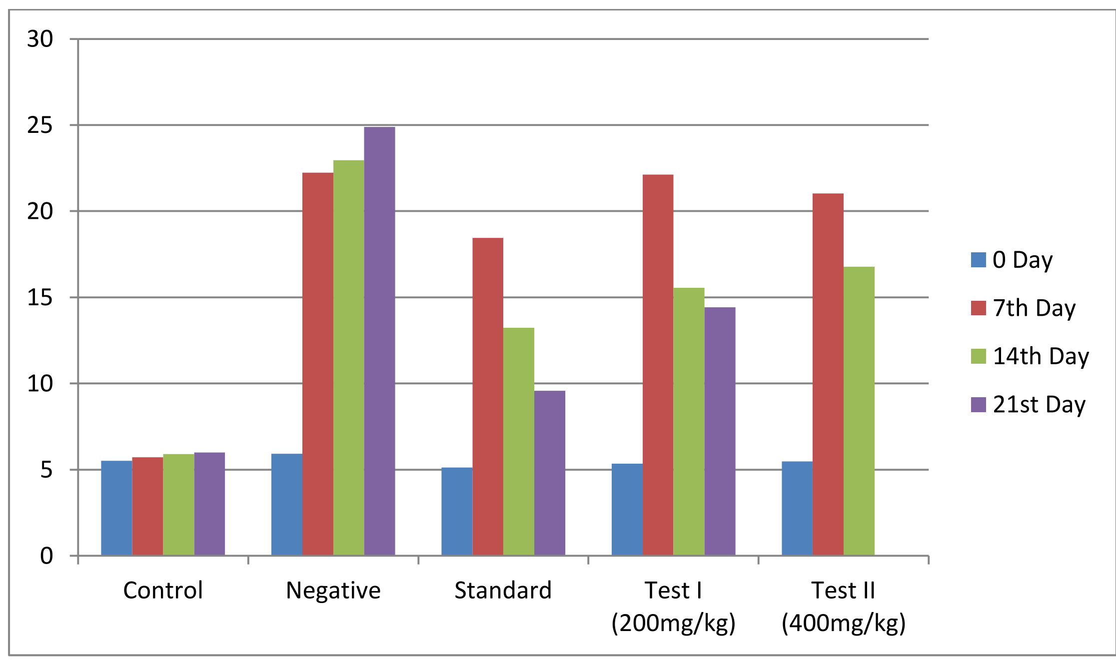


FIGURE NO.27 PAW VOLUME MEASUREMENT – ADJUVANT INDUCED ARTHRITIC RATS

TABLE NO. 25 PERCENTAGE INHIBITION OF PAW VOLUME IN ADJUVANT INDUCED ARTHRITIC RATS

TREATMENT	7th DAY	14th DAY	21st DAY
Group III (Diclofenac sodium)	38.51	63.25	84.45
Group IV (200mg/kg)	31.90	52.45	58.98
Group V (400mg/kg)	35.19	62.8	79.27

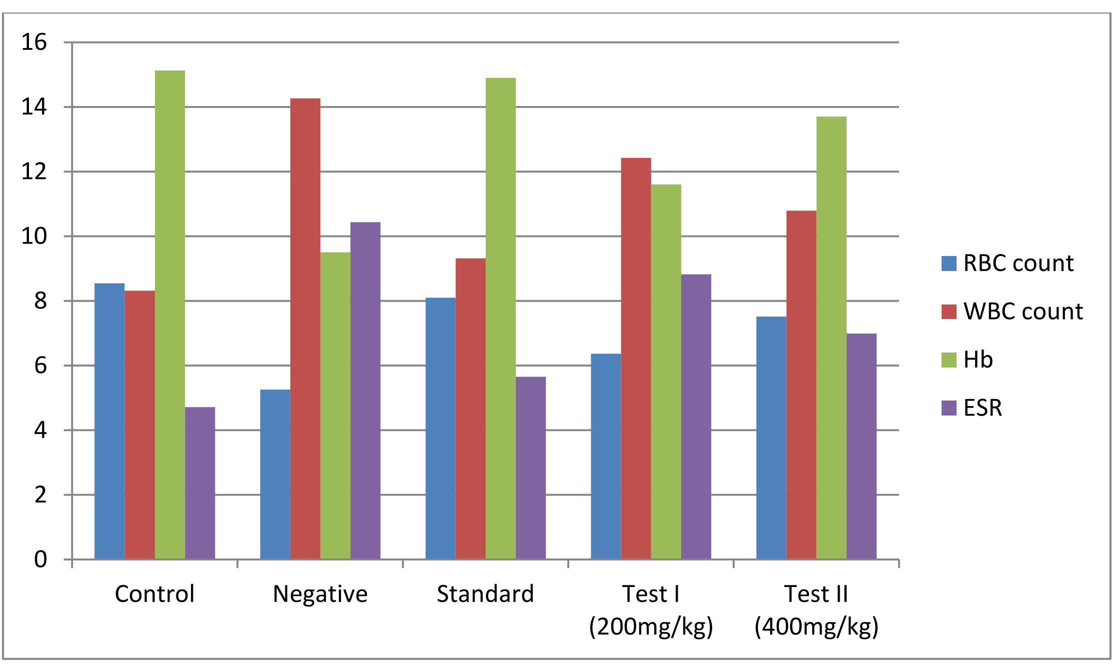
The percentage inhibition of paw volume of all the groups were found. The standard group, test dose 200mg/kg and test dose 400mg/kg were studied for the different intervals of time. The standard drug showed maximum inhibitory action when compared to test drug. Test dose 400mg/kg showed better percentage inhibition than test dose 200mg.kg. It indicates that high dose (400mg/kg) showed significant arthritic effect on rats.

TABLE NO. 26 EFFECT OF HEMATOLOGICAL PARAMETERS – ADJUVANT INDUCED ARTHRITIC RATS

TREATMENT	RBC count (cells/cu.mm)	Total WBC count (cells/cu.mm)	Hb (gm%)	ESR (mm/hr)
Group I (Normal control)	8.54±0.20	8.31±0.29	15.12±0.17	4.71±0.38
Group II (Disease control)	5.26±0.26	14.26±0.34	9.5±0.24	10.43±0.44
Group III (Standard)	8.10±0.29	9.31±0.39	14.9±0.47	5.95±0.51
Group IV (200mg/kg)	6.37±0.13	12.42±0.44	11.6±0.28	8.82±0.56
Group V (400mg/kg)	7.51±0.25	10.79±0.48	13.7±0.42	6.99±0.69

$p < 0.01$, $p<0.001$ as compared to the positive and arthritic control. The data was analysed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

FIGURE NO. 28 GRAPHICAL DATA FOR EFFECT OF HEAMOTOLOGICAL PARAMETERS- ADJUVANT INDUCED ARTHRITIC RATS



The result indicated that the arthritic group of animals showed decreased RBC, and hemoglobin levels, and WBC, ESR level showed the increased values which was compared with control group. In standard group all the blood parameters were brought back to normal levels. In test I (200mg/kg) group showed improvement of blood parameters and test II (400mg/kg) group showed significant improvement in blood parameters than the test I group.

HISTOPATHOLOGICAL STUDIES

The disease severity and the effect of ethanolic extract of *Momordicacharantia*Linn., at various concentration (200 and 400mg/kg) were illustrated by photomicrographs of sections stained with Haemtoxylin and Eosin.

No inflammation, cartilage erosion and pannus formation were shown in section from normal rats. In contrast, the disease control group was marked extensive inflammation, cellular infiltration and pannus formation with resultant erosion of articular cartilage. Test drug I treated rats exhibited moderate cartilage degradation and mild pannus formation, and test drug II ameliorated joint destruction.

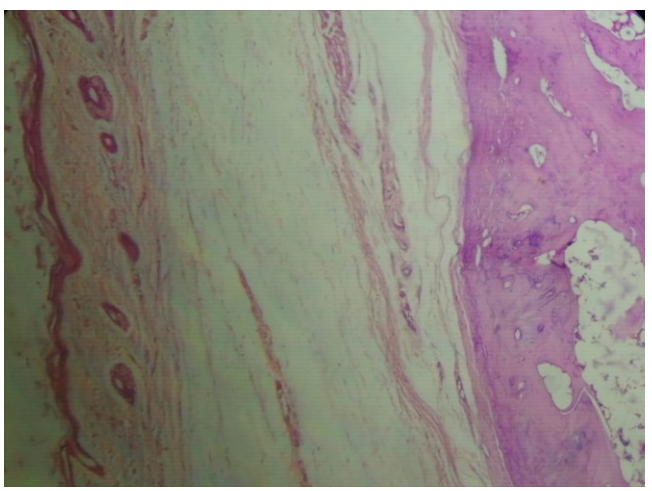


FIGURE NO.29CONTROL GROUP

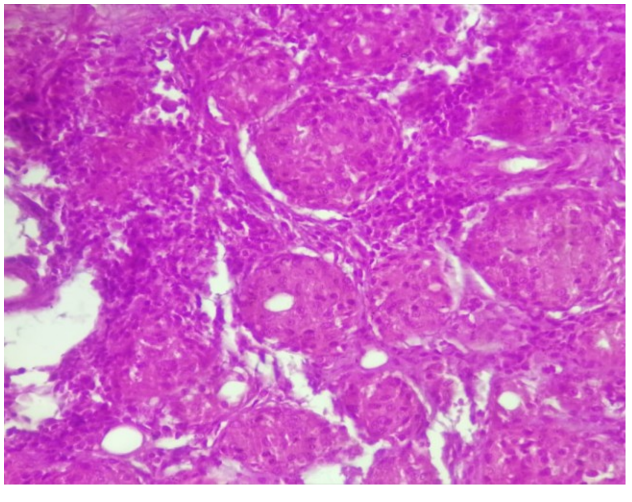


FIGURE NO.30DISEASE CONTROL

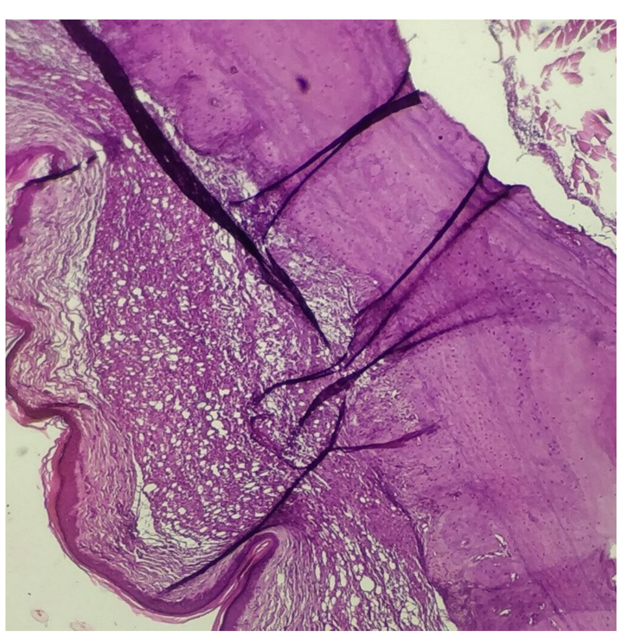


FIGURE NO. 31 STANDARD GROUP

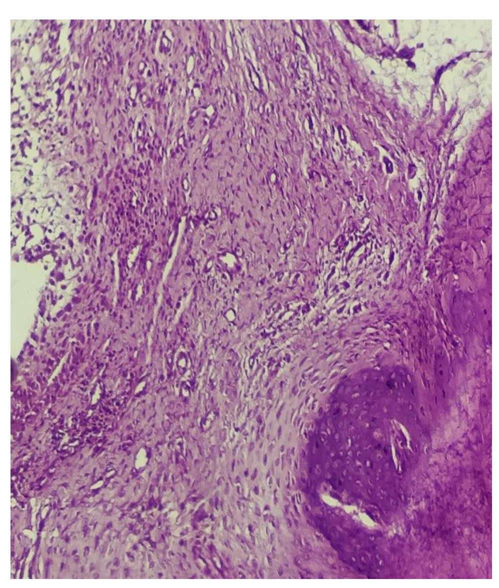


FIG NO. 32TEST DRUG I GROUP

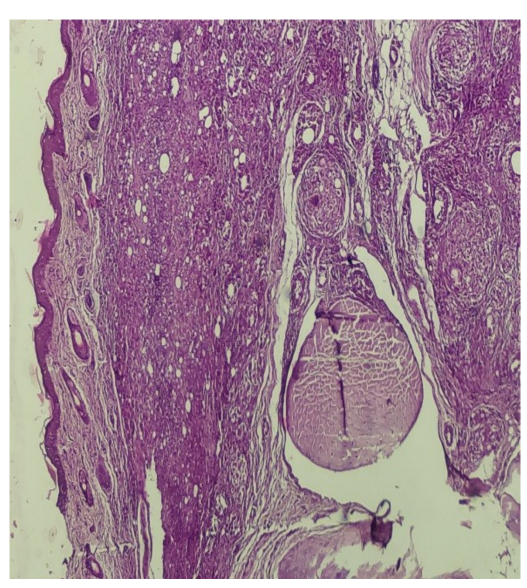


FIG NO. 33TEST DRUG II GROUP

SUMMARY AND CONCLUSION

PHARMACOGNOSTICAL STUDIES

The pharmacognostical studies on the leaves of *Momordica charantia* Linn., was carried out, which showed the unique features of the leaves which is used to differentiate it from other species.

Macroscopical studies states the characteristic features of the leaves like green colour, intensely bitter in taste and had characteristic odour. The leaves are simple and alternate, smooth and thick.

Anatomical studies of leaves showed the presence of epidermis, palisade parenchyma, spongy parenchyma and vascular bundles.

Powder analysis of the leaves showed the presence of non glandular trichomes, anomocytic stomata, idioblasts, fibres and palisade cells.

Linear measurement for trichomes and **leaf constant** of stomatal number, stomatal index, vein islet number and vein termination number were evaluated for the leaves of *Momordica charantia* Linn.,

Physiochemical studies: Various physiochemical constants were evaluated such as ash values, extractive values, loss on drying, foaming index and swelling index.

These pharmacognostical parameters evaluated are useful for the establishment of standards of leaves which essential for its identity and purity.

PHYTOCHEMICAL STUDIES

In phytochemical study, the powdered leaves is successively extracted with n-hexane, chloroform, ethyl acetate, ethanol and aqueous by using soxhlet apparatus.

Preliminary phytochemical studies was done for the powdered leaves and all the extracts. It was found to contain flavonoids, saponins, alkaloids, triterpenoids, phenolic compounds, steroids, etc.,

Quantitative estimation of the phytoconstituents was carried out for phenolic compounds, flavonoids and saponins.

Fluorescence analysis was done to find out characteristic fluorescent substance present in the powdered leaves and all the extracts, and no fluorescent substance was found.

IR spectrum analysis was carried out for identify the functional groups present in ethanolic extract of leaves of *Momordica charantia* Linn.,

TLC analysis of leaves extract and **HPTLC analysis** of ethanolic extracts were carried out to identify phytoconstituents present.

PHARMACOLOGICAL STUDIES

Invitro studies

All the extracts were subjected to *invitro* anti arthritic activity to select the most bioactive extract. Based on this ethanolic extract showed maximum inhibition of protein denaturation and membrane stabilization activity. Hence the ethanolic extract was selected for the *invivo* studies.

Invivo studies

Arthritic activity was assessed by method of Complete Freund's adjuvant induced arthritis. The ethanolic extract of dose 200 and 400mg/kg were given orally to the rats, it is compared with standard Diclofenac sodium.

The parameters studied were rat paw swelling, body weight changes, hematological parameters and histopathological studies. The ethanolic extract at the dose 400mg/kg showed significant anti-arthritic effect on the arthritis induced rats which was comparable with the standard.

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Introduction

Review of Literature

Plant Profile

Rationale for Selection

Aim and Objective

Plan of Work

Materials and Methods

Result and Discussion

Summary and Conclusion

References

Annexure

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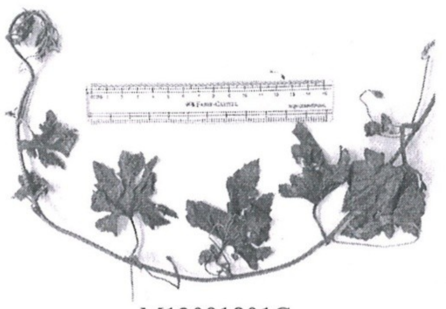
27.09.18

AUTHENTICATION CERTIFICATE FOR 97.M12091801C

Certified that the sample submitted by Megala. S, M.Pharm Final year, Dept of Pharmacognosy, Madras Medical College, Chennai-03 is identified as

SN	Botanical/Chemical Name	Part	Code
1.	*Momordica charantia* L.	Leaf	M12091801C



M12091801C

Sunil Kumar KN 27/9/18

Dr. K.N. Sunil Kumar
Research Officer and HOD Pharmacognosy

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Madras Medical College,Chennai-600 003
Institutional Animal Ethics Committee
Proceedings
Present: Dr.Sudha Seshayyan, M.B.B.S, M.S (Anatomy)
Roc . No. 24 /AEL/IAEC/MMC,Date: 16.11.2018
Sub: MMC-IAEC approval-regarding.
Ref: IAEC meeting held on 09.11.2018

The following order is issued based on the approval of the Institutional Animal Ethics Committee held on 09.11.2018.

Project ID	24/18
CPCSEA registration number	1917/ReBi/S/16/CPCSEA/25.10.2016
Name of the Researcher	S.MEGALA, M.Pharm II year, Department of Pharmacognosy.
Name of the Guide	Dr.R.Radha,M.Pharm,Ph.D.,
Title of the Project	Pharmacognostical , Phytochemical studies and evaluation of Anti-arthritic activity of leaves of *Momordica charantia*.
Date of submission of proposal to IAEC	07.08.2018
Date on which IAEC conducted	09.11.2018
Date of submission of modified proposal (if applicable)	09.11.2018
Date on which approved	09.11.2018
Validity of the approved proposal	1 year
Remarks : **Wistar albino rats of either sex -24** numbers approved	

Chairperson
Institutional Animal Ethics Committee
Madras Medical College
Chennai-03

To
Dr.R.Radha,M.Pharm,Ph.D.,
Head of the Department, Dept. of Pharmacognosy,
College of Pharmacy,
MMC,Chennai-03.

Copy to
Special Veterinary Officer, Animal Experimental Laboratory,
Madras Medical College,Chennai-03.

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Kattankulathur-603203, Kancheepuram District, Tamilnadu, India

(Recognized by Pharmacy Council of India)

Certificate

This is to Certify that Dr. / Mr. / Ms. S MEGALA *has Participated as a Resource Person / Delegate / Organizing Member in the one day seminar on* ***"CLINICAL RESEARCH PROTOCOL DEVELOPMENT ON HERBAL MEDICINES"*** *organized by Department of Pharmacognosy, SRM College of Pharmacy, SRM Institute of Science & Technology, held on 2nd February, 2018.*

Dr. P.R. Kumar
Organizing Secretary

Dr. K.S. Lakshmi
Convener

Dr. N. Chandraprabha
Director, Medical & Health Sciences

Accreditated by The Tamilnadu Dr.M.G.R. Medical University, Chennai with 5 CREDIT POINTS

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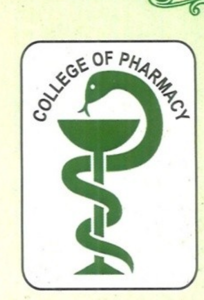

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This is to certify that Mr. / Ms. MEGALA . S ,

a student of I M.PHARM *, College of Pharmacy, Madras Medical College, Chennai has attended the* **"Lecture series-2018"** *conducted by the College of Pharmacy, Madras Medical College, Chennai - 03 on 11.01.2018.*

Dr. N. Jayshree, M.Pharm, Ph.D.,
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This is to certify that Dr./Mr./Ms. Megala S.

has participated as a ~~Resource person~~ / ~~Oral~~ / ~~e poster Presenter~~ / Delegate in the National Conference on ***"Current Perspectives in Herbal Drug Regulations - Global Scenario"*** *held on 28th - 29th June 2017, organized by Sri Ramachandra University, Porur, Chennai.*

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This is to certify that Dr./Mr./Ms. Megala S.

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K.V. Somasundaram

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Prof./Dr./Mr./Ms. ....MEGALA .S..........

has participated as Delegate / Volunteer

in the 69th Indian Pharmaceutical Congress

held at Chitkara University, Rajpura from December 22nd to 24th, 2017.

Dr. Mahesh Burande	**Dr. Shailendra Saraf**	**Dr. Dhirender Kaushik**	**Dr. Ashish Baldi**
President - IPCA	Chairman - LOC	Organizing Secretary	Chairman, Registration Committee - LOC

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REVIEW ON *MOMORDICA CHARANTIA* LINN.

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ABSTRACT

Momordica charantia Linn., is also known as bitter gourd and bitter melon belonging to Cucurbitaceae family. This plant cultivated throughout the India as vegetable crop and used in folk medicine. Plant has a important role as source of carbohydrates, fats, proteins, minerals, vitamins and the leaves are nutritious source of calcium, magnesium, potassium, phosphorus and iron. The fruits and leaves of this plant contain a variety of biologically active compounds such as alkaloids, glycosides, saponin, flavonoids, phenolic compounds and tannins. In traditional medicine, it used as antidiabetic, anticancer, anthelmintic, antimalarial, analgesic, antipyretic, antifertility and antimicrobial. This article aims to provide a comprehensive review on pharmacological aspects of *Momordica charantia*.

KEYWORDS: *Momordica charantia*, bitter gourd, vitamins, flavonoids, antidiabetic, antimalarial.

INTRODUCTION[4,6,19,24]

The plant *Momordica charantia* L., family Cucurbitaceae, is also known as bitter gourd, bitter melon, balsam pear, bitter cucumber and African cucumber. The word *Momordica* is derived form the Latin word *Mordeo* which means to bite and the species name is derived from Greek word and it means beautiful flower. This is a monoecious climber found throughout India upto an altidude of 1500 m. The plant is cultivated in India as vegetable crop, it also grown as ornamental and is used extensively in folk medicine. Two types are commonly found, “Jethua” which comes in a hot summer, and “Barmasiya” which bears fruits throughout the year. *Momordica charantia*, known in India as karela is used in the form