

Amino acid Profile of the Watermelon, *Citrullus vulgaris* and Detection of its Antimicrobial Activity

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ABSTRACT

Seventeen free amino acids from the various parts of watermelon plant, including essential and non-essential amino acids were found by using Automatic Amino Acid Analyzer. In addition, the antimicrobial activity study of the susceptibility of watermelon (*Citrus vulgaris*L) against tested microorganisms was investigated. The identified amino acids included: phenylalanine, histidine, tryptophan, lysine, ornithine, arginine, aspartic acid, threonine, serine, glutamine, citrulline, alanine, valine, isoleucine and leucine. The essential amino acids (histidine, leucine, lysine, methionine, phenylalanine, tyrosine, and valine ranged between 1.8%-3.6%, 5.5-9.1%, 3.7-6.7%, 0.4-1.1%, 4.1, 6.4%, 0.8-7.2% and 5.5-7.6% in the various parts of watermelon plant, respectively. There were notable variations between the various parts of watermelon plants in their contents of amino acids. However, the highest contents of most of the essential amino acids were concentrated in the leaves, while the highest contents of most of the non-essential amino acids were concentrated in the roots. The investigated amino acids had antibacterial and antifungal activities against the tested organisms.

KEY WORDS: AUTOMATIC AMINO ACID ANALYZER, HPCL, GLUTAMINE, VALINE, ISOLEUCINE.

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INTRODUCTION

Strains of the significant human pathogen have demonstrated expanded from all accessible traditional antibiotics (Aloush, 2006; Manchanda *et al.*, 2010). A lot of systems have been engaged in bacterial resistance procedure including the mutation or enzymatic activation that results in the modified objective protein, gaining genes from other bacteria to express less susceptible focused on proteins, and furthermore obtain portable elements including transposons or plasmids (Baltzer and Brown 2011). In spite of nonstop endeavors, multiple drug resistance is as yet a genuine worldwide concern, especially a serious restorative danger to creating countries bringing about monetary weight and increment death rate. It is assessed that worldwide death because of antimicrobial resistance would reach to 10 million by 2050 (Wang *et al.*, 2016). Antimicrobial peptides (AMPs) have turned into a promising competitor and have gotten outstanding consideration as a novel class of antibiotics (Antimicrobial Resistance Benchmark 2018). They are peptides normally

delivered by all organisms including prokaryotes to human beings in response to foreign microbes and have a role in inborn explicit safeguard defense system and provide instant non-specific defense against infections. Amino acids are found either in the free state or as linear chains in peptides and proteins. There are 22 commonly occurring amino acids in proteins. Amino acid analysis has vital for the study of the composition of proteins, foods, and feedstuffs. Free amino acids are additionally determined in organic material, for example, plasma and urine, and in fruit juice and wine. When it is performed on a pure protein, amino acid analysis is equipped for distinguishing the protein (Hobohm *et al.*, 1994; Schegg *et al.*, 1997), and the analysis is likewise utilized as an essential for Edman degradation and mass spectrometry and to determine the most reasonable enzymatic or chemical digestion technique for further investigation of the protein. Watermelon juice may give a novel source of the essential amino acid, arginine. Arginine is a precursor for nitric oxide, which has been appeared to lower blood pressure lessen blood

thickening and ensure against myocardial localized necrosis and strokes (Collins *et al.*, 2003; Cutrufello *et al.*, 2015; Fall *et al.*, 2019). Free amino acids such as phenylalanine, histidine, tryptophan, lysine, ornithine, arginine, aspartic acid, threonine, serine, glutamic acid, glutamine, citrulline, alanine, valine, isoleucine and leucine are accounted to have been extracted from watermelon and quantitatively analyzed (Tedesco *et al.*, 1984; Perkins-Veazie *et al.*, 2012; Jordan *et al.*, 2019)). This study aimed to analyze the free amino profile of various parts of watermelon plant and detection of their antimicrobial activity.

MATERIALS AND METHODS

Amino Acid Analysis by Automatic Amino Acid Analyser S-433 Automatic Amino Acid Analyser S-433: The innovative automatic Amino Acid Analyzer S 433 combines the advantages of the classical ion exchange separation method with the modern technique of high performance liquid chromatography. The complete package of sophisticated instrumentation, a wide variety of preplaced and tested separation columns, combined with optimized ready-to-use buffer solutions and chemicals, create the right answer for any amino acid determination

High Performance Liquid Chromatography (HPLC): HPLC instrument consists of a reservoir of mobile phase, a pump, an injector, a separation column and a detector (Karen *et al.*, 2019). Sykam chromatographs are modular systems optimized for customer's needs. Every single system can be configured to suit a certain application. Any existing Sykam HPLC can be upgraded by adding additional components.

ION Chromatography System – S 135

Ion Chromatography is one of the most important methods for the determination of alkaline, alkaline earth, transition metals, inorganic anions, sulphuric compound of different oxidation levels, organic acids and various tensides. Sykam S 135 is a compact system with modular setup. Even the basic system is designed for most sensitive anion analysis employing suppression of eluent conductivity.

Reagent organizer S 7131: The S 7131 Reagent Organizer is the optimal solutions for storing your solvents. The integrated gas supply with pressure regulator let you store the eluent under inert-gas pressure or for degassing purposes using Helium. The organizer can hold up to four eluent bottles which can be closed individually with integrated value bottle caps.

Conductivity Detector S 3115: The S 3115 Conductivity Detector's outstanding features are high background suppression, baseline stability, and signal linearity over a range of several decades. These characteristics become especially important when single column techniques are employed, e.g. for the determination of alkaline ions and alkaline earths. The HPLC Pump S 2100 is a compact eluent dosing system, upgradable from a high performance isocratic pump to a quaternary gradient pump with outstanding features.

Solvent Delivery System S 2100-Ex: The HPLC Pump S 2100 is a compact eluent dosing system, upgradable from a high performance isocratic pump to a quaternary gradient pump with outstanding features.

Sample preparation: For preparation of sample hydrolysate, 200 milligram of watermelon (roots, stems, leaves and three maturation stages of its fruit and seeds), was weighted in a hydrolysis tube to prepare hydrolysate sample. Then 5 ml of 6N HCl were added and the tube was tightly closed. The sample was incubated for 24 hours at 110°C. The sample was allowed to cool. 125 ml was filtered using 200 micro-liters of the filtrate were taken into another tube and evaporated at 140°C ovens for about 1 hour. 1 ml of diluting buffer was then added to the dried sample and transferred to amino acid analyzer vial and then injected for analysis. After the separation of the injected sample with a temperate cation separation column, ninhydrin was added continuously to the system. An integrated reagent dosing pump was responsible for the delivery of this reagent, while an external buffer pump was delivering the eluent. After adding ninhydrin, the eluent was lead through a high temperature

reactor coil of about 16 m length. With a typical flow rate of 0.7 ml/min the buffer / ninhydrin mixture was stayed in the heated reactor for about 2 min. maximum absorption at 570 nm), while the secondary amino acids (have their maximum at 440 nm). After the reaction the mixture was lead through a dual-channel photometer where both wavelengths (570 nm and 440 nm) were measured continuously.

Tested microorganisms: The standard microorganisms used for this study were received from the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Sudan and from the Department of Microbiology, Beijing University of Chemical Technology, China. They were *Bacillus subtilis*, B.s. (NCTC 8236, ACCC), designated as Gram +ve, *Staphylococcus aureus*, S.a. (ATCC 25923 & CVCC), designated as Gram +ve, *Escherichia coli*, *E. coli* (ATCC 25922, ACCC), designated as Gram -ve, and one fungus, *Candida albicans*, Ca.a. (ATCC 7596, CVCC), designated as Gram +ve. The microorganisms were produced a stock preparation containing a log-phase cell density of approximately 10⁷ colony forming units (CFU)/ml as evaluated initially by measurements of the optical density at 600 nm.

Antibacterial Assay

Determination of antibacterial activity: After separation of amino acids in small pre-sterilized containers, they were taken for determination of antimicrobial activity. The antibacterial activity was assessed by the agar-well diffusion method (Kinsbury and Wagner, 1990). The inoculum size of each tested bacterium was adjusted to a suspension of 10⁶ cells. The inoculum suspension was spread over a Mueller Hinton agar (MHA) plate to achieve confluent growth and allowed to dry. 10 mm-diameter wells were bored in the agar using a sterile cork borer. A 100 µL-aliquot of the reconstituted extract was placed into a well with standard Pasteur pipette and the plate was held for 1 hr at room temperature for diffusion of extract into the agar. Subsequently, the plate was incubated for 18 hr at 37°C. After incubation, the diameters of the zones of inhibition were measured to the nearest mm. Three replicates were performed and results were recorded.

RESULTS

Amino acid composition

Using the amino acid analyzer, the retention time and the amount of amino acid in the various parts of the watermelon plant as obtained from the chromatograms are indicated in Table (1) and in Figures (1 and 2). 17 essential and non-essential amino acid were identified. The identified amino acids included: phenylalanine, histidine, tryptophan, lysine, ornithine, arginine, aspartic acid, threonine, serine, glutamic acid, glutamine, citrulline, alanine, valine, isoleucine and leucine. The results also indicated that the highest amount of amino acids present were in this order, Glutamine (39.0%) then Ammonia (9.2%) and Arginine (7.7%) in the roots (SP_{1a}). The results indicated in Fig. 2 shows the identification of 17 essential and non-essential amino acids by using the amino acid analyzer to determine the retention time and the amount of amino acid in watermelon various parts. Results revealed that Glutamine has the highest amount among detected amino acids (39.0%) followed by Ammonia (9.2%)

and Arginine (7.7%) in the plant roots (SP_{1a}) (Fig. 2 -A). Also the highest amount of amino acids present the stems of watermelon (SP_{1b}) was for Glutamine (21.90%) then Aspartic Acid (9.9%) and the Ammonia (8.2%) as indicated in Fig. 2 (B). On the other hand, Glutamine (19.5%) has the highest amount among amino acids present in the leaves of watermelon (SP_{1c}), followed by Aspartic Acid (9.5%) and Leucine (9.1%) as shown in Fig. 2 (C). The results shown in Fig 2 (D) indicated that Glutamine Acid highest amount (20.4%) among amino acids in the green crust of watermelon (SP_{1d}), followed by Arginine (12.3%) and Aspartic Acid (8.1%). Fig. 2 (E) also shows the identification of 17 essential and non-essential amino acids using the amino acid analyzer to determine the retention time and the amount of amino acids in white crust of watermelon. The results showed the that the highest amount found was Glutamic Acid (39.0%) followed by Ammonia (9.2%) and Arginine (7.7%) in the white crust of watermelon (SP_{1e}). As for the watermelon kernel (SP_{1f}), the highest amount present of was that of Glutamine Acid (18.7.0%), Arginine

Table 1. Essential and non-essential amino acids contents of various parts of watermelon plant

NO.	Amino acids	SP1a	SP1b	SP1c	SP1d	SP1e	SP1f	SP1h
1	Aspartic Acid	6.0	9.9	9.5	8.1	6.0	6.4	8.3
2	Threonine	2.8	4.0	4.7	3.8	2.8	3.0	3.3
3	Serine	1.8	2.9	3.0	3.7	1.8	2.5	2.7
4	Glutamine	39.0	21.9	19.5	20.4	39.0	18.7	22.7
5	Glycine	5.3	5.0	5.5	3.8	5.3	0.6	5.0
6	Alanine	2.6	6.6	6.1	4.0	-	7.7	5.1
7	Cysteine	-	0.1	0.7	0.2	2.6	0.5	1.0
8	Valine	5.1	7.6	7.2	6.9	5.1	7.3	5.9
9	Methionine	0.4	0.2	0.6	0.2	0.4	1.1	1.1
10	Isoleucine	4.3	5.7	5.7	5.3	4.3	5.8	4.7
11	Leucine	5.5	8.0	9.1	7.9	5.5	7.3	7.1
12	Tyrosine	0.8	0.5	7.2	0.7	0.8	1.0	1.3
13	Phenylalanine	4.1	5.6	6.4	5.6	4.1	5.4	6.0
14	Histidine	1.8	2.4	2.6	3.6	1.8	2.1	2.7
15	Lysine	3.7	6.4	6.4	6.7	3.7	2.5	4.4
16	Ammonia	9.2	8.2	4.3	6.9	9.2	11.9	4.0
17	Arginine	7.7	4.9	6.1	12.3	7.7	16.0	14.6

Watermelon plant = (SP1); SP1a= Roots; SP1b=Stem; SP1c= Leaves; SP1d = Green crust; SP1e = White crust; SP1f = kernel; SP1h = seed

Figure 1. Amino acid profile of the various parts of watermelon plant

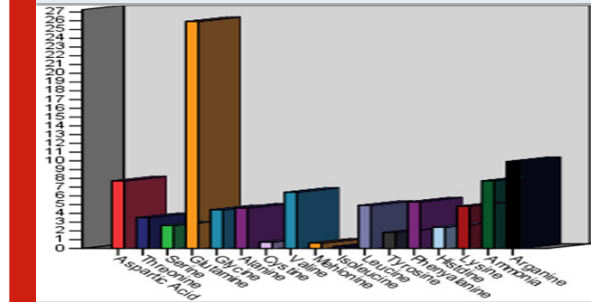


Figure 2. Free amino acids of watermelon (A) roots , stems (B), leaves (D), white crust (E),

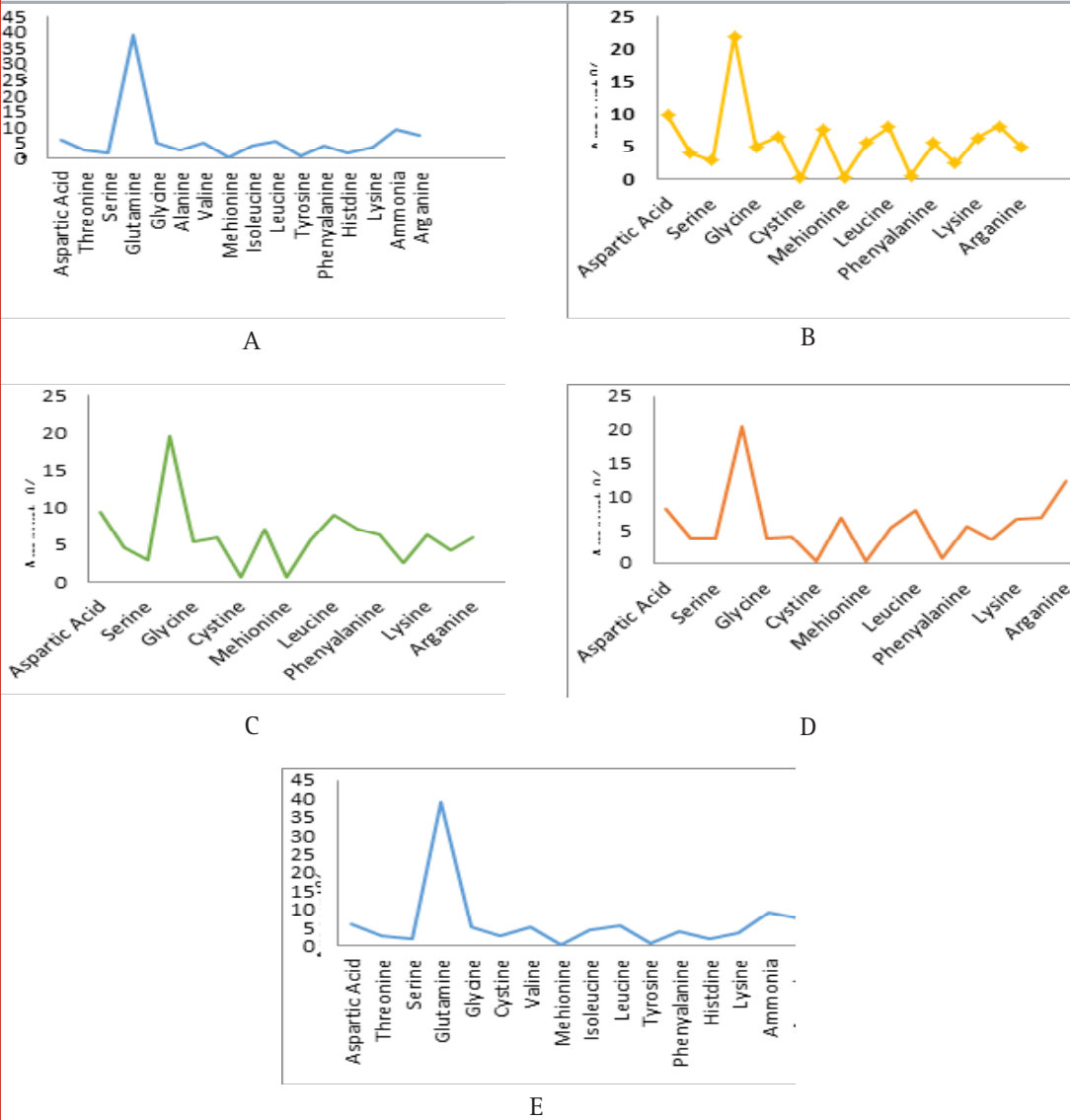
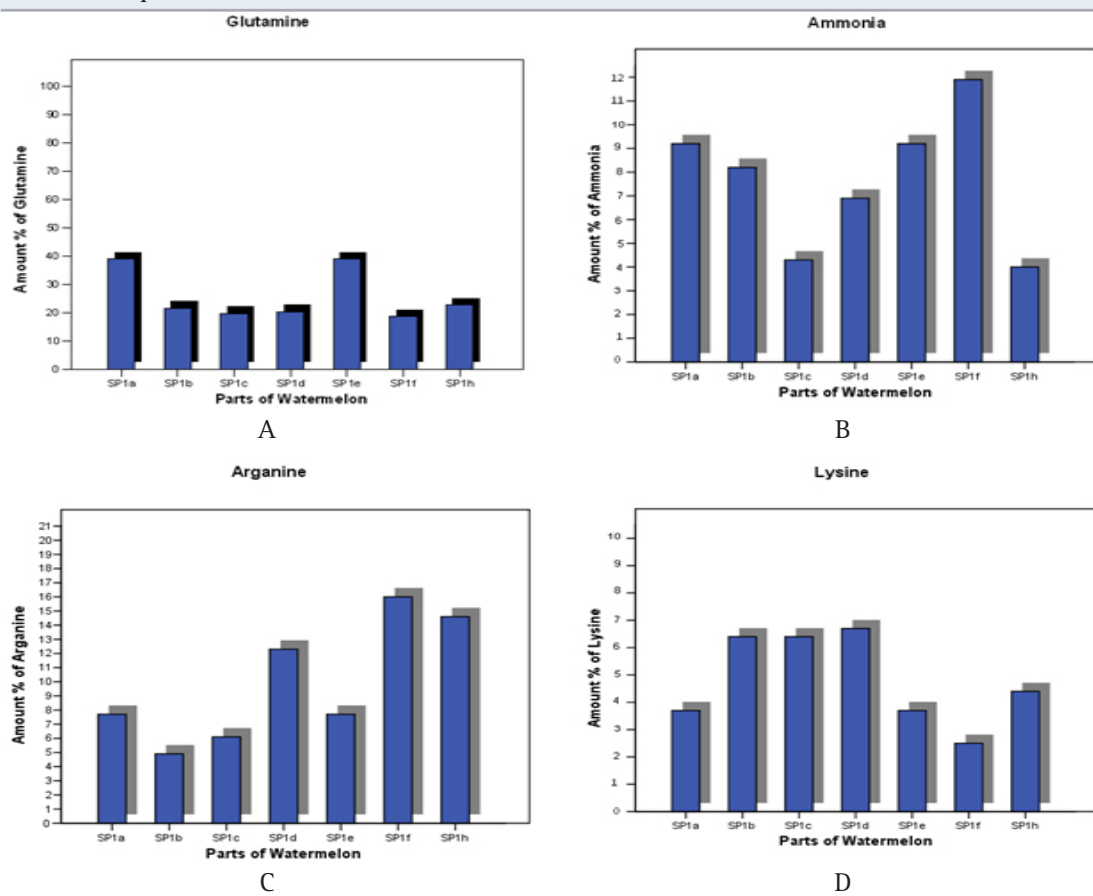


Figure 3. Contents of Glutamine (A), Ammonia (B), Arginine (C), Lysine (D) in the various parts of watermelon plant



(16.0%) and Ammonia (11%). The results showed that the highest amount of amino acids present in the seeds of watermelon (SP1h) was Glutamic Acid (22.7.0%), Arginine (14.6%) and Aspartic acid (8.3%).

Antimicrobial activity of the amino acids

The results of the antimicrobial activity of the free amino acids as shown in Fig. (4) shows that the investigated amino acids had antibacterial and antifungal activities against the tested organisms. However, the antimicrobial varies with the nature of amino acids, and the part of the plant and organisms. Fig. (4) also shows that the amino acid glutamine shows the highest antimicrobial activity against the fungus *Candida albican* which had the highest inhibition zone diameter (28 mm) followed by the bacteria *Staphylococcus aureus*, then *E.coli* and lowest activity was

against *Bacillus subtilis* which had the lowest inhibition zone diameter (13 mm). The bacteria *Staphylococcus aureus* was highly inhibited by all detected amino acids with exception to serine and threonine. However, *E.coli* was inhibited by all detected amino acids with exception to serine. *Bacillus subtilis* was inhibited by all detected amino acids with exception to serine, lysine and citrulline. The fungus *Candida albican* was highly inhibited by all detected amino acids with exception to serine amino acid.

DISCUSSION

Amino acids contents: Amino acids are the building blocks of peptides and proteins. They possess two functional groups, the carboxylic acid group gives the acidic character, and the amino group provides the basic character. Proteins are

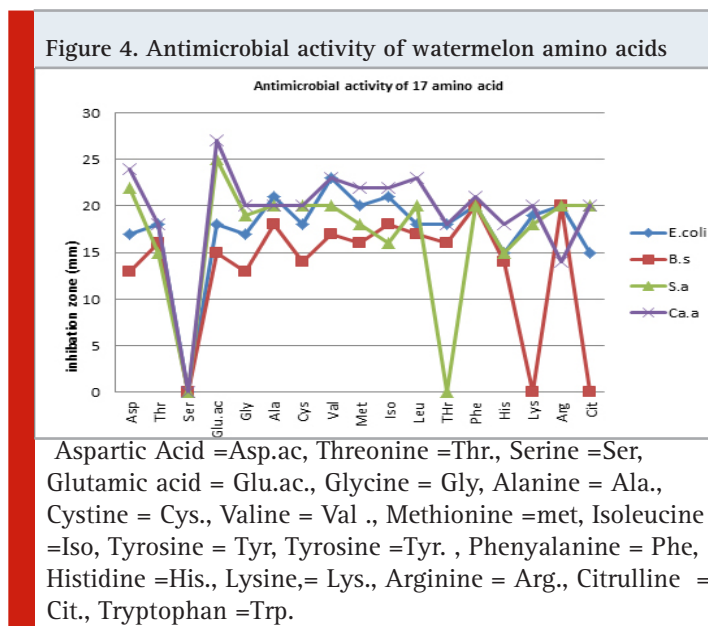
composed of different amino acids and hence the nutritional quality of a protein determined by the content, proportion, and availability of its amino acids (Becker, 2007). To date, more than 300 amino acids have been found in nature, of which 20 are engaged in protein synthesis and are known as proteinogenic amino acids. Proteinogenic amino acids exist in two structures: in a free state in physiological liquids (e.g., plasma, urine) and food (e.g., wine, beverage) and bound in peptides or proteins (Yu and Mou 2005). Table 1 shows the amino acid profile of watermelon various parts. This result suggested that the water melon plant parts were rich in amino acids, which might be served as good source of nutrition. Of 22 amino acids, almost 17 amino acids were present. However, some of the amino acids were present in lower concentration in comparison to that reported by FAO pattern (FAO 2018). The essential amino acids (Histidine, Leucine, Lysine, Methionine, Phenylalanine, Tryptosine, and Valine) ranged between 1.8%-3.6%, 5.5-9.1%, 3.7-6.7%, 0.4-1.1%, 4.1, 6.4%, 0.8-7.2% and 5.5-7.6% in the various parts of watermelon plant, respectively.

Valine essential amino acid was the dominant essential amino acid in all watermelon plant parts. Moreover, watermelon stem contained the highest value, while both the root and white kernel contained the least value. On the other hand, Methionine amino acid was the least in concentration among all essential amino acids in all watermelon plant. Nonessential amino acids (Alanine, Aspartic acid, Glutamine, and Serine) ranged between 1.8 – 39% for all watermelon plant parts. Glutamine was the dominant non-essential amino acids in all watermelon parts. Moreover, it was the most dominant amino acid investigated in this study. Most of the amino acid values are comparable with those of most vegetable protein determined by many investigators (El- Adawy *et al.*, 2001; Mune *et al.*, 2011; Ogunlade *et al.*, 2011; Sánchez- Vioque *et al.*, 1999). Generally the average percentage of nonessential amino acids was higher in concentration than essential amino acids in watermelon plant parts. In comparison, Usman *et al.* (2010) revealed the prevalence of glutamic acid and aspartic acid in watermelon seeds. This suggests variety happens

as indicated by genotype and the geographical and environmental conditions in which watermelons are developed. Glutamine is an important amino acid with numerous capacities in the body. It is a building block of protein and critical part of the immune system. Likewise, glutamine has an extraordinary role in intestinal health. Your body naturally produces this amino acid, and it is also found in numerous foods. There were notable variations between the various parts of watermelon plants in their contents of amino acids (Table 1).

However, the highest contents of most of the essential amino acids were concentrated in the leaves (SP1a), while the highest contents of most of the non-essential amino acids were concentrated in the roots (SP1b). Watermelon plant = (SP1); SP1a= Roots; SP1b=Stem; SP1c= Leaves; SP1d = Green crust; SP1e = White crust; SP1f = kernel; SP1h = seed. A high- quality protein contains essential amino acids in ratios proportionate with human needs. This can be dictated by comparing the amino acid contents of different proteins with the FAO reference pattern. The FAO reference pattern dependent on the essential amino acid requirements of young children (1–2 years) is viewed the preferred reference protein (Andini *et al.*, 2013). Methionine and Tyrosine were found in limited amount for most of the watermelon plant parts, however, this shortage might be explained by their denaturation during analysis or their values are very limited in the plant. To compensate this limitation in watermelon, additional Utilization of animal or plant proteins, for example, milk, egg, lentils, and heartbeats are profoundly prescribed (Silva *et al.* 2014).

Antimicrobial activity: Antimicrobial peptide (AMPs) is a part of inborn safeguard framework in most multicellular life forms, from people to plants to insects. To date more than 2200 common or synthetic AMPs have been accounted for through the antimicrobial peptide database (APD) (Silva *et al.* 2014). Antimicrobial peptides (AMPs) are commonly composed of short sequences of 10–100 amino acids deposits and profoundly and membrane active (Reddy *et al.* 2004). AMPs have a wide range of antibacterial, antifungal, antiviral, and anti-tumor activity at low concentrations. In



contrast to traditional antibiotics, antimicrobial peptides can direct the host resistant immune system and kill bacteria directly (Gottler and Ramamoorthy 2009). Moreover, other biological activities of AMPs have also been described as following: neutralization of endotoxins, immune-modulating properties, chemokine-like activities, and induction of both angiogenesis and wound repair (Guani-Guerra et al. 2010). It has been reported that Gram-positive bacteria are known to be more susceptible to amino acids complexes than gram-negative bacteria. The weak antibacterial activity against gram negative bacteria was attributed to the existence of an external film which stances hydrophilic polysaccharides chains as a boundary to the amino acids complexes.

It has also been reported that the antibacterial action of a complex is affected by its dependability. The lower strength of the amino acid complex, the more prominent is the antibacterial action. This is likely because they have more free ions in the solution, which can improve the cooperative association between the metal particles and the ligands, (Marcu et al., 2008; Stanila et al., 2007). The variety in the adequacy of various compounds against various organisms depends either on the impermeability of the cells of the microorganisms or the distinction in ribosomes of

microbial cells. It has additionally been suggested that concentration assumes a fundamental role in expanding the level of inhibition; as the concentration increases, the activity increments as it was accounted for likewise in the present cases (El-Wahab et al., 2005). In this context further investigations are expected to understanding the antibacterial or antifungal mechanism Antimicrobial peptides which are composed of a great number of amino acids have a broad spectrum of activity against a wide range of microorganism including Gram negative and Gram positive bacteria, fungi, parasites and viruses (Brown et al., 2007; Hancock (2001); Andrea and Rivas (1998); Bradshaw 2003).

CONCLUSION

There are a total of 17 free amino acids that were found in all organs of watermelon plant, out of which glutamic acid, arginine and aspartic acids were found to be in higher concentration in all organs of the plant. However, the highest contents of most of the essential amino acids were concentrated in the leaves, while the highest contents of most of the non-essential amino acids were concentrated in the roots. The results of antimicrobial activity tests, show that amino acids complexes have inhibitory effect against the bacteria: *Staphylococcus aureus*, *Escherichia*

coli, and the fungus, *Candida albicans*, and less efficient against *Bacillus subtilis*. Studies regarding the mode of action for the amino acids in the microbial bacterial cell should be investigated, since these substances are natural, their hazardous potential is lower when compared with other products.

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