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Research Article

Pharmacognostical and Pharmacological Evaluation of leaves of *Citrullus lanatus L.*

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Abstract:
Citrullus lanatus (Watermelon) is commonly available throughout India and traditionally used in treatment of various ailments. This plant is well-explored for its fruit but the potential of its leaves is still unexplored. Thus, the present study focuses on pharmacognostical and pharmacological evaluation of leaves of *Citrullus lanatus*. In pharmacognostical evaluation, all macroscopic, microscopic, qualitative, quantitative and microbiological analysis were performed. In pharmacological evaluation, the study mainly focuses on the potential of leaves for antioxidant and antidiabetic activities. The antioxidant activity has been performed by various methods such as DPPH and phospho-molybdenum method. The antidiabetic activity has been performed by non-enzymatic glycosylation of hemoglobin assay method, glucose uptake in yeast cells method, alpha amylase inhibition assay method and alpha glucosidase inhibition assay method. The results of antioxidant activity shows that the plant extract possess good free radical scavenging properties due to the presence of vitamin C, polyphenol and flavonoid content. The results of antidiabetic activity by non-enzymatic glycosylation method shows that the plant extract of *C. lanatus* exhibit significant inhibition of glycosylation as compared with standard drug alpha tocopherol. In glucose uptake by yeast cells method, the extract showed greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug acarbose. In alpha amylase inhibition method, Alpha amylase inhibitors bind to alpha bond of polysaccharide and prevent breakdown of polysaccharide in mono and disaccharide which shows significant activity of plant extract as compared to an acarbose standard drug. Thus from the above findings, it can be concluded that *C. lanatus* leaves possess a great potential for antioxidant and antidiabetic activity and this plant should be more researched and formulation should be marketed in the recent future.
Keywords: *Citrullus lanatus* (Cucurbitaceous), Watermelon, Anti-oxidant Activity, Anti-diabetic Activity

Introduction

1.1 Medicinal Plants

A plant or different parts of plant which, in one or more of its organs contain substances that can be used for multiple purposes such as healing, diagnostic or therapeutic which are out rider for synthetic or semi-synthetic compounds. When a plant is termed or specified as 'medicinal', it is said that it is useful as a drug or therapeutic agent or an active ingredient of a herbal or ayurvedic preparation. Healthy plants are described as a

group of plants that acquire some special qualities or molarity that mark them as essay of drugs and remedial agents, that can be used for medicinal purposes.[1]

1.2 Characteristics of medicinal plants

Medicinal plants have many characteristics which are used for essential medicinal purposes such as:

- **Bilateral medicine-** The components of plants that are interacted synchronously, so their uses can supplement or damage others or counteract their negative effects.
- **Support of official medicine-** The supplements of the plants proved to be quite productive in the therapy of intricate conditions like neo plastic diseases.
- **Social or Preventive medicine-** It has been proven that the supplement of the plants also has the potential to prevent the aspect of some diseases which can help to reduce the use of the chemical based drugs and minimize the side effect of synthetic or chemical based treatment.

1.3 Medicinal plants in India

About 60 percent of the entire worldwide population uses herbal or ayurvedic medicines. Herbal medicines are not only used for primary health care not just in rural areas and in developing countries, but also in developed countries as well where modern medicines are predominantly used.

In India there are about 45500 medicinal plant species, spread in the Eastern and Western region. According to protocol plants with herbal ability there are about 3000 medicinal plants but classical practitioners use more than 6000 medicinal plants. India is the largest manufacturer of ayurvedic species which are known as botanical garden on the earth.

Ayurveda and herbal medicine have dominant principle of complementary medicine that is broadly usable in India. The indigenous system of medicine predominantly, Ayurveda form of medicine, is believed to be existent in India for thousands of years.

The modified traditions have about 25,000 plant drug formulations that have emerged from such studies. All over 50,000 formulations are concluded to be obtainable in the folk and tribal traditions. All these point to the fondness for an overtime knowledge about herbal plants that can be existent in this home land from time ancient. [2]

1.4 Future of medicinal plants

Medicinal plants are auspicious as well as most necessitated for coming time because there are about half million plants around the world, and most of their therapeutic evaluation have not been analyzed yet, and their pharmacological activities could be very effective in the treatment of present or for coming time research works.

2. Plant Profile- *C.lanatus*

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**.**

Figure 2.1 Leaves of *C. lanatus*

2.1 Classification

- **Common Name:** Watermelon
- **Kingdom:** Planate
- **Subkingdom:**Tracheobionta
- **Super division:** Spermatophyta
- **Division:**Magnoliophyta
- **Subclass:**Dilleniidae
- **Family:** Cucurbitaceae
- **Genus:** Citrullus Schard
- **Species:***Citrullus lanatus (Thunb.)Mastum. & Nakaivar lanatus*[3]

2.2 Geography and Distribution

Citrullus lanatus (Watermelon) is thought to be native to Africa. It is found in grassland and bush land, mostly on sandy soils, and often along water courses 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.

2.3 Ethnomedical Uses

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.

The other uses are described below:

a. The root is purgative and in high dose it canals observes as emetic.
b. These have 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. Preliminary research indicates that the consumption of water melon may have antihypertensive effects.
c. Fatty oil in the seed, as well as aqueous or alcoholic extracts has been reported to paralyze tape worms and round worms.
d. The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes.

3. Pharmacognostical Evaluation

3.1 Material and Methods

A. Macroscopic 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. The leaves of *Citrullus lanatus* were collected and the macroscopical characters like shape, structure, colour and pattern were studied[4-5]. The macroscopic characteristic of leaves has been shown in **Fig 3.1, 3.2 & 3.3.**

Figure 3.1: Herbarium of *Citrullus lanatus*

Figure 3.2: Dorsal View of Leaf of *C. lanatus*

Figure 3.3: Ventral View of Leaf of *C. lanatus*

B. Microscopic Studies

The microscopical evaluation allows more detailed examination of the plant material to identify the organized drug by its histological character. It provides detailed information about the crude drugs by virtue of its property to magnify the fine structures of minute objects to be visualized and there by confirm the structural details of the plant drugs under evaluation.

(I) Collection of specimens

a) The plant specimen for the proposed study was collected from *Citrullus lanatus* leaf. Care was taken to select healthy plant. The leaf was cut and removed from the plant and fixed in FAA (Formalin-5ml+Acetic acid-5ml+70% Ethyl alcohol-90 ml) after 24hr suffixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule

given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60˚C) until TBA solution-stained super saturation. The specimens were cast into paraffin blocks

b) The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12µm. Dewaxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method published by O'Brien et al. (1964). Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. whenever necessary sections were also stained with safranin and Fast green and iodine in potassium iodide.

c) For studying the stomatal morphology, venation pattern and trichome distribution, para dermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jaffrey's maceration fluid were prepared. Glycerin mounted temporary preparations were made form a curated /cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell component were studied and measured.

(II) Photomicrographs

Microscopic description soft tissues are supplemented with micrographs where is necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For normal observations bright field was used for the study of crystals, starch grains, and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books[6-7].The microscopic features observed for leaves is represented in **Fig. 3.4**.

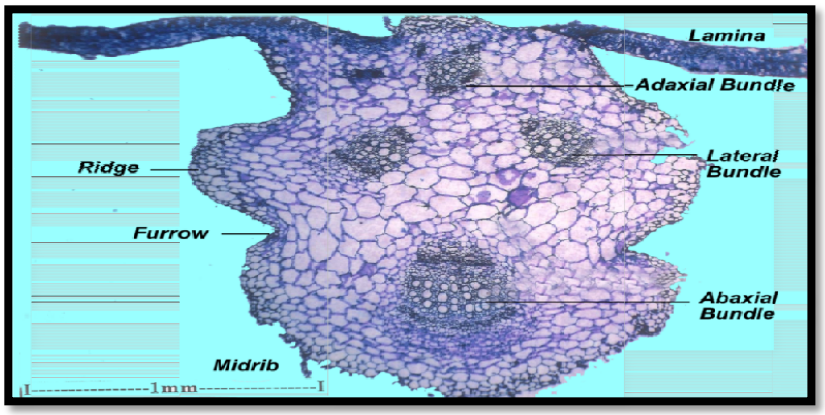


Figure 3.4: T.S. of Leaf through Mid-rib

C. Quantitative Microscopy

Quantitative analytical microscopy issue measuring the cell content soft the crude drugs and help in their identification, characterization and standardization. A cleared the number so obtained can be compared with a standard value to find out whether it is within the range. It helps to determine the purity of the plant material.

(i) Stomatal number: Stomatal number is the average number of stomata/sq.mm of epidermis of the leaf.

(ii) Stomatal index: Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells.

(iii) Vein islet number is defined as the number of vein islet per sq.mm of the leaf surface mid way between the midrib and the margin.

(iv) Vein termination number is defined as the number of vein termination per sq.mm of the leaf surface midway between the midrib and margin.

The results of quantitative microscopy have been illustrated in **table 3.1.**

Table 3.1: Quantitative Microscopical Parameters of the leaf of *Citrullus lanatus*

Sr. No.	Parameters*	Values obtained
1	Stomatal number in upper epidermis	21.83 ± 0.300
2	Stomatal number in lower epidermis	32.25 ± 0.629
3	Stomatal index in upper epidermis	19.68± 0.396
4	Stomatal index in lower epidermis	22.12 ± 0.093
5	Vein islet number	12.66 ± 0.333
6	Vein termination number	19.66 ± 0.881

***mean of three readings ± SEM**

D. Standardization Parameters

The determination of the foreign organic matter, loss on drying, ash values and extractive values etc. gives a clear idea about the specific characteristics of crude drug under examination, besides its macro-morphological or cyto-morphological, microscopical nature in both its entire and its powder form. These diagnostic features enable the analyst to know the nature and characteristic of crude drugs.

(i) Foreign Organic Matter: The part of organ or organs other than those specified in the definition or description of the crude drugs is defined as foreign organic matter.

(ii) Ash Value: The ash values for air dried powdered leaves of *Citrullus lanatus* were determined as per official method. The determination of ash is useful for detecting low grade products, exhausted drug and excess of sandy or earthy matter.

Different types of ash values are used in detection of crude drugs like, total ash, acid insoluble ash, water soluble ash and sulphated ash.

(iii) Loss on Drying: The moisture content of a drugs hold is minimized to prevent decomposition of plant material due to chemical or microbial contamination. It may be determined by heating a material at constant temperature to constant weight.

(iv) Extractive Values: The extractive values are the important factor to determine the amount of active principle or Phyto-constituents present in the plant materials, when extracted with suitable solvents. The extraction of crude drug with a particular solvent yields a solution containing different Phyto-constituents. The composition of these Phyto-constituents in that particular solvent depends upon the nature of the drug and solvent used.

(v) Foaming Index: Some plant materials when shaken with water cause persistent foam which may be attributed to the presence of saponins in that material. The foaming ability of an aqueous solution of plant materials and their extracts is measured in terms of foaming index.

(vi) Swelling Values: Swelling index is the volume in mL taken up by the swelling of plant material under specified conditions. The medicinal plant materials like gums, mucilage, and pectin haves welling property.

The results of standardization parameter has been mentioned in table 3.2

Table 3.2: Standardization parameters of *Citrullus lanatus*

Sr. No	Parameters*	Values* expressed as %
1	Foreign organic matter	0.04 ± 0.180
2	Loss on drying	7.29 ± 0.012
3	Ash value	
	Total ash	14.73 ± 0.080
	Acid in soluble ash	1.28 ± 0.074
	Water soluble ash	4.03 ± 0.062
	Sulphated ash	17.76 ± 0.292
4	Extractive values	
	Petroleum ether	29.33 ± 0.360
	Chloroform	7.39 ± 0.101
	Ethyl acetate	8.09 ± 0.210
	Ethanol	13.65 ± 0.413
	Methanol	26.57 ± 0.268
	Water	24.99±0.671
	Acetone	11.25 ± 0.145
	Benzene	5.87±0.153
	Hexane	5.64 ± 0.046
5	Foaming index	<100
6	Swelling index	2.4±0.493(ml)

*mean of three readings ± SEM

The powdered crude drug analysis was aimed to study and also to assess the quality of herbal drugs for therapeutic value which are generally studied by classical pharmacognostical studies. The authenticity of herbal drugs was confirmed by comparison of their powder characteristics.

(i) Reaction of chemicals with powdered crude drugs: The raw leaf powder of C.*lanatus* was treated with different chemical reagent such as iodine solution, 10% potassium hydroxide solution, acetic acid etc. for the identification of secondary metabolites. The results shown in **table 3.3.**

(ii) **Fluorescence analysis:** The fluorescence nature of powder drugs was analyzed to find out whether any fluorescent compound as present in the sample and the observations with different fluorescent chemicals were so carried out and recorded. The air-dried plant material so both plants were taken in clean was glass and subjected to different chemicals has acids, alkalis and some reagents are observed under day light and UV light.

Table 3.3: Reaction of chemical with powdered drug *Citrullus lanatus*with various chemical reagents

Drug powder + reagent	Colour In day light (Visible)	Colour in UV light	
		254nm	365nm
Powder	Yellowish green	Dark green	Yellowish green
Powder + 1M sodium hydroxide	Yellowish green	Fluorescent green	Dark green
Powder+ Iodine	Yellowish green	Dark green	Dark brown
Powder+ 10% potassium hydroxide	Yellow	Fluorescent Green	Dark brown
Powder+ 1M Hydrochloric acid	Yellowish brown	Dark green	Dark brown
Powder+ Glacial acetic Acid	Yellowish green	Green	Orange
Powder+ 50% sulphuric Acid	Yellowish green	Dark green	Dark brown
Powder+ 50% nitric acid	Brown	Greenish	Dark brown
Powder+ 50% Hydrochloric acid	Yellowish brown	Dark green	Dark brown

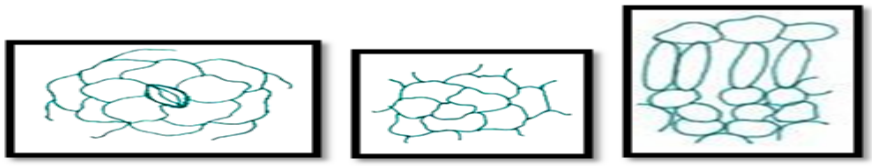
Note: Colour reactions are viewed under natural light by naked eye

Detailed fluorescence behavior of crude drug powder has been shown in **Table 3.4.**

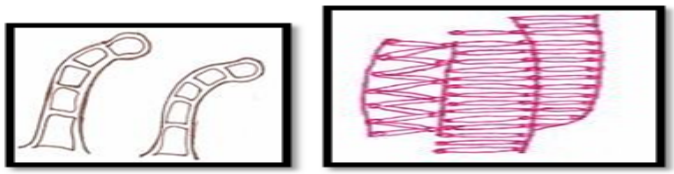
(iii) Powder microscopy: The dried leaf was powdered and the powder was passed through sieve no.60 for the study of powder microscopy. Chloral hydrate, water, iodine, phloroglucinol and hydrochloric acid (1:1) etc. were employed as mounting medium[8]. The pictorial representation is represented in **Fig 3.5.**

Table 3.4: Fluorescence analysis of extracts of *Citrullus lanatus*

Extract	Consistence	Colour in day light (Visible)	Colour in UV light	
			254nm	365nm
Ethanol	Semisolid	Light green	Dark green	Orange
Methanol	Semisolid	Yellowish green	Dark green	Light orange
Benzene	Semisolid	Yellowish green	Dark green	Dark orange
Petroleum ether	Semisolid	Light yellow	Dark green	Fluorescent green
Ethyl acetate	Semisolid	Light green	Dark green	Dark orange
Water	Semisolid	Yellowish green	Greenish black	Fluorescent green
Chloroform	Semisolid	Light green	Green	Dark orange
Acetone	Semisolid	Light green	Green	Dark orange
Hexane	Semisolid	Light green	Dark green	Orange



ActinocyticStomat Fragments of Parenchyma CellsFragments of Palisade Cells



Multicellular uniseriate Xylem Vessels (Spiral and Annular) unbranched Epidermal Trichomes

Fig. 3.5. Powder Microscopy of *Citrullus lanatus*

4. Pharmacological Evaluation

4.1 Invitro Antioxidant Activities

Method 1: Free radical Scavenging activity using diphenyl picrylhydrazyl (DPPH) free radical

The free radical scavenging activity of the extracts is evaluated by assessing their ability to reduce the colour of DPPH in ethanol according to Brand Williams [69]. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts.

Procedure: A stock solution of 0.5mg/mL concentration of methanolic extract of *C. lanatus* was prepared. To 1mL of various concentrations of test samples, 4mL of DPPH was added.

Control was prepared without sample in an identical manner. DPPH was replaced by ethanolic case of blank. The reaction was allowed to be completed in the dark for about 30 min. Then the absorbance was measured at 517nm. Vitamin C was used as standard. The percentages scavenging was calculated using the formula [(Control-Test)/Control] x 100.

Table 4.1: Percentage inhibition of methanolic extract of *C. lanatus*and standard ascorbic acid against DPPH at 517nm

Sr. No.	Conc. In µg/mL	Percentage inhibition by ascorbic acid	Percentage inhibition by *Citrullus lanatus*
1	10	48.91± 0.60	32.55 ± 0.32
2	20	58.03± 0.50	42.32 ± 0.48
3	40	67.86± 0.27	64.14 ± 0.61
4	60	79.49± 0.30	71.63 ± 0.29
5	80	85.36± 0.29	78.16 ± 0.29
	IC_{50}	27.29µg/ml	37.12µg/ml

*mean of three readings ± SEM

A graph was constructed by plotting concentration versus percentage inhibition and a linear regression equation calculated. The concentration of the sample required for 50% reduction in absorbance (IC50) was calculated using linear regression analysis. A triplicate reading was taken and average was calculated. The results obtained are presented in **Table 4.1.**

Method 2: Total antioxidant activity by Phosphomolybdenum Method

An aliquot of 0.3mL of different concentrations of sample solution was combined with 2.7mL of the reagent solution (H_2SO_4, sodium phosphate and ammonium molybdate). In case of blank, 0.3mL of ms ethanol was used in place of sample. The tubes were incubated for 95°C for 90 min. After the mixture was cooled to room temperature, the absorbance was measured at 695 nm against blank. Ascorbic acid was used as a standard and was treated in a similar manner. The total antioxidant activity is expressed as the number of equivalents of ascorbic acid (µg/g). The results were tabulated in **table 4.2.**

Table 4.2: Absorbance of methanolic extract of *C. Lanatus*and standard ascorbic acid in Phosphomolybdenum method

Sr. No.	Conc. In µg/mL	Absorbance of Ascorbic acid	Absorbance of *Citrullislanatus*
1	16.66	0.085±0.005	0.061±0.002
2	33.33	0.165±0.004	0.132±0.004
3	50.00	0.206±0.008	0.189±0.009
4	66.66	0.323±0.004	0.306±0.004
5	83.33	0.371±0.005	0.357±0.005

*Mean of three readings ± SEM

4.2 Invitro Antidiabetic Activity

Method I: Non-enzymatic Glycosylation of Hemoglobin Assay

Procedure: Anti diabetic activity of methanolic extract of the leaves of *Citrullus lanatus* were investigated by estimating degree of non-enzymatic hemoglobin glycosylation, measured spectrophotometrically at 520nm. Glucose (2%), hemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in 0.01M phosphate buffer (pH 7.4). 1mL each of above solution was mixed with 1mL of various concentrations of the extract. The mixture was incubated in dark at room temperature for 72h. The degree of glycosylation of hemoglobin was measured spectrophotometrically at 520nm. α-tocopherol (Trolax) was used as a standard drug for assay. The percentage inhibition was calculated. All the tests were performed in triplicate. [9]

The % inhibition was calculated using the following formula,

$$\% \, Inhibition = \frac{Abs_{sample} - Abs_{control}}{Abs_{sample}} \times 100$$

Where **Abs control** is the absorbance of the control and **Abs sample** is the absorbance of the test sample. The results are tabulated in **Table 4.3.**

Method 2: Invitro Glucose Uptake in Yeast Cells

Procedure: Yeast cells were prepared by commercial baker's yeast was washed repeated centrifugation (3,000rpm; 5min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. 1mL of various concentrations of methanolic extract of *Citrullus lanatus* were added to 1mL of glucose solution (5 and 10mM) and incubated together for 10min at 37°C. Reaction was started by adding 100µL of yeast suspension, vortexed and further incubated at 37°C for 60min. After 60min, the tubes were centrifuged (2,500 rpm, 5min) and glucose was estimated in the supernatant by glucose oxidase method. The optical density 520 nm was measured. Acarbose was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula,

$$\% \, Increase \, in \, glucose \, uptake = \frac{Abs_{sample} - Abs_{control}}{Abs_{sample}} \times 100$$

Where, **Abs control** is the absorbance of the control and **Abs sample** is the absorbance of the test sample. All the experiments were carried out in triplicates. The results are tabulated in **Table 4.4 & 4.5.**

Table 4.3: *In-vitro* Non-enzymatic glycosylation of hemoglobin method

Sr. No	Conc. in µg/ml	α-Tocopherol		Methanolic extract of Citrulluslanatus	
		Abs	% inhibition	Abs	%inhibition
1	20	0.159 ± 0.003	23.270 ± 1.674	0.147 ± 0.000	17.006 ± 0.496
2	40	0.187 ± 0.002	34.759 ± 1.016	0.169 ± 0.004	27.810 ± 1.847
3	60	0.258 ± 0.004	52.713 ± 0.813	0.233 ± 0.002	47.639 ± 0.589
4	80	0.386 ± 0.004	68.393 ± 0.342	0.346 ± 0.005	64.739 ± 0.623
5	100	0.512 ± 0.004	76.171 ± 0.213	0.442 ± 0.003	72.398 ± 0.221
IC_{50} Value		59.762µg/mL		65.648µg/mL	

*mean of three readings ± SEM

Table 4.4: Percentage inhibition of Glucose uptake in 5mM glucose concentrations

Sr. No	Conc. µg/ml	Acarbose		Methanolic extract of Citrulluslanatus	
		Abs	% inhibition	Abs	% inhibition
1	40	0.102 ± 0.001	60.78 ± 0.220	0.099± 0.002	59.60 ± 0.386
2	80	0.117± 0.000	65.81 ± 0.222	0.106±0.002	62.26 ± 0.030
3	120	0.132± 0.001	69.70 ± 0.271	0.124± 0.003	67.74 ± 0.204
4	160	0.150 ± 0.002	73.33 ± 0.101	0.140± 0.001	71.43 ± 0.177
5	200	0.188±0.002	78.72 ± 0.248	0.152 ± 0.006	73.68 ± 0.362
IC_{50} Value		74.083µg/mL		80.218µg/mL	

* Mean of three readings ± SEM

Table 4.5 : Percentage inhibition of Glucose uptake at 10mM concentration

Sr. No	Conc. µg/ml	Acarbose		Methanolic extract of *Citrulluslanatus*	
		Absorbance	%inhibition	Absorbance	%inhibition
1	40	0.200 ± 0.003	65.00 ± 0.242	0.170 ± 0.004	58.82 ± 0.257
2	80	0.214 ± 0.002	67.28 ± 0.163	0.220 ± 0.001	65.00 ± 0.282
3	120	0.249 ± 0.004	71.88 ± 0.178	0.248 ± 0.001	68.54 ± 0.081
4	160	0.303 ± 0.003	76.89 ± 0.206	0.311 ± 0.003	73.28 ± 0.077
5	200	0.379 ± 0.001	81.53 ± 0.181	0.335 ± 0.003	76.74 ± 0.214
IC_{50} Value		67.408µg/mL		77.031µg/mL	

*Mean of three readings ± SEM

Method 3: Invitro Alpha Amylase Inhibition Assay

Procedure: Various concentrations of methanolic extract of *Citrullus lanatus* 500µL

were added to 500µL of 0.02M sodium phosphate buffer (pH 6.9 containing 6mM sodium chloride) containing 500µL of α-amylase solution and were incubated at 37°C for 10min. Then 500µL soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15min. 1M HCl (20µL) was added to stop the enzymatic reaction, followed by the addition of 100µL of iodine reagent. The colour change was noted and the absorbance was read at 620nm. Acarbose was used as reference. Inhibition of enzyme activity was calculated as:

$$\%\ Inhibition\ of\ Enzyme\ Activity = \frac{C - S}{C} \times 100$$

Table 4.6: *In-vitro* Alpha amylase inhibition

Sr. No	Conc. µg/ml	Acarbose		Methanolic extract of *Citrulluslanatus*	
		Absorbance	%inhibition	Absorbance	%inhibition
1	20	0.099 ± 0.001	44.44 ± 0.646	0.077 ± 0.003	28.57 ± 3.185
2	40	0.116 ± 0.004	52.58 ± 1.751	0.103 ± 0.002	46.60 ± 1.020
3	60	0.152 ± 0.003	63.81 ± 0.844	0.128 ± 0.004	57.03 ± 1.341
4	80	0.193 ± 0.002	71.50 ± 0.340	0.154 ± 0.003	64.28 ± 0.768
5	100	0.232 ± 0.006	76.29 ± 0.658	0.186 ± 0.002	69.44 ± 0.410
IC_{50} Value		47.880 µg/mL		58.558 µg/mL	

* Mean of three readings ± SEM

Where S is the absorbance of the sample and C is the absorbance of blank (no extract).[10]The results are tabulated in **Table 4.6.**

Method 4: Invitro Alpha Glucosidase Inhibition Assay

Procedure: The enzyme α- glucosidase inhibitory activity is determined by incubating solution (0.1 ml) of an enzyme preparation with 0.2 M Tris buffer, pH 8.0 (1.0ml) containing 1mL of various concentrations of extract at 37 ºC for 60 minutes. The reaction mixture is heated for two minutes in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidation method. Acarbose was used as reference. the results are tabulated in **Table 4.7.**

$$\%\ Inhibition = \frac{(Enzyme\ activity\ of\ control - Enzyme\ activity\ of\ extract)}{Enzyme\ activity\ of\ control} \times 100$$

Table 4.7:In-vitro α-glucosidase inhibition assay

Sr. No.	Conc. µg/ml	Acarbose		Methanolic extract of Citrulluslanatus	
		Absorbance	% inhibition	Absorbance	% inhibition
1	200	0.809 ± 0.004	24.46 ± 0.457	0.896 ± 0.002	16.34 ± 0.189
2	400	0.519 ± 0.002	51.54 ± 0.188	0.780 ± 0.001	27.17 ± 0.136
3	600	0.388 ± 0.001	63.77 ± 0.113	0.517 ± 0.002	51.73 ± 0.270
4	800	0.216 ± 0.001	79.83 ± 0.136	0.408 ± 0.002	61.90 ± 0.188
5	1000	0.106 ± 0/002	90.10 ± 0.243	0.204 ± 0.002	80.95 ± 0.273
IC_{50}Value		482.188µg/mL		627.270µg/mL	

* mean of three readings ± SEM

5. Summary and Conclusion

The aim of the present study was to evaluate *C. lanatus* (Watermelon) which is commonly available throughout India and traditionally used in treatment of various ailments. There were very few studies on its leaves hence to exploit its potential use prompted the present study to investigate the leaves of this plant with clear

scientific protocol.

In pharmacognostical evaluation, 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, multicellular uniseriate unbranched epidermal trichomes. Vascular system of the midribs multi stranded, a large abaxial median bundle, two adaxial bundles. All the bundles are bicollateral having phloem strand both outer a dinner side of the xylem. The epidermal cells are small elliptical or rectangular and thin walled. Spongy parenchyma cells small and spherical.

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.

In Pharmacological studies evaluation the Antioxidant activity by various methods has been studied and the extract possessed good antioxidant property due to the presence of Vitamin-C, polyphenolic, flavonoid content.

The antidiabetic activity of extract of the leaves of *Citrullus lanatus* was carried out by non-enzymatic glycosylation of hemoglobin Assay method. The extract exhibits significant inhibition of glycosylation as compared with the standard drug alpha tocopherol. Decreases the formation of the glucose-hemoglobin complex and thus amount of free hemoglobin increases.

The extract showed greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug acarbose. After the treatment of the yeast cells with the leaf extracts, the glucose uptake was found to increase in a dose dependent manner.

Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent breakdown of polysaccharide in mono and disaccharide. The result showed extract of *Citrullus lanatus* significant activity as compared to an acarbose standard drug.

Alpha-glucosidase inhibitors have a potential for the treatment of diabetes because they reduce diet-induced hyperglycemia. The extract showed better alpha-glucosidase inhibition property.

The i*n vitro* assays of the present study indicated the methanolic extract of *Citrullus lanatus* possess good anti diabetic activity.

Citrallus lanatus (water melon) is popular in indigenous system of folk medicine. The leaf extract of *Citrullus lanatus* contain bioactive compounds such as flavonoid, phenolic compound, tannin, triterpenes, sterols and alkaloids, vitamins. The extract may serve as a lead medicinal plant to synthesis various semi-synthetic drugs to treat various lives threatening disease.

6. References

1. Upadhyay, B., Singh, K.P. and Kumar, A., 2011. Ethno-veterinary uses and informants consensus factor of medicinal plants of Sariska region, Rajasthan, India. *Journal of Ethnopharmacology*, *133*(1), pp.14-25.
2. Dinesh kumar, B., Analava, M. and Manjunatha, M., 2009. A review on common Indian medicinal plants with anti-diabetic activities. *Journal of Medicinal and Aromatic Plant Sciences*, *31*(2), pp.152-158.
3. Kurniawaty, D., 2019. Effect of Cadmium in Biosorption of Lead by Lengkeng Seed and Shell (Euphoria logan lour). *Journal of Chemical Natural Resources*, *1*(2), pp.23-29.
4. Hussain, M.S., Rehman, R. and Imran, M., 2022. Comparative evaluation of the adsorption performance of citric acid-treated peels of *Trapa natans* and *Citrullus lanatus* for cationic dyes degradation from water. *Journal of Chemistry*, *2022*.

5. Cherif, A. and Verheggen, F., 2019. A review of *Tuta absoluta* (Lepidoptera: Gelechiidae) host plants and their impact on management strategies.*23*(4).

6. Mehra, M., Ojha, A., Meghwal, M. and Pareek, S., 2023. Watermelon and Muskmelon by-products: An under-utilized Source of Biodegradable Packaging.

7. Fonseca, A.M., Geraldi, M.V., Junior, M.R.M., Silvestre, A.J. and Rocha, S.M., 2022. Purple passion fruit (Passiflora edulis f. edulis): A comprehensive review on the nutritional value, phytochemical profile and associated health effects. *Food Research International*, p.111665.

8. Mali, V.R. and Bodhankar, S.L., 2010. Cardioprotective effect of Lagenaria siceraria (LS) fruit powder in isoprenaline-induced cardiotoxicity in rats. *European Journal of Integrative Medicine*, *2*(3), pp.143-149.

9. Deshmukh, P.B. and DM, R., 1982. A study of insecticidal activity of twenty indigenous plants. Mahapatra, S., Rao, E.S., Hebbar, S.S., Rao, V.K., Pitchai muthu, M. and Sriram, S., 2023.

10. Evaluation of rootstocks resistant to gummy stem blight and their effect on the fruit yield of watermelon. *The Journal of Horticultural Science and Biotechnology*, pp.1-14.