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## STUDY THE EVALUATION AND STANDARIZATION PARAMETERS OF CITRULLUS LANATUS

Rishi Chandrashekhar. Lodh<sup>1</sup>, Miss.Madhuri B.Wankhade<sup>2</sup>, Prof. Sawti P.Deshmukh<sup>3</sup>

Shraddha Institute of Pharmacy, Washim(MS) INDIA 444505

### Abstract

Watermelon (*Citrullus lantanas*) is a nourishing and popular fruit in the world. Watermelon is grown in favourable climates from tropical to temperate regions worldwide belong to the family Cucurbitaceae. Watermelon contains phytochemicals such as polyphenols, flavonoid, stilbenes/lignans, and is rich source of carotenoids, lycopene. Watermelon contains cucurbitacin E which is anti-inflammatory phytonutrient and amino acids such as L-arginine and citrulline. It is the major source of vitamin C and vitamin A. In the nutritional composition of watermelon includes carbohydrates, sugar, soluble and insoluble fibres. Moreover, is contains good amount of potassium and magnesium. Watermelon by-products which can be produced such as juice, smoothies, dips, seed oils, medicines, ethanol etc., consumption of watermelons imparts various health benefits such as chemical components of watermelon enhance its capacity to scavenge the low-density lipoprotein (LDL) and high-density lipoprotein (HDL) in a cell membrane. A plethora of evidence shows that it can be effective for weight loss. Several epidemiological studies showed it has phytochemicals that can reduce risk of Cardio Vascular Disease (CVD) aging related ailments, obesity, diabetes, ulcers, and various types of cancers. Watermelon pomace is reported to be a concentrated source of lycopene as compared to the juice. This review shows up to date information on nutritional and Phytochemical constituents of watermelon, its wastage and by products and different methods used for dehydration, extraction and analysis of its chemical constituents. *Citrullus lanatus* has been reportedly used widely in traditional herbal medicine. The leaves of *Citrullus lanatus* is analgesic, anti-inflammatory, mosquitocidal, gonorrhoea and anti-microbial property. The fruits of *Citrullus lanatus* are eaten as a febrifuge when fully ripe or even when almost putrid. The fruit is used as a diuretic, anti-cancer, for treatment of high BP, antiviral and is effective in the treatment of dropsy and renal stones. The seed is also a good vermifuge and has a hypotensive action. It is demulcent, pectoral and tonic. It is sometimes used in the treatment of the urinary tract infections as well as bed wetting. The root is purgative and in high dose it can also serve as emetic. Fatty oil in the seed, as well as aqueous or alcoholic extracts, had been reported to paralyze tapeworms and roundworms. The seed oil has an anthelmintic action which is better than that of pumpkin seed oil.<sup>[1]</sup>

### Introduction

## **Medicinal Plants**

A plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for semi-synthetic compounds. When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes.

### **History of medicinal plants**

Plants have been used for medicinal purposes from 5000 BC with the emergence of the Indus Valley Civilization. The indigenous system of medicine, viz.-Ayurvedic, Siddha and Unani, have been in existence for several centuries. The country has 45,000 different plant species and 15000 medicinal plants that include 2000 plants used in Ayurveda, 700 in Unani, 600 in Siddha, 450 in Homoeopathy and 30 in modern medicines. The drugs are derived either from the whole plant or from different parts like leaves, stem, bark, root, flower, seed etc. Some drugs are prepared from excretory plant product such as gum, resins and latex.<sup>[2]</sup>

### **Significance of medicinal plants to human beings.**

- (1) Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- (2) Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- (3) Many food crops have medicinal effects, for example garlic.
- (4) Medicinal plants are resources of new drugs. It is estimated there are more than 250,000 flower plant species.

Hence studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants.<sup>[3]</sup>

### **Medicinal plants in India**

About 60 percent of the world's population use herbal medicines. Herbal medicines are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used.

There are about 45,000 medicinal plant species in India, concentrated in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000 plants. India is the largest producer of medicinal herbs and is called the botanical garden of the world.

Ayurveda and Kabirizi (herbal medicine) are two important forms of alternative medicine that is widely available in India. Ayurveda form of medicine is believed to be existent in India for thousands of years.

The codified traditions have about 25,000 plant drug formulations that have emerged from such studies. In addition to this, over 50,000 formulations are believed to be available in the folk and tribal traditions. All these point to the deep passion for an exhaustive knowledge about medicinal plants that have existed in this land from time immemorial.<sup>[4]</sup>

### **Importance of Medicinal Plants**

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs.

Integrating the use of Traditional medicine (TM) in the treatment of incurable disease such as AIDS to boost immunity is wiser than waiting for the immune system to weaken to begin antiviral therapy as is the common practice, especially when evidence exists that 11 of the anti-infective herbs in Chinese TM have shown to be anti-HIV.

According to the WHO, 25% of modern medicines are made from plants first used traditionally. One recent example is the use of Artemisinin based drugs for treating malaria due to the malaria parasite exhibiting drug resistance to previously prescribed drug therapies. Traditional Chinese medicine has been used to effectively treat malaria with cultivated Artemisia plants for over 2500 years.

In South Africa, the medical research council is conducting studies on the efficacy of the plant Sutherland microphyll in treating AIDS patients. Traditionally used as a tonic, this plant may increase energy, appetite and body mass in people living with HIV.

Diabetes mellitus is another area where a lot of research is going on. Ajuga reptans (the active principle is said to potentiate effects of insulin), Galagea officinalis (galagine), Bougainvillea spectabilis (pinitol), Momordica charantia (chirantin), Gymnema sylvestre (gymnemic acid) are some medicinal herbs that have shown effectiveness in non-insulin dependent diabetes. Recently extract of Tecoma stans has shown potent anti diabetic activity.

Alkaloid tecomonine is considered to be active principle of the herb.

Arthritis is another potential disease where no satisfactory answer is present in modern medicine. Commiphora mukul (guggulsterones), Boswellia serrata (boswellic acid), Withania somnifera (withanolides), Ruscus aculeatus (ruscogenin) are prominent plants with anti-arthritic activity.

Croton sublyratus (plaunoyol) has potent and wide spectrum anti peptic ulcer action.

Ancistrocladus korupensis (michellamine-b), Caulophyllum langigerum (calanolide-A), Caulophyllum teymani (costatolide-A), Homalanthus natans (prostratin) are the medicinal herbs from African countries that are being employed in research for finding a suitable cure for Aids.<sup>[5]</sup>

### **CITRULLUS LANATUS (Watermelon)**

Citrullus lanatus (water melon) produces a fruit that is about 93% water, hence the name “water” melon. The “melon” part came from the fact that the fruit is large and round and has a sweet, pulpy flesh. The scientific name of the watermelon is derived from both Greek and Latin roots. The Citrullus part comes from a Greek word “citrus” which is a reference to the fruit. The lanatus part is Latin, and has the meaning of being woolly, referring to the small hairs on the stems and leaves of the plant (Baker, et al., 2012). Watermelon is thought to have originated in southern Africa because it is found growing wild throughout the area, and reaches maximum diversity of forms there. It has been cultivated in Africa for over 4,000 years. Citrullus lanatus was brought to America by Spanish and quickly became very popular crop (Robinson and Decker, 1997).

- Citrullus lanatus (Water melon) has been reportedly used widely in traditional herbal medicine. The fruit is used as a febrifuge, diuretic, purgative and used in treatment of diarrhoea, gonorrhoea, dropsy and renal stones.
- The leaves of Citrullus lanatus is analgesic, anti-inflammatory, mosquitocidal, gonorrhoea and anti-microbial property.

- The fruit is also diuretic, anti-cancer, high BP, antiviral and is effective in the treatment of dropsy and renal stones. The seed is also a good vermifuge and has a hypotensive action. Preliminary research indicates that the consumption of watermelon may have antihypertensive effects.
- The root is purgative and in high dose it can also serve as emetic.
- The seed is a good vermifuge and has a hypotensive action. It is a demulcent and used in the treatment of the urinary tract infections as well as bed wetting.
- Fatty oil in the seed, as well as aqueous or alcoholic extracts, had been reported to paralyze tapeworms and roundworms.
- The rind of the fruit is prescribed in cases of alcoholic poisoning and
- The plant *Citrullus lanatus* has been selected (specially the leaves) for the present investigation on the basis of the ethnomedical information and the review of literature as the plant is widely cultivated throughout India.<sup>[6]</sup>

## **AIM AND SCOPE OF THE PRESENT STUDY**

The use of herbal products is global importance because of their low side effects, accessibility and affordability when compared with conventional medicine. *Citrullus lanatus* (watermelon) is popular in indigenous system of folk medicine and it is known to contain bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids, vitamins, minerals.

*Citrullus lanatus* has been reportedly used widely in traditional herbal medicine. The leaves of *Citrullus lanatus* is analgesic, anti-inflammatory, mosquitocidal, gonorrhoea and anti microbial property. The fruits of *Citrullus lanatus* are eaten as a febrifuge when fully ripe or even when almost putrid. The fruit is used as a diuretic, anti-cancer, for treatment of high BP, antiviral and is effective in the treatment of dropsy and renal stones. The seed is also a good vermifuge and has a hypotensive action. It is demulcent, pectoral and tonic. It is sometimes used in the treatment of the urinary tract infections as well as bed wetting. The root is purgative and in high dose it can also serve as emetic. Fatty oil in the seed, as well as aqueous or alcoholic extracts, had been reported to paralyze tapeworms and roundworms. The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes. *Citrullus lanatus* used in Northern Sudan for burns, swellings, rheumatism, gout and as laxative.

The biological activities reviewed include antimicrobial, antioxidant, anti-plasmodial, anti-inflammatory, anti-prostatic hyperplasia activity, antiviral activity, anti-oxidant, analgesic properties, its effects on the histology of the kidney of adult Wistar rats, antisecretory, antidiabetic, laxative, antiulcerogenic and hepatoprotective activities. In view of its wide pharmacological and biological activities, its traditionally reported therapeutic potential such as, antihypertensive, anti diarrhoeal, as well as its in-depth toxicity studies, among others, are yet to be experimented.

The species of *Citrullus* such as *Citrullus colocynthis* have already reported anti- cancer (breast cancer) activity.

Based on the ethnomedical information and studies available, the present research work has been framed to carry out the following studies on the leaves of *Citrullus lanatus*.

### **1. Pharmacogenetic Evaluation**

- Macroscopical evaluation and Microscopical Evaluation.
  - Microscopical evaluation
  - Standardization parameters
  - Quantitative Analytical parameters.
  - Powder Microscopy and Fluorescence analysis of powder and extracts.
- ### **Phytochemical Evaluation**
- Preliminary phytochemical screening.
  - Quantitative estimation of some secondary metabolites presents in the plant.
  - TLC and HPTLC finger print analysis.<sup>[7]</sup>

## PLANT PROFIL

### DESCRIPTION

It is an annual climbing or trailing herb, with hairy stem up to 10m long. Tendrils divided at the tip into two or three parts. Separate male and female flowers are borne on the same plant.

**Botanical Source:** *Citrullus lanatus* (Thunb). Matsum. & Nakai

**Family:** Cucurbitaceae

**Synonyms:** *Citrullus vulgaris* Schrad., *Colocynthis Citrullus* Linn., *Citrullus Citrullus* (L.),

*Cucubertia citrullus* L., *Anguria citrullus* Mill., *Momordica lanata* Thunb.

**Common Names:** Watermelon, wild watermelon, sweet melon (English); Egusi melon(English, Kenya); pastèque, melon d'eau (French).

### Vernacular Names

**Malaysia:** Tembikai

**English:** Watermelon

**India:** Karingda

**Chinese:** Da zi gua zi xi gua.

**Tamil:** Pitcha.

**Sanskrit:** Tarambuja.

**Hindi:** Tarbuj

### General

**Symbol:** CILAL

**Group:** Dicot

**Family:** Cucurbitaceae

**Duration:** Annual Growth

**Habit:** Vine Forb/ herb

### GEOGRAPHY & DISTRIBUTION:

*Citrullus lanatus* is thought to be native to Africa. It is found in grassland and bushland, mostly on sandy soils, and often along watercourses or near water, up to 1,785 m above sea level. It flourishes in dry climates and requires only limited rainfall. Some say that the Kalahari region (Botswana, Namibia and South Africa) as the area of origin, whereas others suggest it is native to north eastern Africa.

**HABITAT:** Grassland and bushland, often along watercourses.

### Classification:

**Kingdom:** Plantae - Plants

**Subkingdom:** Tracheobionta - Vascular plants

**Superdivision:** Spermatophyta - Seed plants

**Division:** Magnoliophyta - Flowering plants

**Class:** Magnoliopsida – Dicotyledons

**Subclass:** Dilleniidae

**Order:** Violales

**Family:** Cucurbitaceae<sup>[8]</sup>

### **ETHNOMEDICAL USES**

- (1) Citrullus lanatus has been reportedly used widely in traditional herbal medicine. The fruits of Citrullus lanatus are eaten as a febrifuge when fully ripe or even when almost putrid. The fruit is also diuretic and is effective in the treatment of dropsy adrenal stones.
- (2) The root is purgative and in high dose it can also serve as emetic.
- (3) The seed is demulcent, pectoral and tonic. It is sometimes used in the treatment of the urinary tract infections as well as bed wetting. The seed is also a good vermifuge and has a hypotensive action.
- (4) Preliminary research indicates that the consumption of watermelon may have antihypertensive effects
- (5) Fatty oil in the seed, as well as aqueous or alcoholic extracts have been reported to paralyze tapeworms and roundworms.
- (6) The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes
- (7) Citrullus lanatus is used in Northern Sudan for burns, swellings, rheumatism, gout and as laxative.
- (8) The fruits are used as a drastic purgative in Senegal; they are also used to treat diarrhoea and gonorrhoea in Nigeria.
- (9) Tar is extracted from the seeds and used for the treatment of scabies and for skin tanning. The seed oil has an anthelmintic action which is better than that of pumpkin seed oil.<sup>[9]</sup>

### **MATERIALS AND METHODS**

#### **MACROSCOPICAL STUDIES**

Macroscopical studies include aspects of the outward appearance (shape, structure, colour and pattern) as well as the form and structure of the internal parts like cells etc. Some of these gross morphological characters of drugs such as shape, size, margin, apex and venation are identification features of drugs. these features give valuable information about the drugs.

#### **Collection of plant material**

The leaves of Citrullus lanatus were collected and the macroscopical characters like shape, structure, colour and pattern were studied. The photographic representations of the macroscopic features are presented.

#### **MICROSCOPICAL STUDIES**

Microscopical evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, and in detection of hydroxide solution, glacial acetic acid etc. On a clean watch for the identification of secondary metabolites. The colours Obtained with various reagent presented.<sup>[10]</sup>

## **Procedure**

### A) Reaction of chemicals with powdered crude drugs:

The raw leaf powder of *Citrullus lanatus* was treated with different chemical reagent such as iodine solution, 10% potassium hydroxide solution, glacial acetic acid etc. on a clean watch glass for the identification of secondary metabolites. The colours obtained with various reagents are presented.

### B) Fluorescence analysis:

The fluorescence nature of powder drugs was analysed to find out whether any fluorescent compound was present in the sample and the observations with different chemicals were also carried out and recorded. The air-dried plant materials of both plants were taken in clean watch glass and subjected to different chemicals such as acids, alkalis and some reagents are observed under day light and UV light. Detailed fluorescence behavioural crude drug powder has been shown in.

### C) Powder microscopy:

The dried leaf was powdered and the powder was passed through sieve no.60 for the study of powder microscopy. Chloral hydrate, water, iodine, phloroglucinol and hydrochloric acid (1:1) etc.<sup>[11]</sup>

## **MACROSCOPICAL EVALUATION**

Macroscopical characters of *Citrullus lanatus* (Thunb.) Matsum. & Nakai

Whole plant: *Citrullus lanatus* is an annual climbing or trailing herb, with hairy stem up to 10 m long. Tendrils divided at the tip into two or three parts. Separate male and female flowers are borne on the same plant.

Leaves: Leaf blades up to about 20 × 20 cm, more or less hairy, usually deeply 3–5-lobed, the central lobe being the largest. The lobes themselves are further divided. Leaf stalks (petioles) up to about 19 cm long, more or less hairy.

Flowers: Solitary, borne in leaf axils. Both male and female flowers are yellow, up to 3 cm in diameter, and borne on pedicels (flower stalks) up to 45 mm long. Flowers are usually pollinated by honey bees.

Fruits: Fruits of wild plants up to about 20 cm in diameter, greenish mottled with darker green. Fruits of cultivated plants up to about 70 × 30 cm, rounded, oval or oblong, with a golden- yellow to dark green skin, the skin being uniform, mottled or striped. Flesh usually red or yellow, sometimes orange, pink or white.

Seeds: Flat, smooth, variable in size and colour (white, tan, brown, black, red, green or mottled).<sup>[12]</sup>

## MICROSCOPICAL EVALUATION

**Anatomy of the leaf:** The leaf has very thick abaxially hanging midrib and thin lamina. The midrib is 1.9mm thick and 1.7mm wide. It has four thick ridges alternatively deep furrows. The adaxial part of the midrib has short, tick cone.



## CHROMATOGRAPHY

Chromatography is a non-destructive procedure for resolving a complex mixture into its individual fractions or compounds. "Chromato" "graphy" derives its name from two words as chromo= colour and graphy= writing. i.e., colour bands are formed in the procedure which are measured or analysed. It is defined as the process of separation of the individual components of a mixture based on their relative affinities towards stationary and mobile phases. These two phases can be solid-liquid, liquid-liquid or gas-liquid.

### **Principle**

The samples are subjected to flow by mobile liquid onto or through the stable stationary phase. The sample components are separated into fractions based on their relative affinity towards the two phases during their travel. The fraction with greater affinity to stationary layer travels slower and shorter distance while that with less affinity travels faster and longer. Overall available chromatography techniques for regular analysis

- a) Column chromatography.
- b) High performance liquid chromatography.
- c) Gas chromatography.
- d) Ion-exchange chromatography.
- e) Size exclusion chromatography.
- f) thin layer chromatography.
- g) High performance thin layer liquid chromatography.
- h) Paper chromatography.
- I) Affinity chromatography.



## **THIN LAYER CHROMATOGRAPHY**

The term “thin-layer chromatography”, introduced by E. Stahl in 1956, means a chromatographic s. TLC is a quick, inexpensive microscale technique that can be used to:

- determine the number of components in a mixture
- verify a substance’s identity
- monitor the progress of a reaction
- determine appropriate conditions for column chromatography.

analyse the fractions obtained from column chromatography separation process in which the stationary phase consists of a thin layer applied to a solid substrate or “support”. Thin layer chromatography (TLC). Thin-layer chromatography or TLC, is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents

### **Principle**

TLC is based on the principle of adsorption. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) travel over the surface of stationary phase and during this movement, the compounds with higher affinity to stationary phase

travel slowly while the others travel faster. Thus, separation of components in the mixture is achieved.

### **Preparation of TLC Plates**

The adsorbent (silica gel G) slurry was prepared in water in the ratio of (1: 2). The glass plates (20cm x 5cm) were cleaned and laid in a row as a template, the suspension was poured into Stahl TLC spreader, which was adjusted to 0.25mm thickness and coated in a single passage of the spreader over them. These plates were air dried and activated in hot air oven at 105°C for 30min and kept in a desiccator. The plates were used as the stationary phase or pre-coated aluminium plates coated with silica gel G F254 (Merck) were also used for analysis.

### **Sample application**

The extracts were dissolved in mobile phase and the spot was applied on the TLC plates using capillary tube.

### **Development of the chromatogram**

After drying of the spot, the plates were developed in a chromatographic tank containing the solvent system. After one third of the plate was developed the plates were taken outside and dried. The TLC plate was examined visually or under UV light.

#### **Solvent system I**

Stationary phase - Silica gel G

Mobile phase - Toluene: Ethyl Acetate: Methanol (7:2:1) Detecting agent - visual & UV light

#### **Solvent system II**

Stationary phase - Silica gel G

Mobile phase - Chloroform: Methanol (9.5:1) Detecting agent - visual & UV light

The R<sub>f</sub> values were calculated using the formula [Distance travelled by solute/ Distance travelled by solvent]. The phytochemical evaluation of methanolic extract of Citrullus lanatus was carried out by using TLC studies. The results are presented.<sup>[13]</sup>

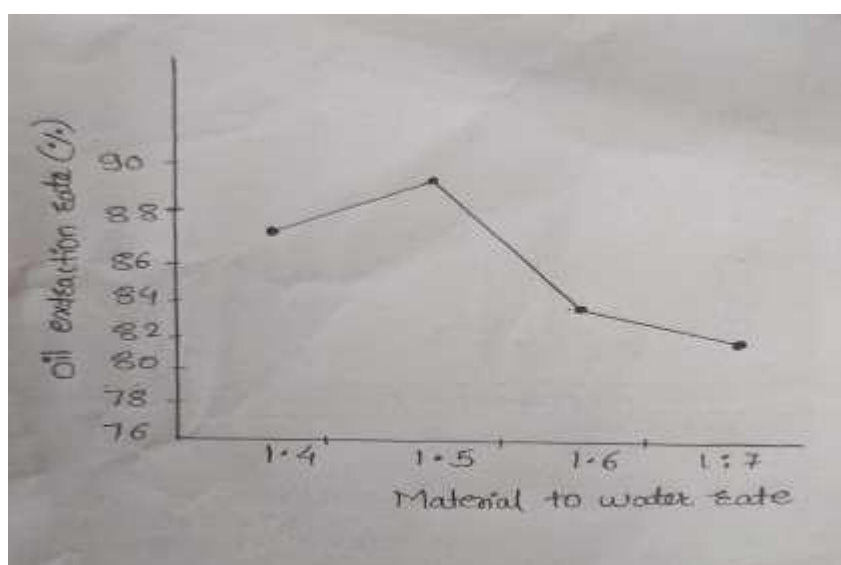
## Herbal formulations in the treatment of diabetes mellitus

Diabetes mellitus in Ayurveda is known as Madhu-meha. Several Ayurvedic formulations have been used in the treatment of Diabetes mellitus for centuries. In addition to herbs, minerals find wide application in Ayurvedic prescription for diabetes. Medicinal herbs like Momordica charantia, Gymnema sylvestre, Enicostemma littorale, Pterocarpus marsupium, Salacia reticulate, Coccinia gluaca and Trigonella foneum graceum are prescribed as single powder drugs or in combination (poly-herbal).

## Result And Discussion

- The analysis of single factor experiment
- The effect of materials to water rate on oil extraction rate

shows that the oil extraction rate is increased with the ascending of materials to water rate and reaches the highest point at 1:5. The oil extraction rate falls when the materials to water rate beyond 1:6.



TLC

$$\text{R.F. Value} = \frac{\text{Distance Travelled by Solute}}{\text{Distance Travelled by Solvent}}$$

Mixture Of 25% HCL and 96 % Ethanol Observation and Calculation of RF Values

R.F. Value = 5/6

R.F. Value = 0.83<sup>[14]</sup>

## SUMMARY AND CONCLUSION

The present study entitled the “Pharmacogenetic, Phytochemical evaluation of the Leaves of *Citrullus lanatus* (Thunb.) Matsum. & Nakai. (Cucurbitaceae)” focuses on a plant which is commonly available throughout India and traditionally used in treatment of various ailments.

Studies on the leaves of *Citrullus lanatus* are still lacking. Hence to exploit its potential use prompted the present study to investigate the leaves of this plant with clear scientific protocol.

The chapter on Literature Review deals with the information regarding the pharmacogenetic, phytochemical evaluation of the *Citrullus lanatus* plant and other species of *Citrullus*.<sup>[15]</sup>

### **The chapter on Pharmacogenetic studies highlights on**

- Macroscopical features were studied and the adherence of general characters to the family *Citrullus lanatus* was found.
- Microscopical study reveals the presence of actinocytic stomata, multi cellular uniseriate unbranched epidermal trichomes. Vascular system of the midribis multistranded, a large abaxial median bundle, two adaxial bundle. All the bundles are bicollateral having phloem strand both outer and inner side of the xylem. The epidermal cells are small elliptical or rectangular and thin walled. Spongyparenchyma cells small and spherical. Palisade zone consist of single layer of cylindrical cells, loosely arranged.
- Quantitative microscopical studies namely stomatal number, stomatal index, vein islet number, vein termination number, ash value, extractive value, loss on drying value etc.,
- Also studied cell powder microscopy, fluorescence analysis of powder and the results helps in achieving a trouble-free identification and authenticity of the plant leaf or in powder form in future.
- **The chapter on Phytochemical Evaluation deals with**
  - Preliminary phytochemical screening reveals the presence of carbohydrate, alkaloids, flavanoids, protein & aminoacids, glycosides etc.,
  - Quantitative determination of secondary metabolites (phenol, flavanoid, tannin content) has been carried out.
  - TLC<sup>[16]</sup>

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