# Screening of *Citrullus lanatus* (Linn.) seeds for its biological activity and metabolite profiling

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***Abstract:*** *Citrullus lanatus (Linn.) with known therapeutic potential has immense value for its seeds, roots, leaves and flowers. The authors have attempted to study the biological activities of seed solvent extracts. The solvent extracts of hexane, chloroform, diethyl ether and methanol were prepared. The tannins were reported in hexane, chloroform and methanol extracts, whereas saponins were in hexane, diethyl ether and methanol extracts. The phenolic and flavonoids content in C. lanatus plant extracts varied from 75µg GAE, ($R^2$= 0.88) and flavonoid 10µg in 51µg ($R^2$=0.96). Methanolic extract exhibited higher antioxidant activity ($IC_{50}$ = 30 µg/ml) when compared to other extracts. The C. lanatus showed significant antibacterial activity against pathogenic E. coli. The C. lanatus seed extract tested inhibited lipoxygenase in a concentration dependent manner. The highest inhibitory effect was observed for C. lanatus with an $IC_{50}$ of 64 µg. Identification of metabolites based on UPLC-PDA-ESI/HDMS and in-house MS data identified compound A as chlorogenic acid; Compound B was identified palmitic acid; compound C cinnamic acid; Compound D was identified as 7-O-glucosyl apigenin; compound E as pinostrobin; compound F as liquiritin; compound G as Myricelin-glucoside; compound H as penta-O-galoyl-β-D-glucose.*

**Keywords:** *total tannins and saponins, phytochemical analysis, anti-bacterial property, lipoxygenase inhibition,*

## 1. Introduction

*Citrullus lanatus* (water melon) is the fruit of a plant originally from a vine of Southern Africa. It produces about 93% water; hence name "water" melon [1]. *Citrullus lanatus* is a prostrate plant with several herbaceous, firm and stout stems. The leaves are herbaceous but rigid, becoming rough on both sides. The leaf stalks are upto 150 mm long. The tendrils are rather robust and usually in the upper part of the plant. They are monoecious with the flower stalk up to 4mm long and to 20mm in diameter; the fruit upto 50mm long [2]. In the world this plant biodiversity is the largest and is the source of herbal medicine. The world population (60 –80 %) rely on plant based medicines which were being used since the ancient ages in the traditional health care system. Plants continue to be used world-wide for the treatment of disease and novel drug entities continue to be developed through research into their constituents [3]. The amino acid citrulline was first extracted from watermelon. The nutritional quality of watermelon shows that it is very rich in vitamins A 3%, Vitamin C 14%, different vitamins from vitamin B complex like Thiamine (Vit. B1), Riboflavin (Vit. B2), Niacin (Vit. B3), Pantothenic acid (B5), vitamin B6 and Folate (Vit. B9), which ranges between 1-3%,. The mineral composition is Calcium 1%, Iron 2%, and Magnesium 3%, Phosphorus 2%, Potassium 2% and Zinc 1%. Along with it, it also contains highly unsaturated fatty acids and oils. It is also rich in essential amino acids like arginine, glutamine and aspartic acid**.**

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [4]. Phytochemical are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents. The secondary compounds include terpenoid, alkaloids and phenolic compounds [5]. Melon belongs to the family Cucurbitaceae and is an edible fruit. Melons have their origin in Africa and Southwest Asia, but they later started appearing in Europe at the end of the Roman Empire. Melons are a nourishing food. Its seeds are used to treat tuberculosis [6]. They have high levels of potassium. Melons are considered diuretics due to their high water content. It has been researched that melons possess the ability to lower the risk of cancer. They contain lycopene, an antioxidant found in some fruits and vegetables.

It is referred as a pepo by botanists, which is a berry having a thick rind (exocarp) and fleshy center (mesocarp and endocarp). Watermelons are a good source of Vitamin C. These have also been investigated for their potential as significant diuretic agents [7]. C. *lanatus* can be used for smoothes, sorbets or granite depending on the

texture whether smooth or coarse. The fruit is also diuretic, being effective in the treatment of dropsy and renal stones [8].

Watermelon seeds are known to be highly nutritional, rich sources of protein, vitamins B, minerals (such as Magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others as well as phytochemical ⁅9]. They are known to have economic benefits especially in countries where cultivation is on the increase. The seeds are for instance used to prepare snacks, milled into flour and used for sauces. Oil from the seeds are used in cooking and incorporated into the production of cosmetics [10]. The immature fruit is usually consumed as a vegetable, but the seed from the mature fruit is used in the form of roasted and salted food items. The kernel, extracted from the seed in the whole form, traditionally called ‘‘Magaz’’, is in great demand as an adjunct or condiment to many Indian confectionery items. The seed, which is 10 to12% of the mass of the mature fruit, is estimated to contain 20 to 30% oil. Consumptions of varieties of plant food including watermelon seeds in diabetes may provide additional health benefits. The current project is an attempt to describe some pharmacognostic, physicochemical and phytochemical characteristics of *Citrullus lanatus* seeds for its identification, evaluation, characterization, and standardization [11].

In spite of the various potential applications, the watermelon seeds are often discarded while the fruit is eaten. There is also limited literature on the effect of variety on the nutritional, phytochemical and antioxidant properties of the watermelon seeds. In this study, the proximate and antioxidant activity were determined in the seeds of three of watermelon varieties. The seeds were also screened for the presence of some phytochemical and minerals content [9]. Their antimicrobial capacity was analyzed. Inhibition of 15-lipoxygenase (LOX) as an indicator of their anti-inflammatory potential is reported.

## 2. Material and Methods

### 2.1. Chemicals and reagents

Linolenic acid, 1, 1-diphenyl-2picrylhydrazyl (DPPH) catechin (Sigma ALDRICH MO, USA), 15 Lipoxygenase, Ascorbic acid ,Gallic acid, sodium bicarbonate, potassium ferric cyanide, nutrient agar, peptone, beef extract, Phosphate buffer, borate buffer, aluminum chloride, potassium ferric cyanide, trichloro acetic acid (TCA), ferric chloride, hexane, methanol, chloroform, diethyl ether, borate salt, sodium dihydrogen phosphate, disodium hydrogen phosphate are laboratory chemicals.

### 2.2. About plants

#### 2.2.1. Processing of plant samples

The *Citrullus lanatus* (watermelon) was purchased from Yelwal market Mysore, Karnataka India. The seeds were separated manually and were washed under tap water. They were shade dried and crushed with liquid nitrogen using mortar and pestle. Fractionation of the bioactive were carried out using solvent to increase the polarity of the solvents like Hexane, Diethyl ether, Chloroform and Methanol, for 48 h in dark with continuous stirring at room temperature. After each fractionation the respective solvents are carefully separated by decantation using muslin cloth to prevent contamination by seed residue. The clear extract was air dried to get fine paste. They were weighed and stored at 4°C in dark till further use.

### 2.2. Extraction of plant material

Weigh coarsely powdered (25g) watermelon seeds was suspended in (200ml ) methanol for fractioning followed by solvent increasing order of polarity i.e., hexane, chloroform, diethyl ether, methanol. Solvent evaporation was by using incubator shaker at 30°C 60rpm for 48 h. And the solvent is collected back using rotator evaporator at 160rpm. The obtained yield was measured and stored at room temperature for further use.

### 2.3. QUALITATIVE ANALYSIS OF SAPONINS AND TANNINS

#### 2.3.1.Test for saponin:

Add methanolic extract (5ml; 0.1g in 1ml) was shaken with distilled water (5ml). Formation of frothing (appearance of creamy miss of small bubbles) shows that presence of saponin.

#### 2.3.2.Test for tannins:

To methanolic extract (2ml;0.1g in 1ml), alcoholic ferric chloride(10%) was added; formation of green color indicates the presence of tannins.

### 2.4. Determination of total phenol content

The total phenol content was analyzed using Folin-Ciocalteu. Standard was prepared using gallic acid (50µg in 10µl). The extract (5mg) was weighed and diluted to make (1ml). The extract (20-100µl) was mixed with Folin Ciocalteu reagent (250µl,1:1 dilution) and incubated at room temperature for 5 min. Sodium bicarbonate (1.5ml, 20%) was added to the mixture and incubated against at room temperature for 1 h. Absorbance was measured at 765nm using UV-VIS spectrophotometer (Beckman Coulter, DU 730 Life Sciences). The total phenol content in all the extract was estimated and reported as Gallic acid equivalents(GAE).

**2.5. Total flavonoid content**

The total flavonoids were measured using the aluminum chloride by colorimetric method. Weighed catechin (2mg;10-50µl) was dissolved in methanol (1ml) was used as standard. The methanol extract ( 50-250µl) was added to distilled water (4.5ml) , followed by $NaNo_2$ (0.03ml, 5%). Incubate for 5min after incubation , add $AlCl_3$ (0.03 ml, 10%) was added at 25°C. At the sixth minute, the reaction mixture was treated with NaOH (2ml, 1M) and absorbance was measured at 510 nm.

2.6. **DPPH radical scavenging assay**

The traditional DPPH method was followed. DPPH was used to determine the radical scavenging activity of extracts. Methanolic extract (1, 2, 3, 4, 5µl) were plated out in duplicate in a 96- well micro titer plate. The methanolic DPPH (2.9mg in 25ml methanol; 95µl) solution was added to test samples and methanol was used as a control of test sample in the remaining columns. The percentage of decolourisation was recorded spectrophotometrically at 517nm using the Thermo Scientific Varioskan Flash micro titer plate reader. Ascorbic acid (10-50µg) was used as anti oxidant standards and positive controls. Three independent tests were performed for each sample. Seed extracts of hexane, diethyl ether were also analyzed for DPPH scavenging capacity. The DPPH absorbance decreased with an increase in DPPH radical scavenging activity. Results were expressed as $IC_{50}$, concentration where 50% inhibition of DPPH radical is scavenged. This activity is given as the percentage of DPPH radical scavenging which is calculated by this equation

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs [control]} - \text{Abs[sample]} \times 100}{\text{Abs(control)}}$$

**2.7. Reducing power**

This estimation of reducing power was carried out as described previously [20] with slight modifications. Test sample solution (0.1ml, 1 mg/ml) was mixed with equal volume of phosphate buffer (0.2M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). It was incubated at 50° C for 20 min. Trichloroacetic acid (TCA; 10 %, 2.5 ml) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. After centrifugation of the supernatant solution (1.5ml) were mixed with equal volume of distilled water and added $FeCl_3$ (0.5ml, 0.1%). Ascorbic acid was used as standard and phosphate buffer was used as blank solution. Absorbance was measured at 700 nm using UV-VIS spectrophotometer (Beckman Coulter, DU 730 Life Sciences). Increased absorbance of the reaction mixture indicates stronger reducing power.

**2.8. Anti bacterial activity**

Anti bacterial activity of methanolic extracts of seeds was determined by disc diffusion method on nutrient agar medium. Cultures of *Escherichia coli (E. coli), Klebsiella pneumonia,* and *Pseudomonas vulgaris* were spread on separate nutrient agar plates. Methanolic extracts (1, 2.5, 5, 10 µg) were loaded separately on sterile discs, allowed to dry and placed on bacteria inoculated nutrient agar media. Negative control was prepared by loading the discs with solvents and positive control was by streptomycin (10 µg). The plates were incubated at 37°C for 24-48 h and zone of inhibition around the disc were measured.

**2.9. Anti-inflammatory activity**

**2.9.1. Lipoxygenase assay**

*C. lanatus* seed extracted with methanol and tested for in vitro anti inflammatory activity Spectrophotometric assay for determination of Lox activity for watermelon 15- LOX (5µg) activity with linolenic acid (0.2µM;1.9µl in 5µl methanol and 20ml borate buffer) as substrate in borate buffer (0.2M,Ph, 9.0) was carried out. The methanol extracts (5, 10, 15, 20µg) were used for inhibition studies and ascorbic acid used as positive reference standard (1- 10 µg). The activity was recorded at 234nm using UV-vis spectrophotometer (Beckman coulter, DU 730 life science). The inhibitory effect of the extract was also expressed as percentage of enzyme activity inhibition $IC_{50}$ indicating the concentration required to inhibit 50% LOX activity was also calculated.

$$\text{\% inhibition} = \frac{\left(\text{Abs control} - \text{Abs sample}\right)}{[\text{ Abs control}]} \times 100$$

**2.10. LC-MS analysis**

Retention Time and Mass Spectrometric Profiling of Compounds in *Citrullus lanatus* for the qualitative analysis of the metabolites were analyzed by Synapt G2 (UPLC separations with QuanTof) according to manufacturers protocol. The nebulizer pressure was 60 psi and the nitrogen flow rate 10 L/min at a drying temperature of 350°C. The methanolic seed extract was filtered (0.2 micron syringe filters, Millipore, U.S.A) and an aliquot (5µl) was injected into the system. The mass spectra were acquired from m/z 100-1000 in negative ionization mode. Helium

was used as the collision gas for the fragmentation of the isolated compounds in the ion trap. The detection conditions were as follows: capillary voltage, 3500 V; skimmer voltage, –40 V; cap exit voltage, –158.5 V; Oct 1 DC, –12 V; Oct 2 DC, –2.45 V; trap drive level, 45.0; Oct RF, 150 Vpp; Lens 1, 5.0 V; Lens 2, 60 V [12].

### 3.0 Statistical Analysis

All experiments and measurements were made in triplicate and the values are reported as the mean ± standard deviation (SD). The results were subjected to variance analysis followed by Tukeys` test to analyse differences between the endophyte ethyl acetate extract and controls. Statistically significant differences ($p < 0.001$) were reported.

### 4. Results

The results that are obtained are given in tables and figures as follows under each of its respective topics. All the experiments performed were under standard laboratory conditions with standard protocols. For the coarsely powdered seeds (25 g), different quantities of yield were obtained for different solvents used **(Table 1)**.

**Table 1: Yields of extract of water melon seeds**

| Sample | Solvent | Extract (g) |
|---|---|---|
| *Citrullus lanatus* | Hexane | 1.72 |
| | Chloroform | 0.14 |
| | Diethyl ether | 0.28 |
| | Methanol | 1.52 |

#### 4.1. Phytochemical analysis

Qualitative analysis of Tannins and Saponins were carried out as reported in materials and methods. Their presence or absence in various extracts is reported in **Table 2**.

**Table 2: Phytochemical screening of methanolic extract of *Watermelon* seeds**

| Phytochemicals | Hexane | Chloroform | Diethyl ether | Methanol |
|---|---|---|---|---|
| Tanins | + | + | - | + |
| Saponin | + | - | + | + |

The screening of the seeds showed the presence of saponin, tannins with different solvents. Tannins was present in hexane, chloroform and methanol solvents, and tannins were absent in diethyl ether solvent. Saponin is also present in hexane, diethyl ether and methanol solvents and in chloroform solvent shows saponins were absent [**25**].

#### 4.2. Determination of total phenolic content

The phenolic and flavonoids content in *C. lanatus* plant extracts varied from 75µg GAE **(Figure 1)**, ($R^2$= 0.88) and flavonoid **(Figure 2)** 51µg ($R^2$=0.96). A positive correlation was observed with respect to total phenol content and free radical scavenging capacity. It suggests that the antioxidant capacity of the methanol extract could be attributed largely to the phenolic and flavonoids content of these extract. Our results indicate that the phenolic and flavonoids play an important role in the antioxidant activity.

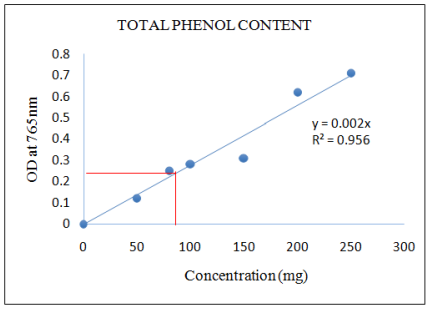



**Figure 1: Total phenol content of 75 µg gallic acid equivalent (GAE) was recorded using standard prepared by gallic acid**

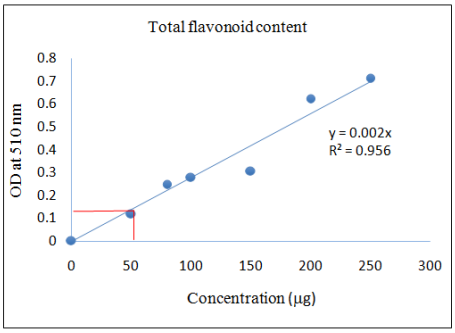



**Figure 2: Total flavonoid content of 51 µg and estimated GAE recorded using standard prepared by ascorbic acid**

**4.3. Free radical scavenging assay**

Our study clearly demonstrated that the seed extract have good antioxidant properties when assessed by DPPH and reducing power models. The percentage of DPPH depolarization is attributed to hydrogen donating ability of test compounds. Reference standard ascorbic acid showed 50% inhibition at 35 µg/ml. Methanolic extract exhibited higher antioxidant activity ($IC_{50}$ = 30 µg/ml) when compared to other extracts. The hexane and diethyl ether extracts of *Citrullus lanatus* had shown moderate activity. Hexane and diethyl ether extracts of *Citrullus lanatus* did not reach up to 50% inhibition. Reference standard showed 50% higher $IC_{50}$ value implies higher antioxidant power.

**4.4. Reducing power estimation**

The reducing capacity of *Citrullus lanatus* seed methanol extracts was compared to standard ascorbic acid. An increase in absorbance at 700 nm indicates the reducing power of the methanolic extracts. Reducing power showed significant correlation for this extracts [$R^2$ = 0.92].

**4.5. Antibacterial assay**

The antibacterial activity of methanolic extracts of *C. lanatus* Seed extracts inhibited the growth of test organisms with some exceptions and the results were recorded at different time intervals of 12, 24 and 48h **(Figure 3)**. The *C. lanatus* seed extract exhibited significant growth inhibition of *E. coli* **(Table 3)** due to a possibility of presence new clinically effective antibacterial compounds.

**Table 3: Antibacterial activity of methanol extract exhibited towards test microbe *Escherichia coli***

| Solvent used | Concentration | Zone of Inhibition 0h | 12 H | 24 H | 48 H |
|---|---|---|---|---|---|
| Streptomycin | Drug | 0.0 | 0.0mm | 0.0mm | 0.0mm |
| Extracts | 1 | 0.0 | 0.01mm | 0.13mm | 0.37mm |
| | 2 | 0.0 | 0.21mm | 0.3mm | 1.37mm |
| | 5 | 0.0 | 1.57mm | 1.75mm | 3.12mm |
| | 10 | 0.0 | 0.36mm | 2.88mm | 5.09mm |

**Figure 3: Inhibition capacity of methanol extracts at concentration of 2 □g; 5 □g and 10 □g in comparison to and drug, streptomycin (Ab).**

**4.6. 15-lipoxygenase inhibition assay**

The LOX activity was monitored as an increase in the absorbance at 234 nm indicating the formation of hydroperoxyl linolenic acid. The *C. lanatus* seed extract tested inhibited LOX in a concentration dependent manner. The highest inhibitory effect was observed for *C. lanatus* with an $IC_{50}$ of 64 µg (**Figure 4**).

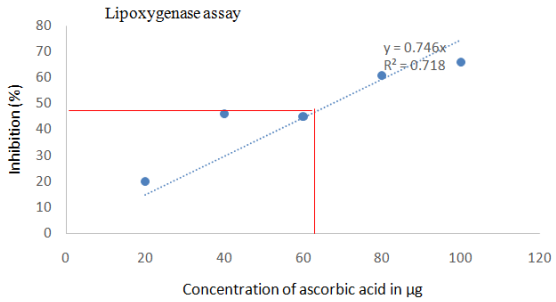


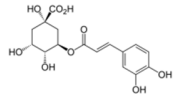
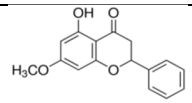
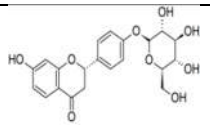
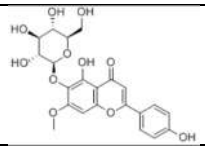
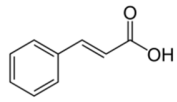
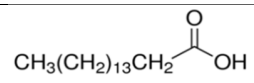
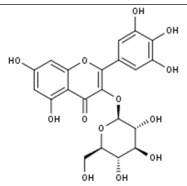

**Figure 4: 15- LOX inhibition by methanolic extract of *Citrullus lanatus* with $IC_{50}$ of 64 µg**

**4.7. Profiling of Compounds in methanol extract of *Citrullus lanatus* seeds determined by UPLC-PDA-ESI/HDMS**

Identification of metabolites based on chromatographic and in-house MS data identified compound A as chlorogenic acid; Compound B was identified palmitic acid; compound C cinnamic acid; Compound D was identified as 7-*O*-glucosyl apigenin; compound E as pinostrobin; compound F as liquiritin; compound G as

Myricelin-glucoside; compound H as penta-*O*-galoyl-β-D-glucose (**Table 4**) by comparison of retention time and MS/MS data [12].

**Table 4: Putative compounds identified by UPLC-PDA-ESI/HDMS**

| m/z RATIO | MOLECULES | STRUCTURE |
|---|---|---|
| 451 | chlorogenic acid derivative |  |
| 269 | pinostrobin |  |
| 417 | liguirilin |  |
| 431 | 7-*O*-glucosyl-apigenic |  |
| 441 | cinnamic acid derivatives | Cinnamic acid  |
| 255 | Palmitic acid |  |
| 272 | Myricetin glucoside |  |

## 5. Discussion

The quantitative estimation of the secondary metabolites may be useful in the analysis of the compounds that would be essential for the growth and development of the plant cell and also the nutritive and therapeutic properties present in the watermelon seed. The current study provides the estimated amounts of the secondary metabolites (Flavonoids, total phenols) present in the methanol seed extract of watermelon. This may provide knowledge on the growth of the seeds in watermelon and its biological activities. Further the quantitative phytochemical screening may aid in the detection of the bioactive elements that are responsible for the therapeutic properties of watermelon seeds.

In recent years, consumers are aware about their health due to heavy burden of non communicable diseases like hypertension, diabetes, cancer, cardiovascular diseases, etc. And there has been a upsurge demand for high quality fruits. Therefore, the investigation on antioxidant composition of watermelon becomes an important field of study facilitating watermelon breeding for developing quality and nutritious fruits. Thus, it is important to characterize different watermelon genotypes for such substances to identify their nutritional value depending on

different cultivars and genotypes, sampling area and fruit ripening stages. However, information on phytochemical and antioxidants is very limited in watermelon cultivars grown in India. Thus, the objective of this study was to determine the phytochemical and antioxidants among the selected genotypes of watermelon under hot arid conditions of India for identification of promising genotypes rich in antioxidants**.**

The hexane, diethyl ether, chloroform and methanol extract of the selected plant seeds of *Citrullus lanatus* exhibited antioxidant, anti-inflammatory and antimicrobial activities. Only methanol extracts of the plant showed interesting and consistent results. The results showed its radical scavenging ability of the seeds (DPPH scavenging was 29µg in methanol extract).

This might be because of wide soluble properties of low molecular and polar substances including the antioxidant active phenolic compound and flavonoids present in the seed. Hence, methanol extract alone were selected to evaluate their total phenolic content, and total flavonoids content, reducing power, antibacterial and anti-inflammatory activities. The methanol extract exhibited concentration dependent inhibitory effects on all tested *in vitro* models.

The transformation of $Fe^{3+}$ to $Fe^{2+}$ in the presence of water melon seed and the reference compound ascorbic acid was used to measure the reductive capability. The samples reduced the radical to the corresponding hydrazine when it reacted with hydrogen donors in the antioxidant principle thus, exhibiting considerable antioxidant activity by the scavenging of the cation radical. The free radical-scavenging activity of the seed constituents could be attributed to the hydrogen-donating ability. Water melon seed constituents with reducing power possess demonstrated by its ability to reduce $Fe^{3+}$ to $Fe^{2+}$.

The result of antibacterial activity of methanolic extract indicated that the plant methanol extracts exhibited the growth inhibitory effect. Antibacterial activities were observed with high concentration of the extracts than at lower concentrations. Activity even at low concentration indicates high potency of the extract against the microorganism.

Lipoxygenases (LOXs) are a family of non-heme iron-containing dioxygenases catalyzing the biosynthesis of leukotrienes. Leukotrienes function as initiators of inflammation and their inhibition is considered to be partly responsible for the anti-inflammatory activity. In the present study methanolic extracts watermelon seeds showed good anti-LOX activity with an $IC_{50}$ value of 55µg. LOX inhibition was used to evaluate anti-inflammatory activity of a few medicinal plants.

Plant phytochemicals with health benefits have been attributed to health as they cannot by synthesized by humans and they have been linked to antioxidant activity. In the present study, UPLC-DAD identified chlorogenic acid; palmitic acid; cinnamic acid; 7-*O*-glucosyl apigenin; pinostrobin; liquiritin; myricelin-glucoside; penta-*O*-galoyl-β-D-glucose among others. These are reported as the strongest natural anti-inflammatory agent. The presence of the phytochemicals in the extract could also support the therapeutic property tamarind seed for mentioned application in traditional literature of India.

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**References**

*[1] T. P. Baker, B. Corwin and L. W. Jeft, "Watermelon bacterial fruit biotechnology", European Journal of Medicinal Plant, vol. 1, no. 4, (**2012**), pp. 171-179.*

*[2] I. U. Nwankwo, C. E. Onwuakor and V. C. Nwosu, "Phytochemical analysis and antibacterial activities of Citrullus Lanatus seed against some pathogenic microorganisms", Global Journal of Medical Research: c Microbiology and Pathology, vol. 14, no. 4, (**2014**), pp. 1-10.*

*[3] M. A. C. Hannah and S. Krishnakumari, "Quantitative estimation of plant metabolites in the hot aqueous seed extract of watermelon (Citrullus vulgaris Schrad.)", Journal of Medicinal Plants Studies, vol. 3. No. 5, (2015) pp. 107-111.*

*[4] A. Nostro, M. P. Germanò, V. D'angelo, A. Marino and M. A. Cannatelli, "Extraction methods and bio autography for evaluation of medicinal plant antimicrobial activity", Letters in Applied Microbiology, vol. 30, no. 2, (2000), pp. 379-384.*

*[5] D. Krishnaiah, R. Sarbatly and A. Bono, "Phytochemical antioxidants for health and medicine: A move towards nature", Biotechnology and Molecular Biology Reviews, vol. 1, no. 5, (2007), pp. 97-104.*

*[6] M. Manika, P. Vani and K. G. Rajinder, "Estimation of nutritional, phytochemical and antioxidant activity of seeds of musk melon (Cucumis melo) and water melon (Citrullus lanatus) and nutritional*

*analysis of their respective oils", Journal of Pharmacognosy and Phytochemistry, vol. 3, no. 6, (2015), pp. 98-102.*
*[7] S. Gul, Z. Rashid and G. Sarwer, "Citrullus lanatus (watermelon) as Diuretic agent: An in vivo investigation on mice", American Journal of Drug Delivery and Therapeutics, vol. 1, no. 4, (2014), pp. 89-92.*
*[8]V. Sumam, R. Narmadha, D. Gomathi, M. Kalaiselvi and K. Devaki, "Phytochemical screening and HPTLC finger printing analysis of Citrullus lanatus (Thumb.) seed", Journal of Acute Disease, vol. 1, no. 3, (2013), pp. 122-126.*
*[9] B. Tabiri, J. K. Agbenorhevi, F. D. Wireko-Manu, D. Faustina and E. I. Ompouma, "Watermelon seeds as food: nutrient composition, phytochemical and antioxidant activity", International Journal of Nutrition and Food Sciences, vol. 5, no. 2, (2016), pp. 139-144.*
*[10] B. D. Jensen, F. M. Toure, F. M., Hamattal, M. A., Toure, F. A. and D. A. Nantoumé, "Watermelons in the sand of Sahara: cultivation and use of indigenous landraces in the Tombouctou region of Mali", Ethnobotany Research and Applications, vol. 9, no. 1, (2011), pp. 151-162.*
*[11] C. D. Deshmukh, A. M. Baheti and A. Jain, "Identification, fractionation, evaluation and physicochemical standardization of Citrullus lanatus seeds (Family: Cucurbitaceae)", International Journal of Research in Pharmacy and Chemistry, vo. 5, no. 4, (2015), pp. 607-614.*
*[12] T. R. Bharathi, S. Shailasree, K. K. S. Kumara, M. C. Madhusudhan, and H. S. Prakash, "Metabolite profiling by UPLC-PDA-ESI/HDMS and antibacterial activity of Memecylon talbotianum Brandis", Pharmacognosy Communications, vol. 6, no.4, (2016), pp. 225-231.*