ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Evaluation Of Antibacterial And Antioxidant Property Of Active Ingredient Of Watermelon Peel Extract.

**Gladvin G*, and K V Santhi Sri.**

Department of Food and Nutritional Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, 522510 Andhra Pradesh, India.

**ABSTRACT**

Watermelon is a member of the *Cucurbitaceae* family and rich in vital minerals, vitamins, and many bioactive phytochemicals. Watermelon fruit rich in phytochemicals exhibit a wide range of pharmacological activities and ideal nutrient-rich fruit. Originally, watermelon is a native of Africa as the dried environment; but in modern times, it cultivated in every part of the world. Now, India and China are leading watermelon producers, and several new hybrid species of watermelon were developed. The key phytochemicals present in watermelon fruit includes lycopene, vitamin C, β-carotene and polyphenols. The native wild type of watermelon species differ in phytochemicals and other nutrients from hybrid species of watermelon. The major bioactive phytochemicals present in watermelon fruit peel includes vitamin C, E, lycopene, and β-carotene responsible for the antioxidant property. Watermelon is also a rich source of B vitamins, especially $B_1$ and $B_6$, as well as minerals such as potassium and magnesium. Flavonoids such as kaempferol, quercetin, and rutin and sterols such as β-sitosterol are vital active phytochemicals present in watermelon fruit peel. The fruit is an ideal source of water and sugars as well. The therapeutic potential of watermelon is mainly due to diverse phytochemicals present in fruit pulp. The initial findings have demonstrated the cholesterol-lowering and antioxidant capacity of watermelon fruit pulp. However, research studies are required to understand the precise compounds responsible for such diverse biological activities. The present study was designed to extract active fruit phytochemicals present in watermelon; the study also emphasizes antioxidant and antibacterial properties of the extract. Phytochemical extraction using various solvents shows methanolic extract is ideal for a wide range of active compounds and extraction yield as well. The extract has demonstrated excellent antibacterial and antioxidant activity of fruit extract. The study provides a scientific basis for the diversity of active compounds present in watermelon fruits and activity.

**Keywords;** Watermelon peel extract, antioxidant, antibacterial, phytochemicals, and free radical scavenging assay.

https://doi.org/10.33887/rjpbcs/2020.11.5.4

***Corresponding author***

ISSN: 0975-8585

## INTRODUCTION

Fruits and vegetables are rich in active plant secondary metabolites with diverse pharmacological properties [1]. These secondary metabolites remain part of traditional medicine for many centuries, and in modern times. The advancement of technology helped in the profiling of each and individual molecules for extended therapeutic applications [2]. Among edible fruits and vegetables, watermelon represents itself as a versatile fruit rich in minerals and vitamins. Watermelon (*Citrullus lanatus)* is a fruit and key member of *family Cucurbitaceae.* Originally, watermelon as the fruit is native of Africa; however, it is also grown commercially in the entire geographical region across the world [3]. The plant ideally grows in a geographical area with high climatic temperature and low annual rainfall. The fruit acquires a high content of water, mineral, and other nutrients such as vitamins and sugars. Watermelon has a close resemblance with cucumber, pumpkin, squash, and gourds and one of the most edible fruit in the world due to its versatile nutritional values. There are several native and genetically engineered variety of watermelons grown in different parts of the world [4]. Study of fruit's nutritional profile reported the rich presence of water (92%), carbohydrates (7.5%), out of which 6.2 % are sugars and 0.4 % dietary fiber. The fruit is rich in carotenoid, vitamin C, citrulline, carotenoid, flavonoids, and fat and cholesterol-free [5]. The presence of lycopene enables fruit as a rich source of B vitamins, especially $B_1$ and $B_6$, as well as minerals such as potassium and magnesium. Though watermelon is cultivated across the globe, however, India and China are the leading producers of different variety of watermelon [6].

Over the last few decades, much emphasis was given in profiling active constituents of watermelon for various therapeutic applications [7]. The extraction of active metabolites present in fruits using different solvent systems results in a series of active secondary metabolites. The antioxidant activity of fruits is mainly due to the presence of high content of vitamin C, and the study shows that vitamin C concentration ranged between 2.50 and 8.30 mg/100 g in watermelon fruit pulp [8]. The composition of various fruit secondary metabolites differs in fresh watermelon pulp, and fresh watermelon rind reported 15 and 76.9 mg/100 g, respectively. The high content of vitamin C, E, lycopene, and β-carotene are associated with antioxidant properties [9]. Research findings have also demonstrated that fruit is rich in flavonoids and sterols content. Fruit contains active flavonoids such as kaempferol, quercetin, and rutin as well. The β-sitosterol one of active sterol (phytosterol) present in fruit varies from species to species and fruit part as well reported 25-76mg/100g of fruit [10, 11]. The anticholesterolemic agent, lowering cholesterol potential of fruit, is mainly due to the presence of β-sitosterol. With the diverse active plant metabolites present in fruit and wide range of therapeutic applications, the present study was designed to extract the active fruit secondary metabolites and evaluation [12, 13]. The study emphasizes antioxidant and antibacterial profiling of extracted fruit secondary metabolites using different bacterial spices.

## MATERIALS AND METHODS

All the consumables and chemicals used in the present work were purchased from Sigma Aldrich India and Hi-Media India of research-grade. During the study, standard microbiological and molecular protocols were followed. The bacterial species used for the study were stored at $-40^0$C. The conventional media and growth parameters were opted during the entire study. For the extraction of active fruit secondary metabolites from watermelon, two species were used i.e. *Citrulus Lanatus* (Indian watermelon) and *Citrullus lanatus* (Chinese watermelon). Three different solvent systems with an increasing concentration of solvents were used in the study for the extraction process.

### Extraction of active fruit metabolites

Fresh and fully grown watermelon fruits from both species i.e. *Citrulus Lanatus* (Indian watermelon wild) and *Citrullus lanatus* (Chinese watermelon hybrid), were selected in this study. The fruit was washed with 70% ethanol and subjected to peel extraction. The spraying 70% ethanol ensures any bacterial and fungal contamination in fruit peel. The peel of fruit was recovered and air-dried in shade for a week. The air-dried peel was powdered using pestle and mortar. The dried peel of both the fruit *Citrulus Lanatus* (Indian watermelon) and *Citrullus lanatus* (Chinese watermelon) with weight 110 gm and 85 gm were used for the extraction process. Two soxhlet extraction apparatus setup was assembled and peel was placed on the extraction column allowed for saturation with solvent. A 200 ml of solvent containing Methanol, Ethyl acetate

and Chloroform (2: 1: 1) was poured into the bottom of the soxhlet extraction apparatus via column that contains watermelon peel powder. The solvent was heated up to 65°C and allowed to cool down via a circulating water system. The extraction process was run for 6-8 hours and fruit active metabolite extract was collected in the bottom of the soxhlet extraction apparatus flask via a siphon tube. The extraction of watermelon fruit active ingredient depends on the affinity of molecules with the solvent system. The collected active metabolites were filtered into separate containers via Whatman filter paper 1. Here, in the study, both the watermelon peel extract was obtained in a separate tube and subjected to further studies. The extraction yield of watermelon peel extract of both the species was calculated using

**% extraction yield = (Weight of extract/Weight of sample) x100**

**Phytochemicals analysis**

Watermelon fruit is rich in phytochemical of different classes, including sterol, polyphenols, saponin, resins, and tannins, etc. Phytochemical analysis and characterization provide a detailed analysis of such compounds in the fruit. Crude extract of fruit collected in soxhlet apparatus using solvent system Methanol, Ethyl Acetate and Chloroform was subjected to phytochemical analysis with several biochemical tests including, alkaloid Test (Dragendroff's), flavonoid test (Shinoda test), saponin test (foam test), quinone test, tannin test, terpenoids and steroids, phenol test, coumarin and test for glycosides. For alkaloid Test 2 ml, watermelon extract was acidified with few drops of dilute hydrochloric acid. To this acidic medium, 1ml of dragendroff's reagent (potassium bismuth Iodide) was added. An orange or reddish-brown precipitate produced indicates the presence of alkaloids. The presence of flavonoids was confirmed by treating the alcoholic plant extract with few fragments of magnesium ribbon and hydrochloric acid. The reaction mixture develops pink, the scarlet or crimson red color indicating the presence of flavonoids. For saponin test, 1 ml of each extract shaken with 10 ml of distilled water and it was agitated in a graduated cylinder for 10 min, the formation of persistent honey-like foam indicated the presence of saponin. For the existence of quinine in watermelon fruit extract, a small amount of extract was treated with concentrated HCL and observed for the formation of a yellow color precipitate. 2 ml of each extract adds few drops of 10% lead acetate were added. The appearance of a white precipitate indicates the presence of tannins. 50% H2SO4 is attached along the sides of the test tube containing a mixture of methanolic HCL and acetic anhydride. If there is any change in color, from green to blue-green (sometimes via red or blue) indicates the presence of terpenoids and steroids. When 0.5 ml of $FeCl_3$ (W/V) solution was added to 2 ml, 0f rest solution formation of an intense color indicated the presence of phenols. To the ethanolic extract, a few drops of alcoholic sodium hydroxide were added. The formation of the yellow color indicated the presence of coumarins. To the ethanolic extract mixed with a little anthrone on a watch glass. A few drops of Conc.$H_2SO_4$ were added and warmed gently over the water bath. The presence of glycosides in watermelon was identified by dark green formation [14].

**Antibacterial activity analysis**

The zone of inhibition analysis evaluated the antibacterial activity of watermelon fruit extract. The fruit extract may contain several compounds, and the antibacterial activity of active constituent is due to one of them. Here in the present study, we have analyzed analysis using the crude extract. We have used six different bacteria species for antibacterial activity analysis. The bacterial species, including *Bacillus subtills, Pseudomonas species Staphylococcus aureus, Klebsiella pneumonia and Proteus mirabilis* were spread on nutrient agar plate and well were created on the plate. The crude extract of both the species of watermelon was added nearly 20µl along with the control solvent alone. We have used different combinations of solvent in the watermelon fruit extraction process, and the hence different solvent extract was added into well on the plates. Plates were incubated overnight at $37^0$C, and the zone of inhibition was measured. The zone of inhibition signifies the presence of active fruit constituents responsible for antibacterial activity. The zone of inhibition was measured and compared with control and both the species of watermelon fruit extract [15].

**Antioxidant activity analysis**

For antioxidant analysis of watermelon, peel extract of both the species was carried out using DPPH radicals scavenging assay. DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution.

The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometer. The watermelon peel extracts were dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution (10 mg/mL) for antioxidant assays. The DPPH radical solutions were prepared with concentration 120µM with 95% ethanol. The watermelon extracts for antioxidant assay were prepared by diluting twice in 96-well microtitre plates. An aliquot of extract (10 µL) was mixed to 195µL of ethanolic DPPH in 96-well microtitre plates. The reaction mixtures were incubated at room temperature for 30 min in the dark and absorbance was measured at 517 nm using a spectrophotometer. The $IC_{50}$ value (µg/mL) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from the linear regression analysis [16]. The free radical scavenging activity was calculated as follows:

**% Free Radical Scavenging activity = [(Blank – Sample / Blank] ×100%**

Where: Blank was the absorbance of without samples, and Sample was the absorbance of the test sample. The values are expressed as the means of triplicate analyses. The antioxidant activity is a function of percentage inhibition calculated for both extracts collected from both species of the watermelon fruit.

## RESULTS AND DISCUSSION

The extraction of active metabolites from watermelon peel was successfully carried out and characterized. The extract yield was calculated for watermelon peel for all solvents and for both of the species. We report here maximum extract yield with methanol in both the species of watermelon Indian (wild) and Chinese (hybrid) i.e. 36.5% and 40.25%, respectively. The yield was calculated using the formula given above. The Chinese/hybrid species of watermelon have shown comparatively higher extraction yield than wild type i.e. Indian species. Further, extract from both the species of watermelon demonstrated the presence of active phytochemical constituents, as shown in the antibacterial and antioxidant analysis [17]. Our findings show the presence of active metabolites in watermelon fruits extract and shown higher extraction yield from previous studies by Hind et al 2017 [18].

**Table 1; Extraction yield of watermelon peel extract from different species and solvent system. Here we have used two species Indian (*Citrulus Lanatus* Wild type) and Chinese (*Citrullus lanatus* hybrid) with three solvent system methanol, ethyl acetate, and chloroform.**

| Watermelon species | Solvent | Yield (%) |
|---|---|---|
| *Citrullus lanatus (Wild type Indian Species)* | Methanol | 26.70 |
| | Ethyl acetate | 11.20 |
| | Chloroform | 7.20 |
| *Citrullus lanatus (Hybrid type Chinese Species)* | Methanol | 40.25 |
| | Ethyl acetate | 13.55 |
| | Chloroform | 9.10 |

### Phytochemical analysis

The phytochemicals analysis was carried out for both the species of wild watermelon type (Indian) and hybrid (Chinese). The study was carried out for the extract obtains from the different solvent systems in both the species. As a result shown in the table below, the methanolic extract of hybrid species of watermelon showed a large number of secondary metabolites. The active watermelon fruit metabolites reported were alkaloids, flavonoids, saponin, quinine, and phenolic compounds. The wild type species of watermelon also had shown the presence of flavonoids, saponins and sterols. These results indicate a higher affinity of watermelon fruit metabolites from hybrid species in a methanolic solvent. Being a genetically modified plant, there is a high possibility of altered phytochemicals in quantity and diversity. Our finding finds a close resemblance with the study carried out in Choksi and Joshi in 2007, where a possible diverse phytochemicals presence in watermelon fruit was suggested [19]. The study in 2001 by Adawy et al showed a pattern of active phytochemicals in watermelon species and other similar species as well [20]. The study also showed the

presence of diverse, active phytochemicals in watermelon, and we report here more diverse active phytochemicals in watermelon fruit extract more precisely in the hybrid plant. The diversity of active phytochemicals in hybrid over wild type is due to genetic modifications for higher nutritional values and habitat purposes also.

**Table 2; The chart represents the presence of various active phytochemicals in watermelon peel extract from different species and solvent systems.**

| Photochemical | HC | HE | HM | WC | WE | WM |
|---|---|---|---|---|---|---|
| **Alkaloid** | - | - | + | - | - | - |
| **Flavonoid** | - | - | + | -- | - | + |
| **Saponin** | - | - | + | - | - | + |
| **Quinone** | - | - | + | - | - | - |
| **Tannin** | - | + | - | - | + | - |
| **Steroids** | - | - | - | - | - | + |
| **Phenolic** | - | - | + | - | - | - |
| **Coumarin** | - | - | + | - | - | - |
| **Resins** | - | - | - | + | - | - |

HC- Hybrid Chloroform, HE- Hybrid Ethyl Acetate, HM-Hybrid Methanol. WC- Wild Chloroform, WE-Wild Ethyl Acetate, WM- Wild Methanol

**Antibacterial activity**

The antibacterial activity of watermelon peel extract from both the species was evaluated using the zone of inhibition assay. As a result, shown in table 3 and figure 1, the zone of inhibition of various exact of both species of watermelon fruit varies among the test bacteria species compare to the control study. The control was used here in the study was the solvent system used for the extraction of active fruit phytochemicals. The large zone of inhibition of control in both the species of watermelon is due to the nature of the solvent itself possess antibacterial activity. However, the study shows here all three sets of solvent possess active phytochemicals as shown significant zone of inhibition against all six tested bacterial species including *Bacillus subtilis, Pseudomonas species staphylococcus aureus, Klebsiella pneumoniae and Protieus mirabilis.*

**Table 3; The chart represents the antibacterial activity of watermelon peel extract from the different solvent with control. The antibacterial activity was measured as a function of the zone of inhibition.**

| Bacterial Species | Zone of Inhibition (mm) | | | | | | Control |
|---|---|---|---|---|---|---|---|
| | MH | MW | EW | CW | CH | EH | |
| *Bacillus subtilis* | 21 | 19 | 21 | 12 | 18 | 18 | 24 |
| *Pseudomonas species* | 20 | 24 | 20 | 15 | 24 | 21 | 24 |
| *Staphylococcus aureus* | 21 | 18 | 26 | 14 | 18 | 19 | 29 |
| *Klebsiella pneumoniae* | 21 | 19 | 21 | 10 | 14 | 18 | 24 |
| *Protieus mirabilis* | 20 | 21 | 18 | 15 | 18 | 18 | 25 |

ISSN: 0975-8585

**Antioxidant activity**

The antioxidant property of watermelon peel extract for both the varieties, including wild type and hybrid, was determined using DPPH radical scavenging assay. The ascorbic acid was used to prepare a standard for DPPH radical scavenging assay, and based on the standard curve antioxidant activity of watermelon peel extract was calculated. As a result, shown in table 4, we report here maximum DPPH inhibition was reported with methanol extract of wild type watermelon. However, hybrid watermelon with methanol also demonstrated a significant DPPH inhibition compare to another solvent for both wild type and hybrid watermelon. The higher content of flavonoids and another phytosterol, along with vitamin C, are responsible for the antioxidant activity of watermelon. As a result, shown in table 4, it is evident that phytochemicals responsible for antioxidant properties have more affinity for methanol. As a result, shown in a recent finding *Lum et al 2019*, we report a similar finding [21]. Previously Hong et al. 2015 demonstrated active phytochemicals present in watermelon, offering a vigorous antioxidant activity [22]. In our finding, we report here a large variety of active watermelon peel phytochemicals might be responsible for intense antioxidant activity analyzed using DPPH free radical scavenging assay [23-26]. The use of enzyme based antioxidant and antimicrobial analysis seems more effective however our finding show similar results [27-29].

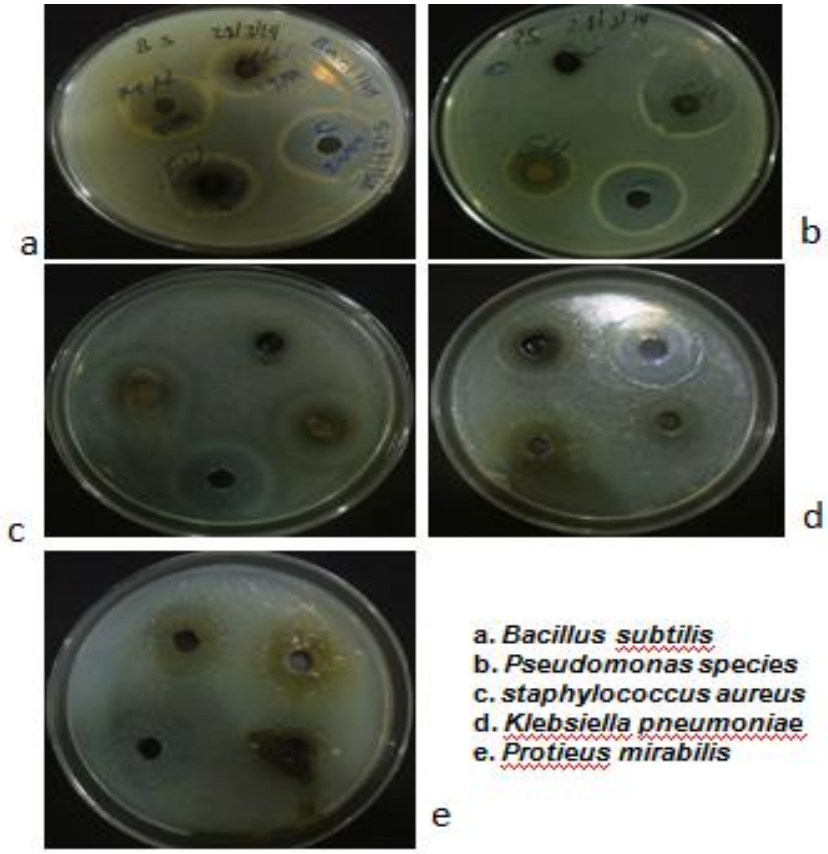


**Figure 1; The figure demonstrates antibacterial activity analysis of watermelon peel extract from wild type and hybrid species. The figure 1a; represent a zone of inhibition for Bacillus subtilis, b; *Pseudomonas species, c; Staphylococcus aureus, d; Klebsiella pneumonia and e; Proteus mirabilis.***

**Table 4; The table represents the antioxidant potential of phytochemicals constituent present in watermelon peel extract. The antioxidant activity was determined using DPPH radical scavenging assay.**

| Concentration in µg/ml | Ascorbic acid | | Absorption | | Watermelon peel Extract | |
|---|---|---|---|---|---|---|
| | Absorbance | % DPPH inhibition | Sample and absorbance (nm) | | Absorbance | % DPPH inhibition |
| Zero | | | | | | |
| 0 | 0 | 0 | Control (water) | | 0.0631 | 0 |
| 5 | 0.902 | 3.632479 | Solvent | 517.0 | 0.1942 | 5.25 |
| 10 | 0.837 | 10.57692 | Solvent | 517.0 | 0.1287 | 10.75 |
| 15 | 0.729 | 22.11538 | MW | 517.0 | 1.4146 | 15.21 |
| 20 | 0.604 | 35.47009 | EH | 517.0 | 0.5956 | 20.60 |
| 25 | 0.483 | 48.39744 | CW | 517.0 | 0.1374 | 25.20 |
| 30 | 0.371 | 60.36325 | CH | 517.0 | 1.8352 | 30.58 |
| 40 | 0.209 | 77.67094 | EW | 517.0 | 1.1042 | 40.65 |
| 50 | 0.073 | 92.20085 | MH | 517.0 | 1.6185 | 50.51 |

## CONCLUSION

Plant based phytochemicals are active in offering several therapeutic potentials and remain part of traditional medicine for many centuries. The watermelon is a classic example of fruit rich in active phytochemicals. Several varieties of watermelon are grown worldwide with varying active phytochemicals. India and China are a leader in producing watermelon, both wild type and hybrid [30]. These varieties of watermelon offer antioxidant, antibacterial, and other therapeutic properties. The fruit pulp and peel are rich in various vital minerals and vitamins, along with a series of active secondary metabolites. In the present study, we have investigated the antioxidant and antibacterial potential of watermelon peel extract using different solvent. The most critical finding of current work is the diversity of active phytochemicals from watermelon fruit extract. The diversity of phytochemicals present in watermelon fruit extract result in antibacterial activity among five dominant bacterial species associated with several diseases to humans. Additionally, the potent antioxidant potential of watermelon peel extract signifies its commercial importance. However, in the present study, we analyzed both antibacterial and antioxidant activities from the crude extract. To establish a more precise role of active ingredient present in watermelon peel extract, detailed analytical studies are required. As far there are several findings demonstrated the beneficial role of watermelon consumption regulates several metabolic disorders and diseases. However, fewer studies are explaining the purpose of each and individual active phytochemicals present in watermelon fruit. Complete profiling of active phytochemicals and activity analysis will allow building a more robust and reliable source of fruit for the therapeutic purpose along with high nutrient food.

**Conflict of Interest;** The author declares no conflict of interest.

## ACKNOWLEDGMENT

The author acknowledges Acharya Nagarjuna University, Guntur, Andhra Pradesh, India, for providing a facility for the current study.

## REFERENCES

[1] Gladvin, G., Santhisri, K.V., Sudhakar G And Somaiah K. Physico-chemical and functional properties of watermelon (Citrullus lanatus) seed-oil, Food Science Research Journal , 2016, 7( 1) 85-88 DOI : 10.15740/HAS/FSRJ/7.1/85-88

[2] Naz A, Butt MS, Sultan MT, Qayyum MM, Niaz RS, Watermelon lycopene and allied health claims. EXCLI Journal. 2014; 13:650-60. eCollection 2014.

[3] Maynard DN. Watermelons: characteristics, production, and marketing. Alexandria, VA: ASHS Press; 2001.

[4] Ahn J, Choi W, Kim S, Ha T. Anti-diabetic effect of watermelon (Citrullus vulgaris schrad) on Streptozotocin-induced diabetic mice. Food Science Biotechnology. 2011;20:251–254

[5] Artés-Hernández F, Robles PA, Gómez PA, Tomás-Callejas A, Artés F. Low UV-C illumination for keeping overall quality of fresh-cut watermelon. Postharvest Biology and Technology. 2010;55:114–120

[6] Bangalore DV, McGlynn WG, Scott DD. Effects of fruit maturity on watermelon ultrastructure and intracellular lycopene distribution. Journal of Food Science. 2008;73:S222–S228

[7] Bruton BD, Fish WW, Roberts W, Popham TW. The influence of rootstock selection on fruit quality attributes of watermelon. Open Food Science Journal. 2009;3:15–34

[8] Oms-Oliu G, Odriozola-Serrano I, Soliva-Fortuny R, Martín-Belloso O. Effects of high-intensity pulsed electric field processing conditions on lycopene, vitamin C and antioxidant capacity of watermelon juice. Food Chemistry. 2009;115:1312–1319

[9] Chandrika UG, Fernando KSSP, Ranaweera KKDS. Carotenoid content and in vitro bioaccessibility of lycopene from guava (Psidium guajava) and watermelon (Citrullus lanatus) by high-performance liquid chromatography diode array detection. International Journal of Food Science Nutrition. 2009;60:558–566

[10] Collins JK, Davis AR, Adams A, Perkins-Veazie P. Sensory evaluation of low sugar watermelon by consumers. Horticulture Science. 2005;40:883

[11] Edwards AJ, Vinyard BT, Wiley ER, Brown ED, Collins JK, Perkins-Veazie P, et al. Consumption of watermelon juice increases plasma concentrations of lycopene and β-carotene in humans. Journal of Nutrition. 2003; 133:1043–1050.

[12] Lucier G, Lin B-H USDA, United States Department of Agriculture, editor. Vegetables and specialties: situation and outlook report. United States Department of Agriculture; 2001. Factors affecting watermelon consumption in the United States; pp. 23–29. (VGS-287

[13] Wu G, Collins JK, Perkins-Veazie P, Siddiq M, Dolan KD, Kelly KA, et al. Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic syndrome in Zucker diabetic fatty rats. Journal of Nutrition. 2007; 137:2680–2685.

[14] Shanely R.A., Nieman D.C., Perkins-Veazie P., Henson D.A., Meaney M.P., Knab A.M., Cialdell-Kam L. Comparison of Watermelon and Carbohydrate Beverage on Exercise-Induced Alterations in Systemic Inflammation, Immune Dysfunction, and Plasma Antioxidant Capacity. Nutrients. 2016; 8:518. doi: 10.3390/nu8080518.

[15] Massa N.M.L., Silva A.S., Toscano L.T., Silva J.D.R., Persuhn D.C., Gonçalves M.D.C.R. Watermelon extract reduces blood pressure but does not change sympathovagal balance in prehypertensive and hypertensive subjects. Blood. 2016; 25:244–248.

[16] Kim C.-H., Park M.-K., Kim S.-K., Cho Y.-H. Antioxidant capacity and anti-inflammatory activity of lycopene in watermelon. International Journal of Food Science Technology. 2014; 49:2083–2091. doi: 10.1111/ijfs.12517

[17] Edwards A.J., Vinyard B.T., Wiley E.R., Brown E.D., Collins J.K., Perkins-Veazie P., Baker R.A., Clevidence B.A. Consumption of watermelon juice increases plasma concentrations of lycopene and beta-carotene in humans. Journal of Nutrition. 2003; 133:1043–1050. doi: 10.1093/jn/133.4.1043

[18] Hind Abd-alluh Abu-Hiamed, Chemical Composition, Flavonoids and β-sitosterol Contents of Pulp and Rind of Watermelon (*Citrullus lanatus*) Fruit. Pakistan Journal of Nutrition, *2017; 16: 502-507.*

[19] Choksi PM, Joshi CVY. A review on lycopene-extraction, purification, stability and applications. International Journal of Food Production 2007; 10:289–298.

[20] El-Adawy TA, Taha KM Characteristics and composition of watermelon, pumpkin, and paprika seed oils and flours. Journal of Agriculture Food Chemistry. 2001; 49(3):1253-9.

[21] Lum T, Connolly M, Marx A, Beidler J, Hooshmand S, Kern M, Liu C, Hong MY, Effects of Fresh Watermelon Consumption on the Acute Satiety Response and Cardiometabolic Risk Factors in Overweight and Obese Adults. Nutrients. 2019; 11(3). pii: E595. doi: 10.3390/nu11030595.

[22] Hong M.Y., Hartig N., Kaufman K., Hooshmand S., Figueroa A., Kern M. Watermelon consumption improves inflammation and antioxidant capacity in rats fed an atherogenic diet. Nutrition Research. 2015; 35:251–258. doi: 10.1016/j.nutres.2014.12.005

[23] Sobha K, V Rao, Ratna K and Mahendra Kumar Verma, An Investigation into phytochemical constituents, antioxidant, antibacterial and anti-cataract activity of *Alternanthera sessilis*, a predominant wild leafy vegetable of South India, Bio-catalysis and Agricultural Biotechnology, 2017; 10; 197-203

[24] Verma MK and Pulicherla KK, Broad substrate affinity and catalytic diversity of fibrinolytic enzyme from *Pheretima posthumous*—Purification and molecular characterization study, International Journal of Biological Macromolecules. 2017, 95:1011-1021 doi: 10.1016/j.ijbiomac.2016.10.090

[25] Verma MK and Pulicherla KK, Enzyme promiscuity in Earthworm serine protease- Substrate versatility and therapeutic potential, Amino Acid. 2016; 48(4); 941-948 DOI 10.1007/s00726-015-2162-3.

[26] Verma MK, Xavier F, Verma MK, Sobha K. Evaluation of the Cytotoxic and Antitumor activity of Serine Proteases Isolated and Purified from the Indian Earthworm *Pheretmia posthuma*, Asian Pac J. Trop. Biomed. 2013; 3 (11): 896-901.

[27] Verma MK and Verma MK, Earthworm- a potential source for stable and potent antimicrobial compounds- isolation and purification study, 2012, Int J Pharm Pharm Sci, 4; 4, 540-543

[28] K Sobha, N Lalitha, N Pavani, R Praharshini, D Pradeep, Verma MK, Statistical optimization of Factors influencing the activity and the kinetics of partially purified Lipase produced from Aspergillus japonicus (MTCC 1975) cultured in protein enriched medium, International Journal of Chem Tech Research, 2014, 6,11,4817-4831

[29] Verma MK and Sobha K, Antioxidant and Anti-Inflammatory Properties of Autolysed Extract of the Indian Earthworm *Pheretima posthuma* after Preliminary Purification - An In Vitro Study, Research Journal of Pharmaceutical, Biological and Chemical Sciences (RJPBCS), 2013, 4, 4, 888-892

[30] Tarazona-Díaz MP, Viegas J, Moldao-Martins M, Aguayo E, Bioactive compounds from flesh and by-product of fresh-cut watermelon cultivars. Journal of Science Food Agriculture. 2011; 91(5):805-12. doi: 10.1002/jsfa.4250.