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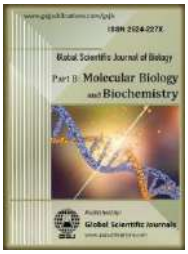

Diagnosis of Some Active Compounds from Three Parts of the (*Annona Muricata*) Plant and Study of their Antioxidant Effects

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ABSTRACT

In this study, (Annona muricata) plant was used to identify its active and antioxidant components and their effects on damages caused by free radicals. **Objective**: Diagnosis of some amino and phenolic for three parts of the (Annona Muricata) plant, including pulp, seeds, and leaves, and studying their antioxidant effects on free radicals. **Methodology**: Amino acids were extracted by taking a weight of 3 grams of the sample and placing it in a 10 ml volumetric flask, 3 ml of 6M hydrochloric acid was added to it along with 0.1% phenol, and it was sealed tightly, and placed in a thermal oven at 45°C for 24 hours. After that, 3 ml of sodium hydroxide and 0.1mg of citric acid were added and mixed well for 15 min. The sample was filtered using a 0.45-micron plastic filter, After That Take 1 ml of the extracted sample and add 200 microliters of 5%) Ortho-phthalate-aldehyde (to it. Mix the sample for two minutes and then take 100 microliters of the final mixture and inject it into the Amino Acid Analysis instrument. The total phenolic content was estimated in seeds of the)Annona Muricata(plant The total phenolic was detected using Gallic acid as a standard and (Folin-Ciocalteu reagent), and the concentration of total phenolic is calculated based on the calibration curve of Gallic acid in milligrams per gram dry weight **Results**: Several amino acids were diagnosed by the amino acid analyzer for three parts of the (Annona Muricata) plant, which are the pulp, seeds, and leaves The quantity of phenols was also measured.

1. Introduction

Free Radical: A molecule is an independent entity that possesses one or more unpaired electrons with different orientations in rotation. This unbound or free electron is the one that is alone in the outer orbit. The hydrogen atom is considered one of the simplest types of free radicals. Electrons are more stable when paired together in the outer orbit, while free radicals are more active and less stable. They can interact randomly with other molecules that are in contact with each other. Once free radicals are formed, they can react with another free radical or a non-free radical from different reactions. Oxygen is an essential element for life, as cells use oxygen to generate energy. Mitochondria produce free radicals, which are generally reactive oxygen species and reactive nitrogen species. These radicals are continuously generated due to the natural use of oxygen **[1]** Free radicals have gained importance in the field of biology due to

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their central role in various physiological conditions and their effects on a variety of diseases. Free radicals can have adverse effects, such as affecting biological molecules like nucleic acids, fats, and proteins, leading to changes in the natural oxidation state and its increase **[2]** Moses Komburg pointed to the presence of free radicals in chemical reactions when he was trying to synthesize (Tetra-Phenyl-Methane) and found that free radicals are molecules characterized by the presence of an unpaired electron in the outer shell. Studies have shown the effect of oxygen on enzymes such as pepsin, catalase, cholinesterase, and carbonic anhydrase **[3]**Also, laboratory rats were exposed to a pure oxygen environment, resulting in their death after three days. This effect could not be attributed to free radicals due to insufficient information and lack of appropriate scientific support. Later, it was proposed that only one electron is transferred to oxygen **[4]** to form a superoxide ion in the mitochondrial respiratory chain.

The harmful effect of X-rays on animal tissues **[5]** occurs through the production of hydroxyl radicals. Subsequently, evidence emerged for the existence of hydroxyl radicals (OH•), superoxide anion radicals (O_2-•), peroxyl radicals (ROO•), and so on, referred to as active oxygen species nitric oxide (NO•) and nitrogen dioxide (NO_2•), known as active nitrogen species; and Thiol radicals (RS•), known as active sulfur species, and so on. Many epidemiological studies show a close relationship between oxidative stress and non-communicable diseases such as cancer, diabetes, psoriasis, and atherosclerosis, among others. Oxidative stress is an imbalance between free radical production and the antioxidant defense system, where the former prevails, and this situation occurs although humans possess a partially effective antioxidant defense system Therefore, it is necessary to consume food that contains antioxidant properties. This narrative review aims to provide knowledge about the processes that lead to free radical production and antioxidant defense systems. Many epidemiological studies show a close relationship between oxidative stress and non-communicable diseases such as cancer, diabetes, psoriasis, and atherosclerosis.

Oxidative stress is the imbalance between free radical production and the antioxidant defense system, where the former prevails; and this situation occurs despite humans possessing a partially effective antioxidant defense system. Therefore, it is necessary to consume foods that contain substances with antioxidant properties. This narrative review aims to provide knowledge about the processes that lead to free radical production and antioxidant defense systems **[6-8]**Free radicals are produced slightly during moderate physical activity and increase during intense physical activity **[9-11]** An example of this is fructose, which is a type of sugar and is primarily metabolized in the liver. Due to the lack of sufficient regulatory metabolic processes, it exerts high pressure of electrons at the mitochondrial level, increasing the production of free radicals. This effect is closely associated with diseases such as obesity, diabetes, and high blood pressure. **[12-16]**

Antioxidant

Antioxidants can be defined as substances whose presence in relatively low concentrations significantly inhibits the role of oxidation of the targets. Due to the continuous generation of partially reduced forms of oxygen by constitutive metabolic pathways, many protective antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), glutathione reductase (GSHRx), glutathione-S-transferase (GST), and non-enzymatic antioxidants, have involved dealing with toxic species. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. Antioxidants are often reducing agents such as Thiols, ascorbic acid, or polyphenols **[17]**. Antioxidants can generally be classified in different ways: natural and synthetic; polar and nonpolar; enzymatic and non-enzymatic; endogenous and exogenous; and according to the mechanisms in which they participate. **[18]**. Antioxidants primarily exhibit activities based on three mechanisms, hydrogen atom transfer, single electron transfer, and metal chelation **[19].** They show their activity through three different pathways: (i) preventive: prevention of free radical formation and

derivatives; (ii) interruption: interrupt radical oxidation reactions; and (iii) inactivation: inactivate free radical/radical derivative reaction products **[18]**. Endogenous antioxidants are primarily enzymes, such as superoxide dismutase , catalase , glutathione reductase, and glutathione peroxidase . On the other hand, non-enzymatic endogenous antioxidants, such as glutathione and lipoic acid, are products of the body's metabolism **[20,21].**

The first-line defense antioxidants (enzymatic) convert reactive superoxide and hydrogen peroxide into water and oxygen. The non-enzymatic antioxidants can act as a second-line defense against ROS by rapidly inactivating radicals and oxidants. The enzymatic antioxidants further act as the third-line defense involved in the detoxification and removal. Dietary Antioxidants Such as vitamins, carotenoids, polyphenols, flavonoids, and bioflavonoids are exogenous antioxidants that have in vivo activity **[18]** Antioxidants are abundant in fruits and vegetables, as well as in other foods including nuts, grains, and some meats, poultry, and fish. β-Carotene is found in many foods, including sweet potatoes, carrots, cantaloupe, squash, apricots, pumpkin, and mangoes. Lutein, best known for its association with healthy eyes, is abundant in green, leafy vegetables such as collard greens, spinach, and kale. Lycopene is a potent antioxidant found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, blood oranges, and other foods. Estimates suggest that 85% of American dietary intake of lycopene comes from tomatoes and tomato products **[22].** Plant antioxidants are a natural reservoir of bioactive compounds. They play important roles in plant acclimation and adaptation to environmental challenges but are also beneficial for human health. As sedentary organisms, plants cannot escape from environmental challenges, originating from natural origin (e.g., temperature, water availability, soil composition, pests or from anthropogenic practices e.g., destruction of habitats, pollution…). Diverse abiotic factors, like pollution as well as nutrient deficiency, temperature regimes (heat/cold), water supply (drought/flooding), light intensity, day/night rhythms, and radiation, modify the balance between the production and scavenging of reactive oxygen species (ROS) and induce a phenomenon well known as oxidative stress **[23–26].** Although ROS are crucial for normal plant growth and development and play important roles in signal transduction, they are also able to induce cellular damage. Therefore, maintaining the oxidative balance is crucial for plant stress adaptation **[27–29].**

2. Methodology

Amino Acid Analyzer: This device is used to analyze the first and second amino acids. It is also used to analyze a variety of solutions easily with superior sensitivity and detection of amino acids in animal tissues, fruit juices, beverages, proteins, collagen, peptides, extracellular fluids, intracellular fluids, plant and animal tissues, fruit juices, beverages, and hydrolyzed amino acids **[30].**

The extraction process of amino acids:

The amino acids were extracted according to the method, Three grams of the sample were taken and placed in a 10 ml volumetric flask, to which 3 ml of 6M hydrochloric acid with 0.1% phenol was added. The flask was sealed and placed in a thermal oven at 45°C for 24 hours. Then, 3 ml of sodium hydroxide and 0.1 mg of tartaric acid were added and mixed well for 15 minutes. The sample was filtered using a 0.45-micron plastic filter and taken to the device for injection. **[31].**

The derivation process :

take 1 ml of the extracted sample and add 200 microliters of a 5% (Ortho-Phthalate-aldehyde) solution. The mixture was stirred for two minutes, after which 100 microliters of the final mixture were taken and injected into a Kori-origin amino acid analyzer **[31]**. The analysis was conducted at the Ministry of Science and Technology/Department of Environment and Water Laboratories, using the method developed by the scientist Scriver. The mobile phase consisting of methanol, acetonitrile, and 5% formic acid in a flow rate of (1ml/min) was used. A separation column (ZORBAX Eclipse AAA 3.5 µl x id=150x4.6mm) was used to separate the amino acids, while a fluorescence detector with excitation and emission wavelengths of 445 nm and 465 nm, respectively, was used to detect the amino acids. The (Clarity 2015) software was used for amino acid analysis. **[32]**

Preparation Standard Curve:

0.1g of a mixture of highly pure amino acids (99.9%) was taken and dissolved in non-ionic water. It was then transferred to a 250 ml conical flask and the volume was completed to the mark, resulting in a concentration of 250 parts per million. Using the dilution law, calibration curve concentrations were prepared and loaded onto the device **[32].**

Total phenolic content estimation:

Sample Preparation:

The seeds of the plant were dried at room temperature for 24 hours, and then ground into a fine powder in an electric blender. 5 grams of it were taken and placed in a Soxhlet apparatus and extracted with 300 ml of ethanol at 50-55C^0 for (3-4) hours. The extract was filtered through a filter paper and evaporated using a rotary evaporator under low pressure at 40°C. The weight of the extract after the concentration process was 2.6 grams.

It was stored in a storage bottle at 4C^0 until analysis was performed, and total phenolics were detected according to the method provided by Slinkard and Singleton**.** Using Gallic acid and (Folin-Ciocalteu reagent), the method involves taking 150 microliters of the alcoholic extract with 500 microliters of Folin reagent and adding 1.5 ml of 20% sodium carbonate. The mixture is then thoroughly mixed and the final volume is made up to 10 ml. After two hours of reaction, the absorbance value is recorded at a wavelength of 765 nm, and the total phenolic concentration is calculated in milligrams per gram of dry weight relative to the calibration curve of Gallic acid **[33].**

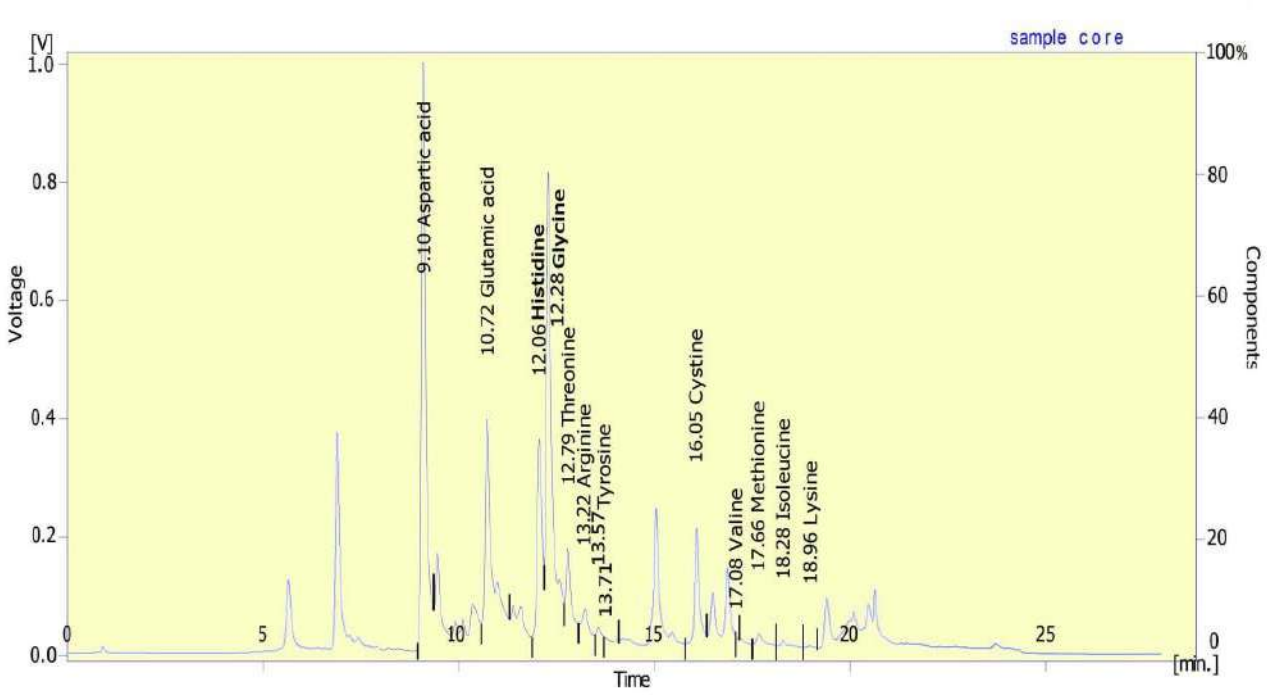

Fig (1) : (ESTD-Sample- Pulp -FID)

3. Result Table (1):

	Reten.Time (min)	Response	Amount (Ul)	Amount %	Peak Type	Compound Name
1	9.100	7538.993	311.076	56.1	Order	Aspartic Acid
2	10.724	3557.019	105.990	19.1	Order	Glutamic Acid
3	12.056	2888.233	13.238	2.4	Order	Histidine
4	12.284	8040.797	53.837	9.7	Order	Glycine
5	12.792	1534.729	16.673	3	Order	Threonine
6	13.220	676.792	11.752	2.1	Order	Arginine
7	13.572	202.190	0000	00	---------	---------
8	13.712	37.311	0.455	0.1	Order	Tyrosine
9	16.048	1464.815	24.315	4.4	Order	Cysteine
10	17.076	1.021	0.022	00	Order	Valine
11	17.656	208.509	11.120	2.0	Order	Methionine
12	18.276	105.429	4.889	0.9	Order	Isoleucine
13	18.960	24.368	0.943	0.2	Order	Lysine
	Total		**554.309**	**100.0**		

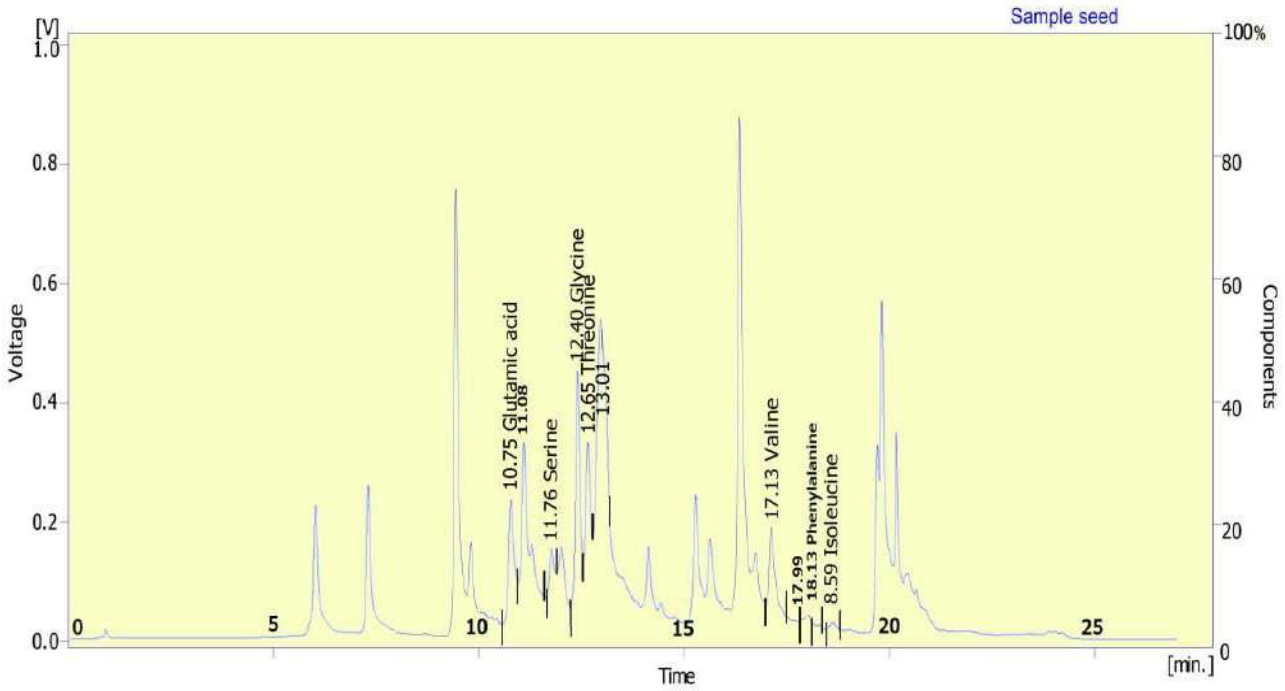

Fig (2): (ESTD-Sample-Seed-FID)

Result Table (2):

	Reten.Time (min)	Response	Amount (Ul)	Amount %	Peak Type	Compound Name
1	10.752	1923.143	57.305	48.1	Order	Glutamic Acid
2	11.080	3522.068	00000	0.0		
3	11.756	368.978	3.775	3.2	Order	Serine
4	12.396	2471.903	16.550	13.9	Order	Glycine
5	12.652	1170.614	12.717	10.7	Order	Threonine
6	13.008	349.522	0000	0.0		
7	17.128	1169.315	24.835	28.8	Order	Valine
8	17.988	115.377	0000	0.0	-------	--------
9	18.132	19.488	0.633	0.5	Order	Phenylalanine
10	18.588	71.223	3.303	2.8	Order	Isoleucine
	Total		119.118	100.0		

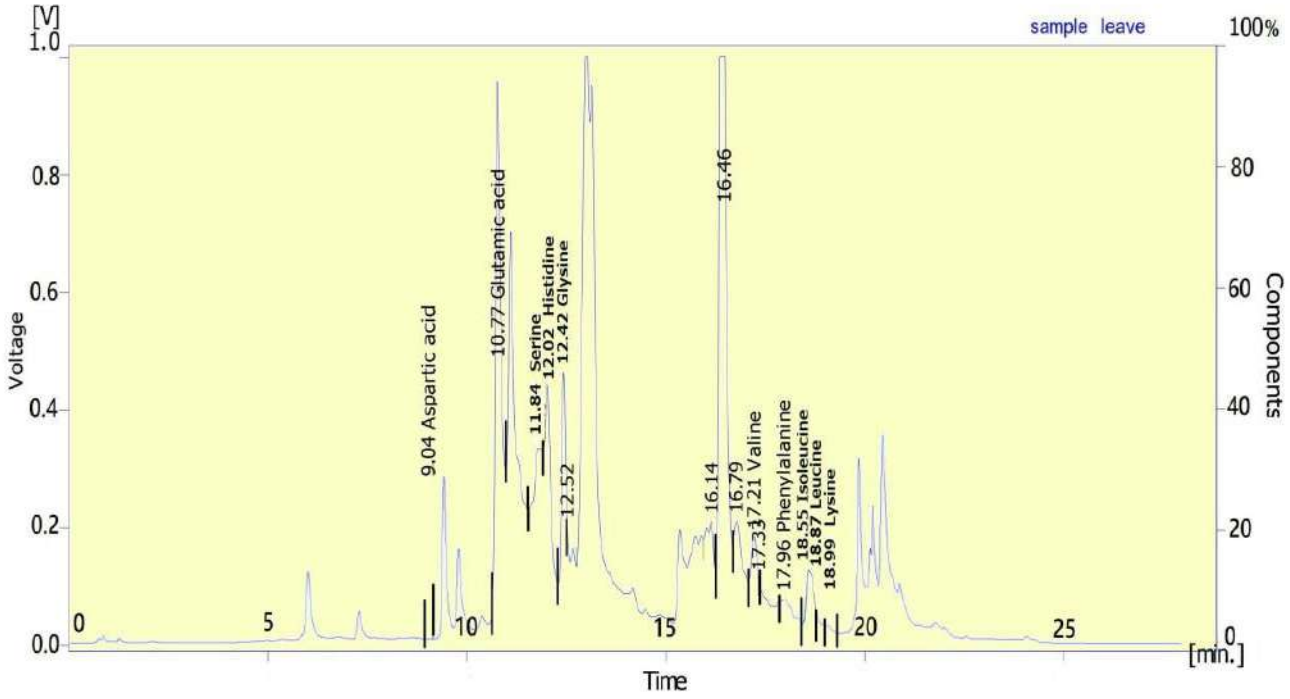

Fig (3): (ESTD-Sample-Leave-FID)

Result Table (3):

	Reten. Time(min)	Response	Amount (Ul)	Amount %	Peak Type	Compound Name
1	9.063	4.648	0.192	0.1	Order	Aspartic Acid
2	10.768	6525.148	194.433	56.1	Order	Glutamic Acid
3	11.840	1920.948	19.651	5.7	Order	Serine
4	12.020	3008.520	13.789	4.0	Order	Histidine
5	12.424	2393.080	16.023	4.6	Order	Glycine
6	12.524	433.822	0000	0.0	--------	---------
7	16.144	646.865	10.737	3.1	Order	Cysteine
8	16.460	11729.611	00000	0.0	--------	--------
9	16.788	1546.907	00000	0.0	---------	--------
10	17.212	1030.078	21.878	6.3	Order	Valine
11	17.332	314.236	0000	0.0	--------	--------
12	17.956	375.007	12.180	3.5	Order	Phenylalanine
13	18.548	1111.660	51.549	14.9	Order	Isoleucine
14	18.780	139.757	3.098	0.9	Order	Lucien
15	18.992	80.223	3.103	0.9	Order	Lysine
	Total		346.633	100.0		

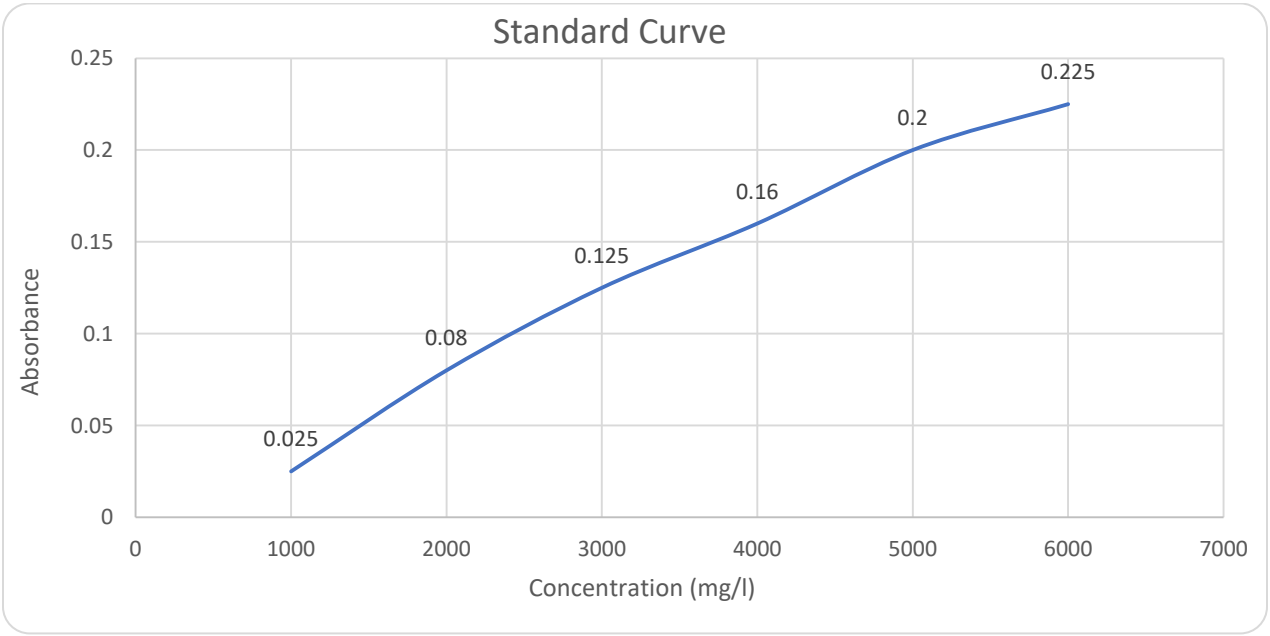

Fig (4): The calibration curve of Gallic acid for estimating total phenols in the seeds of the *Annona muricata* plant.

Table 4: Total phenolic content present in three parts of the Annona Muricata plant: Pulp, leaves, and seeds.

Name	Total Phenolic Compound
Pulp	98.74 mg/gm.
Leave	66.8 mg/gm.
Seed	14.8 mg/gm.

4. Discussion

Several amino acids, including essential and non-essential ones, were diagnosed in three parts of the (*Annona Muricata*) plant, including the pulp, seeds, and leaves, using an amino acid analyzer device. The quantities were as follows: in the pulp, the percentage of amino acids reached 544.309 (Ul/ gm.), in the leaves it reached 346.633 (Ul/gm.), and in the seeds it reached 119.118 (Ul/gm.) . Among the amino acids identified in the pulp of the (*Annona Muricata*) plant, high levels of aspartic acid, glutamic acid, and glycine were found, along with varying amounts of other amino acids, including Histidine, threonine, arginine, tyrosine, Cysteine, Valine, methionine, isoleucine, and Leucine. Previous studies have indicated that these amino acids have significant biological importance. They are utilized for protein synthesis, possess antioxidant properties, contribute to digestion, promote growth and tissue repair, aid in the production of hormones and neurotransmitters in the brain, serve as an energy source, contribute to overall health maintenance, enhance the immune and digestive systems, and play a role in muscle building. **[34].** According to previous studies, fruits are used as a natural remedy for joint inflammation, nerve pain, diarrhea, fever, and malaria, as well as antioxidants, parasites, rheumatism, worms, and skin diseases, and are also used to increase milk secretion after childbirth and to treat various diseases such as high blood pressure, diabetes, liver damage, bacterial infections, and cancer. **[35].** As for the number of amino acids in plant leaves, they include aspartic acid, serine, Histidine, valine, phenylalanine, alanine, isoleucine, and others.

Additionally, there are other amino acids found in plant seeds, such as threonine, glutamic acid, and serine. Plant seeds are used to combat parasitic infections, while the fruit is used to treat joint inflammation, neurological disorders, and diarrhea. The leaves, on the other hand, are used to treat bladder infections, headaches, insomnia, and cancer. **[36] .** The total amount of phenolic in the (*Annona Muricata*) plant was estimated for three parts, the pulp, which was estimated at 98.74 (mg/g), the leaves at 66.8 (mg/g), and the seeds at 14.8 (mg/g). The results indicate a high percentage of phenolic in the pulp of the plant compared to other parts such as the leaves and seeds. This is consistent with previous studies that have indicated the presence of several active components such as phenolic, flavonoids, and alkaloids, which act as antioxidants **[37-38]**. Polyphenols are among the main categories of important natural compounds biologically, showing a wide range of biological and pharmacological activities including antioxidant, anti-inflammatory, immune system stimulant, anti-aging, anti-tumor, antidepressant, and anti-parasitic activities **[39-40]**. The high antioxidant activity of polyphenols is primarily attributed to their oxidative properties, allowing them to act as reducing agents, hydrogen donors, and oxygen quenchers. In this context, oxidative stress plays a crucial role in the development of various neurodegenerative conditions, including rheumatic disorders, heart and vascular diseases, metabolic syndrome, and other diseases. Inflammation is considered a risk factor for high blood pressure, diabetes, and several types of cancer, and may contribute to the development of Alzheimer's disease. **[41]**

5. References

[1]. Sisein, E. A. (2014). "Biochemistry of free radicals and antioxidants." **Scholars Academic Journal of Biosciences 2**(2): 110-118.

[2]. Phaniendra, A., D. B. Jestadi and L. Periyasamy (2015). "Free radicals: properties, sources, targets, and their implication in various diseases." Indian journal of clinical biochemistry 30(1): 11-26.

[3]. Stadie WC, Haugaard N. Oxygen poisoning; the effect of high oxygen pressure upon enzymes; succinic dehydrogenase and cytochrome oxidase. J Biol Chem. 1945;161:153-73.

[4]. Michaelis L. Fundamentals of oxidation and respiration. Am Sci. 1946;34(4):573-596.

[5]. Harmand D. Aging: a theory based on free radical and radiation chemistry. J Gerontol. 1956;11(3):298-300.
[6]. Janciauskiene S. The Beneficial Effects of Antioxidants in Health and Diseases. Chronic Obstr Pulm Dis. 2020;7(3):182-202.
[7]. Janciauskiene S. The Beneficial Effects of Antioxidants in Health and Diseases. Chronic Obstr Pulm Dis. 2020;7(3):182-202.
[8]. Gui-Fang D, Xi L, Xiang-Rong X, Li-Li G, Jie-Feng X, Hua-Bin L. Antioxidant capacities and total phenolic contents of 56 vegetables. J Funct Foods. 2013;5(1):260-6.
[9]. Margaritelis NV, Kyparos A, Paschalis V, Theodorou AA, Panayiotou G, Zafeiridis A, et al. Reductive stress after exercise: the issue of redox individuality. Redox Biol. 2014;2:520–8.
[10]. Margaritelis NV, Theodorou AA, Paschalis V, Veskoukis AS, Dipla K, Zafeiridis A, et al. Experimental verification of regression to the mean in redox biology: differential responses to exercise. Free Radic Res. 2016;50(11):1237-44.
[11]. Yavari A, Javadi M, Mirmiran P, Bahadoran Z. Exercise-induced oxidative Stress and Dietary Antioxidants. Asian J Sports Med. 2015;6(1):e24898.
[12]. Hannou SA, Haslam DE, McKeown NM. Herman MA. Fructose metabolism and metabolic disease. J Clin Invest. 2018;128(2):545- 55.
[13]. Yan-Bo Z, Yan-Hai M, Chang S, Rong-Yuan Z, Shi C. High fructose causes cardiac hypertrophy via mitochondrial signaling pathway. Am J Transl Res. 2016;8(11):4869-80.
[14]. Loza-Medrano SS, Baiza-Gutman LA, Ibáñez-Hernández MA, CruzLópez M, Díaz-Flores M. Alteraciones moleculares inducidas por fructosa y su impacto en las enfermedades metabólicas. Rev Med Inst Mex Seguro Soc. 2019;56(5):491-504.
[15]. Di Nicolantonio JJ, O'Keefe JH, Lucan SC. Added Fructose: A Principal Driver of Type 2 Diabetes Mellitus and Its Consequences. Mayo Clin Proc. 2015;90(3):372-81.
[16]. Jaiswal N, Maurya CK, Arha D, Avisetti DR, Prathapan A, Raj PS et al. Fructose induces mitochondrial dysfunction and triggers apoptosis in skeletal muscle cells by provoking oxidative stress. Apoptosis. 2015;20(7):930-47
[17]. Rasheed, A., & Azeez, R. F. A. (2019). A review on natural antioxidants. Traditional and Complementary Medicine, 1-24.
[18]. Miro´nczuk - Chodakowska, I.; Witkowska, A.M.; Zujko, M.E. Endogenous non-enzymatic antioxidants in the human body. Adv. Med. Sci. 2018, 63, 68–78. [CrossRef]
[19]. Granato, D.; Shahidi, F.; Wrolstad, R.; Kilmartin, P.; Melton, L.D.; Hidalgo, F.J.; Miyashita, K.; Camp, J.V.; Alasalvar, C.; Ismail, A.B.; et al. Antioxidant activity, total phenolics, and flavonoid contents: Should we ban in vitro screening methods? Food Chem. 2018, 264, 471–475. [CrossRef]
[20]. Cömert, E.D.; Gökmen, V. Evolution of food antioxidants as a core topic of food science for a century. Food Res. Int. 2018, 105, 76–93. [CrossRef] [PubMed]
[21]. Fierascu, R.C.; Ortan, A.; Fierascu, I.C.; Fierascu, I. In vitro and in vivo evaluation of antioxidant properties of wild-growing plants. A short review. Curr. Opin. Food Sci. 2018, 24, 1–8. [CrossRef]
[22]. Xianquan S, Shi J, Kakuda Y, Yueming J. Stability of lycopene during food processing and storage. Journal of Medicinal Food. 2005;8(4):413-422
[23]. Meena, K.K.; Sorty, A.M.; Bitla, U.M.; Choudhary, K.; Gupta, P.; Pareek, A.; Singh, D.P.; Prabha, R.; Sahu, P.K.; Gupta, V.K.; et al. Abiotic stress responses and microbe-mediated mitigation in plants: The omics strategies. Front. Plant Sci. 2017, 8, doi:10.3389/fpls.2017.00172.
[24]. García-Sánchez, F.; Simón-Grao, S.; Martínez-Nicolás, J.J.; Alfosea-Simón, M.; Liu, C.; Chatzissavvidis, C.; Pérez-Pérez, J.G.; Cámara-Zapata, J.M. Multiple stresses occurring with boron toxicity and deficiency in plants. J. Hazard. Mater. 2020, 397, 122713, doi:10.1016/j.jhazmat.2020.122713.
[25]. Gou, L.; Zhuo, C.; Lu, S.; Guo, Z. A Universal Stress Protein from Medicago falcata (MfUSP1) confers multiple stress tolerance by regulating antioxidant defense and proline accumulation. Environ. Exp. Bot. 2020, 178, 104168, doi:10.1016/j.envexpbot.2020.104168
[26]. 4- Nadarajah, K.K. ROS homeostasis in abiotic stress tolerance in plants. Int. J. Mol. Sci. 2020, 21, 5208, doi:10.3390/ijms21155208.
[27]. Cuypers, A.; Hendrix, S.; dos Reis, R.A.; De Smet, S.; Deckers, J.; Gielen, H.; Jozefczak, M.; Loix, C.; Vercampt, H.; Vangronsveld, J.; et al. Hydrogen peroxide, signaling in disguise during metal phytotoxicity. Front. Plant Sci. 2016, 7, 1–25, doi:10.3389/fpls.2016.00470
[28]. Gilroy, S.; Białasek, M.; Suzuki, N.; Górecka, M.; Devireddy, A.R.; Karpiński, S.; Mittler, R. ROS, calcium, and electric signals: Key mediators of rapid systemic signaling in plants. Plant Physiol. Biochem. 2016, 171, 1606–1615, doi:10.1104/pp.16.00434
[29]. Hu, C.-H.; Wang, P.-Q.; Zhang, P.-P.; Nie, X.-M.; Li, B.-B.; Tai, L.; Liu, W.-T.; Li, W.-Q.; Chen, K.-M. NADPH oxidases: The vital performers and center hubs during plant growth and signaling. Cells 2020, 9, 437, doi:10.3390/cells9020437
[30]. Fisinin, V., V. Vertiprakhov, A. Grozina and L. Khasanova (2017). "Pancreatic secretion and intestinal digestibility of amino acids in chicken at different dietary protein levels and quality." Agricultural Biology[Sel'skokhozyaistvennaya Biologiya] 52(2): 374-381.
[31]. Dahl-Lassen, R., J. van Hecke, H. Jørgensen, C. Bukh, B. Andersen and J. K. Schjoerring (2018). "High-throughput analysis of amino acids in plant materials by single quadrupole mass spectrometry." Plant Methods 14(1): 8.
[32]. Ali, R. S., W. Chassab Hmood and M. A.-w. Alsirrag (2019). Preparation of dried children's food mixes from local vegetable sources and estimation of their amino and fatty acids content. IOP Conference Series: Materials Science and Engineering, IOP Publishing.

[33]. Slinkard, K. and V. L. Singleton (1977). "Total phenol analysis: automation and comparison with manual methods." American journal of enology and viticulture 28(1): 49-55.

[34]. Kumar, A., & Samanta, K. AMINO ACID PHATHWAY AND ITS SIGNIFICANCE.

[35]. Moghadamtousi, S. Z., M. Fadaeinasab, S. Nikzad, G. Mohan, H. M. Ali and H. A. Kadir (2015). "Annona muricata (Annonaceae): a review of its traditional uses, isolated acetogenins, and biological activities." International journal of molecular sciences 16(7): 15625- 15658.

[36]. Wélé, A.; Zhang, Y.; Caux, C.; Brouard, J.P.; Pousset, J.L.; Bodo, B. Annomuricatin C, a Novel Cyclohexapeptide from the Seeds of Annona muricata. Comptes Rendus Chim. 2004, 7, 981–988. [CrossRef]

[37]. Coria-Téllez, A.V.; Montalvo-Gónzalez, E.; Yahia, E.M.; Obledo-Vázquez, E.N. Annona muricata: A Comprehensive Review on Its Traditional Medicinal Uses, Phytochemicals, Pharmacological Activities, Mechanisms of Action and Toxicity. Arab. J. Chem. 2018, 11, 662–691. [CrossRef]

[38]. Gavamukulya, Y.; Abou-Elella, F.; Wamunyokoli, F.; AEl-Shemy, H. Phytochemical Screening, Anti-Oxidant Activity and in Vitro Anticancer Potential of Ethanolic and Water Leaves Extracts of Annona muricata (Graviola). Asian Pac. J. Trop. Med. 2014, 7, S355–S363. [CrossRef]

[39]. Kumar, S.; Pandey, A.K. Chemistry and biological activities of flavonoids: An overview. Sci. World J. 2013, 2013, 162750. [CrossRef] [PubMed].

[40]. Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouysegu, L. Plant polyphenols: Chemical properties, biological activities, and synthesis. Angewandte Chem. Int. Ed. 2011, 50, 586–621. [CrossRef] [PubMed]

[41]. Uttara, B.; Singh, A.V.; Zamboni, P.; Mahajan, R.T. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr. Neuropharmacol. 2009, 7, 65–74. [CrossRef] [PubMed].

[42]. Al Hatem, J. Y., Sultan, B. S., Diab, F. H., Matar, L. M. (2023). Response of Chrysanthemum Indicum Plant to Treatment with NanoSeaweed Extract and its Imitation on Some Physiological Growth Characetristics of the Plant. *Journal of Global Scientific Research*. 8(2): 2947-2955.