In vitro ANTIOXIDANT SCREENING OF Citrullus lanatus LEAVES

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ABSTRACT

Antioxidant screening of Methanolic extract of Citrullus lanatus (MECL) was done by various in vitro methods. Preliminary phytochemical screening of MECL was done by standard procedure. In-vitro anti-oxidant activity of the MECL was estimated by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity, Phosphomolybdenum Method, Ferric Reducing Antioxidant Power (FRAP) Assay and Reducing power assay. Total Phenolic and Flavonoid content, Tannin content and estimation of Vitamin C were estimated by using Gallic acid, Quercetin, tannic acid and standard Ascorbic acid calibration curve respectively. Preliminary phytochemical screening showed the presence of carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids, sterols and absence of glycosides, volatile and fixed oil. DPPH assay, The IC\textsubscript{50} value calculated was found to be 37.12 and 27.29μg/ml for MECL and Ascorbic acid respectively. Phosphomolybdenum method showed reducing capacity of MECL and Ascorbic acid was found to be 0.357 ± 0.005 and 0.371 ± 0.005. FRAP assay exhibited absorbance of MECL and Ascorbic acid was found to be 0.876 ± 0.003 and 0.936 ± 0.002 at a concentration of 100μg/ml. The extract showed a dose dependent reducing ability. Reducing Power Assay extract possessed a reducing capacity similar to the ascorbic acid. Phenolic content (in terms mg GAE/g of extract), Flavonoids (mg Quercetin equivalent/g of extract) and Tannin content (mg Tannic acid/g of extract) present in the MECL were found to be 47.05±0.338, 89.99±0.30 and 290.9 ± 0.12 mg/g respectively. The amount of Vitamin C of MECL was found to be 34.00 ± 0.009mg/g. The Antioxidant activity of Citrullus lanatus leaves by various showed that the MECL possessed a good antioxidant property due to the presence of Vitamin-C, Poly phenolic, Tannins and Flavonoid content.

Keywords: Citrullus lanatus, In-vitro anti-oxidant, 2, 2-diphenyl-1-picryl hydrazyl (DPPH), Total Phenolic Content.
1. INTRODUCTION

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism. Free radicals may be either oxygen derived (ROS, Reactive Oxygen Species) or nitrogen derived (RNS, Reactive Nitrogen Species). The oxygen derived molecules are $O_2^-$ (Superoxide), $HO_2$ (Hydroperoxyl), ROO$^-$ (Peroxyl), RO (Alkoxyl) as free radical and $H_2O_2$ oxygen as non-radical. Nitrogen derived oxidant species are mainly NO (Nitric oxide), ONOO$^-$ (Peroxy nitrate), NO$^+$ (Nitrogen dioxide) and $N_2O_3$ (Dinitrogen trioxide).

In a normal cell, there is appropriate oxidant: antioxidant balance. However, this balance can be shifted, when production species is increased or when levels of antioxidants are diminished. This stage is called oxidative stress. Oxidative stress results in the damage of biopolymers including nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates. Oxidative stress causes serious cell damage leading to a variety of human diseases like Alzheimer’s disease, Parkinson’s disease, atherosclerosis, cancer, arthritis, immunological incompetence and neurodegenerative disorders, etc.,

Screening of In-vitro antioxidant activity done by various methods such as, Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, Nitric oxide scavenging activity assay, Total Antioxidant activity by phosphomolybdenum method and Ferric Reducing Antioxidant Power Assay (TPTZ method) etc.

Tannins, along with vitamin C help build and strengthen collagen. Flavonoids have antioxidant activity in biological systems and protect the body against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors. The flavonoid quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma while certain flavonoids also can protect low-density-lipoproteins from being oxidized, thereby playing an important role in atherosclerosis. Citrullus lanatus is well known as Watermelon plant (Family - Cucurbitaceae). Water melon is popular in indigenous system of folk medicine. It is a trailing annual plant with several herbaceous, firm and stout stems. The leaves of Citrullus lanatus is used as anti-inflammatory, analgesic, gonorrhoea, mosquitocidal and anti microbial property. Cucurbitaceae plants are known to contain therapeutic compounds such as triterpenes, sterols, curcurbitacin and alkaloids.

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.

In vitro antioxidant screening of C.lanatus seed & fruit was previously reported. But leaves are not given importance and hence the present investigation was conducted to study in-vitro antioxidant activities of leaf extract so as to make researcher to route for other pharmacological activities.

2. MATERIALS AND METHODS

Reagents: 0.1mM Diphenyl Picryl Hydrazyl Radical in Ethanol, 6% Hydrogen peroxide diluted with water in the ratio of 1:10, 0.1M Phosphate buffer (pH 7.4), 0.6M Sulphuric acid, 28mM Sodium phosphate, 4mM Ammonium molybdate, 1% Potassium Ferricyanide, 10% Trichloro acetic acid, Phosphate buffer (pH 6.6), 0.1% Ferric chloride.

FRAP Reagent contains,

a) Acetate buffer 30mM pH 3.6: Weigh 3.1g Sodium acetate trihydrate and add 16 ml of Glacial Acetic acid and make the volume to 1L with distilled water.
b) TPTZ (2, 4, 6-tripyridyl-triazine) (M.W. 312.34) 10mM in 40mM HCl

c) FeCl$_3$. 6H$_2$O (M.W. 270.30) 20mM

FRAP reagent was prepared freshly by mixing a, b & c in the ratio of 10:1:1 at the time of use.

**Instruments:** Shimadzu UV Visible spectrophotometer, Model 1800

### 2.1 Collection and preparation of extract:

The leaves of *Citrullus lanatus* were collected in Thuvarankurichi during the month of August 2013. The plant specimen was identified and authenticated as *'Citrullus lanatus'* (Cucurbitaceae) by Dr. Stephen, Senior Lecturer in Botany and Taxonomist, Dept. of Botany, The American College, Madurai, Tamil Nadu, India. The authenticated herbarium of plant has been kept in the Department of Pharmacognosy, Madurai Medical College, Madurai.

The leaves were washed thoroughly and dried in shade. The shade dried leaves were powdered and used for further studies. Extraction of leaves of *Citrullus lanatus* was carried out by washing the plants and drying at room temperature in 14 days. After that, they were filtered with sieve analyzer to get homogeneous particles and defatted with 2.5L of petroleum ether (60-80°C) by cold maceration method for 72hr. The solvent was then removed by filtration and the marc was dried. The dried marc was re-soaked with 2.5L of Methanol. The steps were performed three times and the combined filtrates were evaporated to a cohesive mass using rota vapour.

### 2.2 Preliminary phytochemical screening

The preliminary phytochemical screening helps us in identifying the type of secondary metabolites present in plants. Preliminary phytochemical screening of Aqueous and Methanolic extract of *Citrullus lanatus* leaf was carried out by using standard procedure.

### 2.3 In vitro ANTIOXIDANT ACTIVITY

#### 2.3.1 Free radical Scavenging activity using Diphenyl picryl hydrazyl (DPPH) free radical

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts.

**Principle:** A simple method that has been developed to determine the antioxidant activity of plants utilizes the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 520nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

\[
\text{DPPH} + \text{AH} \rightarrow \text{DPPH} - \text{H} + \text{A}
\]

**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 Ethanol in case of blank. The reaction was allowed to be completed in the dark for about 30min. Then the absorbance was measured at 517nm. Vitamin C was used as standard. The percentage scavenging was calculated using the formula 

\[
\left(\frac{\text{Control} - \text{Test}}{\text{Control}}\right) \times 100
\]

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 (IC$_{50}$) was calculated using linear regression analysis. A triplicate reading was taken and average was calculated.
2.3.2. Total antioxidant activity by Phosphomolybdenum Method\textsuperscript{31,32}

**Principle:** Phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate Mo (V) complex at acidic pH and the absorbance was measured at 695nm. This method is used to determine the total antioxidant activity of samples.

\[
\text{Mo}^{6+} \rightarrow \text{Mo}^{5+}
\]

**Procedure:** An aliquot of 0.3ml of different concentrations of sample solution was combined with 2.7ml of the reagent solution (H\textsubscript{2}SO\textsubscript{4}, Sodium phosphate and Ammonium molybdate). In case of blank, 0.3ml of Ethanol was used in place of sample. The tubes were incubated for 95°C for 90min. After the mixture was cooled to room temperature, the absorbance was measured at 695nm 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).

2.3.3 Reducing power assay\textsuperscript{31}

**Principle:** This is a spectrophotometric method and is based on the principle that an increase in absorbance of the reaction mixture as concentration increase indicates an increased antioxidant activity. The assay is based on the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the formation of Prussian blue colour complex when treated with ferric chloride. The absorbance of the blue complex is measured at 700nm.

**Procedure:** Various concentration of MECL was mixed with 0.75ml Phosphate buffer and 0.75ml Potassium ferricyanide [K\textsubscript{3}Fe (CN\textsubscript{6})], then the mixture was incubated at 50°C for 20 min. 0.75ml of Trichloro acetic acid was added to the mixture, which was then centrifuged at 3000rpm for 10min. Finally 1.5ml of the supernatant solution was mixed with 1.5ml of distilled water and 0.1ml of Ferric chloride (FeCl\textsubscript{3}) and absorbance was measured at 700nm in a UV-Visible Spectrophotometer. Ascorbic acid was used as standard and Phosphate buffer used as blank. The absorbance of the final reaction mixture of three parallel experiments was expressed as mean ± standard error of mean. Increased absorbance of the reaction mixture indicates stronger reducing power.

2.3.4 Ferric Reducing Antioxidant Power (FRAP) Assay\textsuperscript{32}

The ferric reducing antioxidant power assay measures the potential of antioxidants to reduce the Fe\textsuperscript{3+} and 2,4,6 Tripyridyl-triazine (TPTZ) complex present in stoichiometric excess to the blue coloured Fe\textsuperscript{2+} complex which increases the absorbance at 593nm.

**Principle:** At low pH, reduction of Ferric Tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) can be monitored by measuring the change in absorption at 593nm. The reaction is non-specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe III to Fe II) ion formation. The change in absorbance is therefore, directly related to the combined or “total” reducing power of the electron donating antioxidants present in the reaction mixture.

**Procedure:** MECL was dissolved in Methanol to get a stock solution containing 1mg/ml. Varying quantities of the stock solution were added to 3ml of working FRAP reagent and absorbance was measured at 0min after vortexing at 593nm. Thereafter samples were placed at 37°C in water bath and absorption was again measured after 4min. Ascorbic acid was used as standard.
2.4. Quantitative estimation of phytoconstituents

2.4.1. Estimation of total Phenolic content

Phenols are widespread in nature and are important constituents of medicinal plants. They range from simple structures with one aromatic ring to highly complex polymeric substances such as Tannins, Flavonoids, Anthraquinone and Coumarins. Phenolic substances are water soluble and they have been reported to have multiple biological effects, including antioxidant activity.

The calibration curve of concentration versus absorbance was generated for Gallic acid at different concentrations (2, 4, 6, 8, 10µg/ml) which was used as a standard. The amount of phenol present can be determined by linear regression analysis. The total Phenolic content of the MECL was determined by Folin-Ciocalteau reagent and it was expressed as milligram of Gallic acid equivalent (GAE) per g of extract.

2.4.2. Total Flavonoid content estimation

Flavonoids are widely distributed in nature. It consists of one Benzene–gamma – pyrone structure. They have ability to complex with metal ions and act as an antioxidants and bind to proteins such as structural proteins and enzymes. The different classes within the groups are distinguished by additional oxygen containing heterocyclic rings and hydroxyl groups which includes Flavones, Flavanones, Flavonols, Isoflavones, Catechin, Anthocyanidins, Leuco anthocynadins, Chalcones and Aurones.

The Aluminum chloride colorimetric technique was used for estimation of total Flavonoid content. The intensity of the colour is proportional to the amount of Flavonoids and can be estimated as Quercetin equivalent at wavelength of 415nm. The amount of antioxidant and has been shown to regenerate Flavonoids present was determined by linear regression analysis. The total Flavonoid content in MECL was expressed as mg of Quercetin equivalents per g of extract.

2.4.3 Estimation of Tannins

Tannins are naturally occurring polyphenolic compounds of varying structure. Tannins are having antioxidant and antimicrobial activities and also used as antiseptic and astringents. They are divided into two main groups namely hydrolysable and condensed. Hydrolysable tannins contain a polyhydric alcohol and Condensed tannins are mostly Flavonols.

**Principle:** The tannins are estimated by Folin-Denis Method. This is based on the non stoichiometric oxidation of the molecules containing a phenolic hydroxyl group. Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce a highly colored blue solution. The intensity is directly proportional to the amount of Tannins and measured in a spectrophotometer at 700nm.

**Procedure:** About 0.2ml of MECL was pipetted into test tubes. To this, 0.5ml of Folin-Denis reagent and 0.8ml of distilled water was added. The tubes were kept aside for 15min. To this, 1ml of Sodium carbonate solution was added and the remaining volume was made up with 7.5ml of distilled water. Then the tubes were shaken and the absorbance was recorded at 700nm after 30min. Tannic acid, used as a standard was taken at different concentration i.e 2, 4, 8, 12, 16, 20µg/ml in different test tubes and the procedure adopted above was followed. The calibration curve for Tannic acid was plotted using concentration versus absorbance. A linear regression equation was calculated and the equation was used to calculate the amount of total Tannins as Tannic acid equivalent. The amount of Tannin content is expressed in mg/g of extract.

2.4.4. Estimation of Vitamin C

Vitamin C is also an important physiological other antioxidants within the body, including
α-Tocopherol (Vitamin E). Vitamin C might help to prevent or delay the development of certain cancers, cardiovascular disease, and other diseases in which oxidative stress plays a causal role. In addition to its biosynthetic and antioxidant functions, vitamin C plays an important role in immune function and improves the absorption of non-heme iron, the form of iron present in plant-based foods.

**Principle:** The keto group of Ascorbic acid undergoes a condensation reaction with 2, 4 Dinitro phenyl hydrazine to form a hydrazone which is orange yellow and has an absorbance of about 520nm.

**Procedure:** Ascorbic acid was weighed and dissolved in water to get stock solution of 1mg/ml. Further dilutions were made to get the concentrations ranging from 40-200µg/ml. To 1ml of sample 0.5ml of Dinitro phenyl hydrazine solution was added and incubated for 3hr at 37°C. After 3hr, 2.5ml of 85% Sulphuric acid was added and the absorbance was measured after 30min at 520nm. A calibration curve was constructed by plotting concentration versus absorbance of Ascorbic acid. The procedure was repeated for the plant extract as above and the absorbance was measured at 520nm after 3hr. The amount of Vitamin C can be determined by linear regression analysis and it was expressed as mg/g of extract.

### 3. RESULTS AND DISCUSSION

#### 3.1 Preliminary phytochemical screening

<table>
<thead>
<tr>
<th>S. No.</th>
<th>TEST</th>
<th>Aqueous extract</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Test for Carbohydrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Molisch’s test</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>b. Benedict’s test</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>c. Fehling’s test</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Test for Alkaloids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Mayer’s reagent</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>b. Dragendorff’s reagent</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>c. Hager’s reagent</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>d. Wagner’s reagent</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Test for Phytosterols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Salkowski’s test</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>b. Libermann- burchard’s test</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Test for Glycosides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Anthraquinone glycosides</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>i) Borntrager’s test</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ii) Modified Borntrager’s test</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Test for Proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Millon’s test</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>b. Biuret test</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>Amino acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Ninhydrin test</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Test for Mucilage</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
7. **Test for Flavonoids**
   a. Shinoda test +
   b. Alkali test +
   c. Acid test +

8. **Test for Terpenoids** +

9. **Test for Phenolic compounds**
   a. 5% Ferric chloride solution +
   b. Lead acetate solution +
   c. Bromine water +
   d. Acetic acid solution +
   e. Dilute iodine solution +
   f. Tannic acid +

10. **Test for Tannins**
    FeCl₃ test +

11. **Test for Saponins**
    Foam test +

12. **Test for Volatile oils** -

(+ Present, (-) Absent)

3.2 *In vitro* ANTIOXIDANT ACTIVITY

3.2.1. Free radical Scavenging activity using 2, 2-Diphenyl-1-picryl Hydrazyl (DPPH) free radical

The results obtained for the free radical scavenging activity against DPPH radical was presented in Table 2 and the graphical representation was presented in Fig. 01.

**Table 2: Percentage inhibition of MECL and standard Ascorbic acid against DPPH at 517nm**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. in µg/ml</th>
<th>Percentage inhibition of Ascorbic acid</th>
<th>Percentage inhibition of MECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>48.91 ± 0.60</td>
<td>32.55 ± 0.32</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>58.03 ± 0.50</td>
<td>42.32 ± 0.48</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>67.86 ± 0.27</td>
<td>64.14 ± 0.61</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>79.49 ± 0.30</td>
<td>71.63 ± 0.29</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>85.36 ± 0.29</td>
<td>78.16 ± 0.29</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>27.29 µg/ml</td>
<td>37.12 µg/ml</td>
<td></td>
</tr>
</tbody>
</table>

*mean of three readings ± SEM
From the Table 2, it can be seen that the MECL showed a percentage inhibition of $78.16 \pm 0.29$ while Ascorbic acid showed a percentage inhibition of $85.36 \pm 0.29$ at a concentration of 80µg/ml. The IC$_{50}$ value calculated using the linear regression analysis was found to be 37.12 and 27.29µg/ml for MECL and Ascorbic acid respectively. The extract possessed a good radical scavenging capacity.

### 3.2.2. Antioxidant activity by Phosphomolybdenum method

The results obtained for the phosphomolybdenum activity of MECL and standard Ascorbic acid was tabulated in Table 3 and the graphical representation was presented in Fig. 02.

**Table 3: Absorbance of MECL and standard Ascorbic acid in Phosphomolybdenum method**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. in µg/ml</th>
<th>Absorbance of Ascorbic acid</th>
<th>Absorbance of MECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.66</td>
<td>$0.085 \pm 0.005$</td>
<td>$0.061 \pm 0.002$</td>
</tr>
<tr>
<td>2</td>
<td>33.33</td>
<td>$0.165 \pm 0.004$</td>
<td>$0.132 \pm 0.004$</td>
</tr>
<tr>
<td>3</td>
<td>50.00</td>
<td>$0.206 \pm 0.008$</td>
<td>$0.189 \pm 0.009$</td>
</tr>
<tr>
<td>4</td>
<td>66.66</td>
<td>$0.323 \pm 0.004$</td>
<td>$0.306 \pm 0.004$</td>
</tr>
<tr>
<td>5</td>
<td>83.33</td>
<td>$0.371 \pm 0.005$</td>
<td>$0.357 \pm 0.005$</td>
</tr>
</tbody>
</table>

*mean of three readings ± SEM
From the Table 3 and Fig. 2, it can be seen that the extract possessed a reducing capacity similar to the Ascorbic acid and both of them showed an increase in absorbance.

3.2.3. Reducing Power Assay
The results obtained for the Reducing Power assay of and standard Ascorbic acid was presented in Table 4 and the graphical representation was presented in Fig. 03.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. in μg/ml</th>
<th>Absorbance of Ascorbic acid</th>
<th>Absorbance of MECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>0.745 ± 0.012</td>
<td>0.594 ± 0.005</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>0.820 ± 0.003</td>
<td>0.755 ± 0.006</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>0.930 ± 0.002</td>
<td>0.820 ± 0.003</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>0.958 ± 0.059</td>
<td>0.860 ± 0.003</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>1.052 ± 0.007</td>
<td>0.874 ± 0.007</td>
</tr>
</tbody>
</table>

*mean of three readings ± SEM
3.2.4. Ferric reducing antioxidant power assay (FRAP assay)

The results obtained for the Ferric Reducing Antioxidant Power assay was presented in Table 5.

**Table 5: Ferric reducing anti-oxidant power assay of Ascorbic acid and MECL**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. in µg/ml</th>
<th>Absorbance of Ascorbic acid</th>
<th>Absorbance of MECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.5</td>
<td>0.457 ± 0.001</td>
<td>0.376 ± 0.002</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>0.576 ± 0.004</td>
<td>0.475 ± 0.005</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>0.667 ± 0.003</td>
<td>0.589 ± 0.003</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>0.821 ± 0.001</td>
<td>0.747 ± 0.002</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>0.936 ± 0.002</td>
<td>0.876 ± 0.003</td>
</tr>
</tbody>
</table>

*mean of three readings ± SEM

From the Table 5, it can be seen that the MECL showed an absorbance of 0.876 ± 0.003 for a concentration of 100µg/ml while Ascorbic acid showed an absorbance of 0.936 ± 0.002 at a concentration of 100µg/ml. The extract showed a dose dependent reducing ability. The graphical representation of the reducing power activity of the MECL and Ascorbic acid were presented in Fig. 04.
3.3. Quantitative estimation of phytoconstituents

3.3.1 Estimation of Phenolic content

Phenolic content of MECL and calibration curve of Gallic acid was presented (Table 6 & Fig. 05).

Table 6: Total Phenolic content of MECL in terms of Gallic acid equivalents

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. of Gallic acid in µg/ml</th>
<th>Absorbance at 760nm</th>
<th>Conc. of extract in µg/ml</th>
<th>Absorbance at 760nm*</th>
<th>Amount of total Phenolic content in terms mg GAE/g of extract*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.229 ± 0.010</td>
<td>50</td>
<td>0.256±0.004</td>
<td>43.90±0.304</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.452 ± 0.006</td>
<td>100</td>
<td>0.578±0.004</td>
<td>50.20±0.373</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.695 ± 0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.918 ± 0.031</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>1.162 ± 0.028</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* mean of three readings ±SEM

The linear regression equation was found to be $y=0.116x-0.004$ while the correlation coefficient was found to be 0.9998. The amount of Phenolic content present in the extract in terms mg GAE/g of extract was found to be 47.05±0.338 by using the above linear regression equation.
Calibration curve of gallic acid

\[ y = 0.116x - 0.004 \]

\[ R^2 = 0.9998 \]

-0.2 0 0.2 0.4 0.6 0.8 1 1.2 1.4
0 5 10 15
Conc. in mcg/mL
Absorbance
Absorbance
Linear
(Absorbance)

Fig. 05: Calibration curve of Gallic acid for estimation of total Phenolic content

Polyphenols are naturally occurring compounds largely found in the herbs and medicinal plants. Phenolic compound may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress \(^{[42]}\). Consumption of plant polyphenols offer protection against development of diabetes, cancers, cardiovascular diseases and neurodegenerative diseases.\(^{43,44}\)

3.3.2. Estimation of total Flavonoids

Total Flavonoid estimation MECL & Calibration curve of Quercetin was presented (Table 7 & Fig. 06).

Table 7: Total Flavonoid content of MECL in terms of Quercetin equivalents

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. of Quercetin in µg/ml</th>
<th>Absorbance at 415nm</th>
<th>Conc. of Methanolic extract in µg/ml</th>
<th>Absorbance at 415nm*</th>
<th>Amount of total Flavonoid content in terms mg Quercetin equivalent/ g of extract*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>0.589 ± 0.01</td>
<td>50</td>
<td>0.090±0.001</td>
<td>86.55±0.21</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>1.151 ± 0.04</td>
<td>100</td>
<td>0.243±0.003</td>
<td>93.44±0.39</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>1.710 ± 0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>2.390 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>3.112 ± 0.03</td>
<td></td>
<td></td>
<td>Average 89.99±0.30</td>
</tr>
</tbody>
</table>

* mean of three readings ± SEM
The linear regression equation was found to be 
\[ y = 0.0307x - 0.0432 \]
while the correlation was found to be 0.9974. The amount of Flavonoid content present in the extract in terms mg Quercetin equivalent/g of extract was found to be 89.99 ± 0.30 by using the above linear regression equation. More than 4,000 varieties of flavonoids have been identified, many of which are responsible for the therapeutic activity in humans. Quercetin, Myricetin, Catechin etc., are some most common flavonoids \cite{45}. Quercetin is known to possess strong anti diabetic activity. Recently reported Quercetin has ability to protect the alterations in diabetic patients during oxidative stress. Quercetin significantly protected the lipid peroxidation and offer antioxidant effect in Diabetes.\cite{46}

### 3.3.3 Total Tannin estimation

Total Tannin estimation of MECL & calibration curve of Tannic acid was presented (Table 8 & Fig. 07).

**Table 8: Total Tannin content in MECL in terms of Tannic acid equivalents**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. of Tannic acid in µg/ml</th>
<th>Absorbance at 760nm</th>
<th>Conc. of Methanolic extract in µg/ml</th>
<th>Absorbance at 760nm*</th>
<th>Amount of total Tannin content in terms mg Tannic acid/g of extract*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>0.098 ± 0.020</td>
<td>10</td>
<td>0.060±0.03</td>
<td>260.60±1.51</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0.183 ± 0.010</td>
<td>20</td>
<td>0.131±0.07</td>
<td>292.42±2.00</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>0.203 ± 0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>0.361 ± 0.200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.451 ± 0.100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* mean of three readings ±SEM

\[ \text{Average} \ 276.51±1.75 \]
The linear regression equation was found to be \( y = 0.022x + 0.003 \) while the correlation was found to be 0.9997. The amount of Tannin content present in the MECL in terms of mg Tannic acid/g of extract was found to be 276.51 ± 1.75 by using the above linear regression equation.

There is a clear link between hyperglycemia and active oxygen/nitrogen species in diabetes [47]. Accumulation of Reactive Oxygen Species (ROS) due to oxidative stress which easily oxidize vital cellular components such as lipids, proteins and DNA [48].

### 3.3.4 Estimation of Vitamin C

Vitamin C content of MECL & calibration curve of standard Ascorbic acid was presented (Table 9 & Fig. 08).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. of Ascorbic acid in µg/ml</th>
<th>Absorbance at 520nm</th>
<th>Conc. of Methanolic extract in µg/ml</th>
<th>Absorbance at 520nm</th>
<th>Amt of Vitamin C present mg/ g of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>0.135 ± 0.000</td>
<td>100</td>
<td>0.076 ± 0.004</td>
<td>237.03 ± 0.006</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>0.265 ± 0.015</td>
<td>200</td>
<td>0.137 ± 0.007</td>
<td>253.70 ± 0.006</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>0.346 ± 0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>0.468 ± 0.011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>0.525 ± 0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*mean of three readings ± SEM

The linear regression equation was found to be \( y = 0.0027x + 0.012 \) and a correlation coefficient of 0.9982. The amount of Vitamin C content present in the MECL was found to be 245.37 ± 0.006mg/g by using the above linear regression equation. Oxidative stress may be linked to tissue damage and the development of regenerative disorders. Oxidative stress may be a common pathway linking diverse mechanisms for the pathogenesis of complications in diabetes. Vitamin C act as
antioxidant to scavenge free radicals which responsible for pathogenesis of Diabetes.\textsuperscript{49}

CONCLUSION

The Antioxidant activity by various methods showed MECL possessed a good antioxidant property due to the presence of Vitamin-C, polyphenolic, Tannins and flavonoid content. The leaf extract of \textit{Citrullus lanatus} contain bioactive compounds such as flavanoid, phenolic compound, tannin, triterpenes, sterols and alkaloids, vitamins. The extract may serve as a lead medicinal molecule to synthesize various semi-synthetic drugs to treat various lives threatening disease.

Conflict of interest statement: We declare that we have no conflict of interest.

REFERENCES

19. Rahman H, Priyanka P, Lavanya P, Srilakshmi N, Rajesh Kumar P. A review on ethno botany, phytochemistry and


