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Farklı Ülkelerdeki *Loranthus europaeus*'un Amino Asit Profili

Haval Mohammed ALİ[1], Fikret KARATAS[2*], Dursun ÖZER[3], Sinan SAYDAM[2]

Öne Çıkanlar:
- Aminoasit
- Ökse otu
- HPLC

Anahtar Kelimeler:
- *Loranthus europaeus*
- Amino asitler
- Toplam amino asit
- HPLC

ÖZET:

Bu çalışmada, farklı ülkelerde yetişen *Loranthus europaeus* örneklerindeki aminoasit miktarları Yüksek Performanslı Sıvı Kromatografisi (HPLC) ile tayin edildi. Esansiyel olmayan aminoasitler bakımından, İran'da yetişen *Loranthus europaeus*, aspartik asit, serin, alanin, pirolin ve tirozin; Irak'ta yetişen, glutamik asit, asparajin, glisin, glutamin; Türkiye'de yetişen örneklerin ise sistein yönünden daha zengin olduğu bulunmuştur. Türkiye'de yetişen *Loranthus europaeus* örneklerinin asparajin, serin, glisin, glutamin, alanine, pirolin ve tirozin; Irakta yetişen aspartik asit, İran'da yetişen ise glutamik asit yönünden fakir oldukları söylenebilir.Toplam esansiyel amino asit açısından en zengin İran iken, en fakir Türkiye'de yetişen *Loranthus europaeus* örneklerinin olduğu belirlenmiştir. Toplam aminoasit miktarı en fazla Irak örneklerinde iken, en düşük olan ise Türkiye örneklerinde bulunmuştur. Esansiyel ve non-esansiyel aminoasit miktarlarının bölgeler arasında değişiklik göstermesi, coğrafi ve ekolojik farklılıklardan kaynaklandığı söylenebilir.

Amino Acids Profile of *Loranthus europaeus* from Different Countries

Highlights:
- *Loranthus europaeus*
- Aminoacids
- HPLC

Keywords:
- *Loranthus europaeus*
- Amino Acids
- Total Amino Acid
- HPLC

ABSTRACT:

In this study, the amounts of amino acids in *Loranthus europaeus* samples grown in different regions (Türkiye, Iran and Iraq) were analyzed by High Performance Liquid Chromatography (HPLC).The results indicate that, aspartic acid, serine, alanine, proline and tyrosine were found to be highest in samples grown in Iran, glutamic acid, asparagine, glycine, and glutamine were found in *Loranthus europaeus* samples grown in Iraq, and cysteine was found to be highest in samples grown in Türkiye. It can be said that *Loranthus europaeus* growing in Turkey is poor in terms of asparagine, serine, glycine, glutamine, alanine, proline and tyrosine, on the other hand aspartic acid found to be lowest in samples from Iraq and glutamic acid is the lowest samples from Iran. It was obtained that while *Loranthus europaeus* samples grown in Iran is the richest in terms of total essential amino acids, on the other hand samples grown in Türkiye is the poorest. Total amino acids content was highest in Iraqi samples and the lowest in Turkish samples. It can be said that the variation of essential and non-essential amino acids contents between regions could be due to geographical, and ecological differences.

[1] Haval Mohammed ALI, (**Orcid ID:** 0000-0002-2500-9760), , University of Duhok Chemistry Department, College of Science, Duhok, Iraq
[2] Fikret KARATAŞ (**Orcid ID:** 0000-0002-0884-027X), [2] Sinan SAYDAM, (**Orcid ID**: 0000-0003-1531-5454), Fırat University, Faculty of Science, Department of Chemistry, Elazig, Türkiye
[3] Dursun Özer, (**Orcid ID**: 0000-0002-7225-8903), Fırat University, Department of Chemical Engineering, Faculty of Engineering, Elazig, Türkiye

***Corresponding Author:** Fikret KARATAŞ, e-mail: fkaratas@firat.edu.tr

INTRODUCTION

The hemiparasite *Loranthus europaeus* (Loranthaceae), also known as mistletoe, is a plant native to Southeast Europe, Anatolia, Southern Russia, Iran and Iraq (Sharquie et al., 2017; Benabderrahim et al., 2019). Mistletoe, which is semi-parasitic, produces its own carbohydrates by photosynthesis while living in various host trees, but obtains the mineral and water needs necessary for nutrition from its hosts. (Griggs, 1991; Ferenc et al., 2014). Mistletoe composition and biological activities depend on harvest season and host tree species (Wagner et al., 1996). Mistletoe, which slows down or stops the development of host trees and causes them to weaken, which is used as an animal feed because they do not shed their leaves in winter (Öztürk et al., 2020). Loranthus has traditionally been used to treat schizophrenia, diabetes, blood clots, and as an immune system enhancer. It is also employed to treat heart illness, epilepsy, infertility, rheumatoid arthritis, oedema, menopausal syndrome, burns, eczema, foot ulcers, swellable injuries, and agglutination. Several scientific studies have revealed that this plant contains bioactive constituents such as rutin, epicatechin, and quercetin (Aidy et al., 2022). Studies have reported that *Loranthus europaeus* contains many substances such as flavonoids, rutin, alkaloids, glycosides, carbohydrates, aldehydes, ketones, protein, polysaccharides, terpenes, phenolic acid, palmitic acid, paraffin and sugar (Harvala et al., 1984; Al-Fartosy and Al-Rikaby, 2007; Sharquie et al., 2016).

It has been reported that amino acids are important in the metabolism of nutrients, cellular growth and development, reproduction and health, and abnormal physiological functions and diseases occur when the intake rate is unbalanced or deficient (Song et al., 2018). It has been reported that liver dysfunction is caused by a change in the ratio of aromatic amino acids to branched-chain amino acids (Lake et al., 2002). It has been reported that different amino acids are used to detoxify ammonia in the blood in liver diseases and in the treatment of heart failure, peptic ulcer and male infertility, while some amino acids serve as intermediate precursors in the production of antibiotics (Stoimenova et al., 2013). Since the amino acid content of food and feed is a measure of its nutritional value, that is an important to determine the amino acid amount in plants (Heems et al., 1998).

According to literature survey, there is not sufficient study was found on the amino acid contents of *Loranthus europaeus*. The main goal of this study is to determine the essential and non-essential amino acid content in the seeds of *Loranthus europaeus* grown in three different regions (Türkiye, İran ve Irak) and compare the results. The results obtained from this work may guide researcher to investigate the way to use *Loranthus europaeus* as source of amino acids.

MATERIALS AND METHODS

Materials

Loranthus europaeus seeds were purchased from markets in three different countries (Turkey, Iraq and Iran). Experiments were carried out by SHIMADZU HPLC, Prominence-I LC- 2030C 3D Model equipped with PDA detector, Sonicator (Wise Clean, WUC-AO3H, 170 W), Blender (Fakir Hausgrate 220 W). Double distilled (dd H_2O) water was used throughout the work. All the chemical used are reagent or analytical grade and obtained from Merck or Sigma-Aldrich.

Determination of Amino Acid

According to the methods used by Çakmak et al. (2021) samples dried at 80 ºC then 1.5 gram taken from the homogenized samples, hydrolysed, derivatized and analysed by HPLC.

Statistical Analysis

All measurements were triplicated and Mean ± Standard deviation was determined. The results were subjected to one-way ANOVA by SPSS 22.0 for Windows. Differences between the group's means were analyzed for significance using Tukey's HSD (honestly significant difference) test The level of statistical significance was expressed as $p<0.05$.

RESULTS AND DISCUSSION

Amino acids are the building blocks of proteins and intermediates in metabolic pathways that regulate gene expression, immunity and signal transduction. In addition to their physiological regulatory roles, amino acids are closely related to other metabolic networks (Wu. 2010; Kim et al., 2015). Analysis results of the *Loranthus europaeus* samples taken from different regions are given in Table 1, Figures 1 and 2.

Table 1. The amounts of amino acids in the seeds of the *Loranthus europaeus* plant grown in Turkey, Iran and Iraq (µg/g dw)

Amino acids	Türkiye	Iran	Irak
Aspartic acid	97.81 ± 5.27 ^{b}	271.34 ± 15.76 ^{a}	61.67 ± 6.36 ^{c}
Glutamic acid	530.79 ± 12.30 ^{a}	448.57 ± 26.19 ^{b}	561.75 ± 33.29 ^{a}
Asparagine	2044.38 ± 30.83 ^{c}	2284.67 ± 73.38 ^{b}	2965.00 ± 55.00 ^{a}
Serine	1.80 ± 0.26 ^{b}	3.13 ± 0.25 ^{a}	2.07 ± 0.15 ^{b}
Glycine	178.06 ± 13.42 ^{c}	259.00 ± 13.53 ^{b}	289.00 ± 14.53 ^{a}
Glutamine	688.11 ± 32.80 ^{c}	939.67 ± 35.50 ^{b}	1371.33 ± 73.21 ^{a}
Alanine	3.90 ± 0.59	4.97 ± 0.18	4.31 ± 0.47
Proline	1.88 ± 0.20	2.42 ± 0.28	2.02 ± 0.26
Tyrosine	291.02 ± 21.19 ^{c}	440.67 ± 27.30 ^{a}	372.00 ± 17.09 ^{b}
Cysteine	36.80 ± 3.22 ^{a}	34.50 ± 4.43 ^{a}	19.80 ± 4.85 ^{b}
Histidine*	465.39 ± 50.21	404.33 ± 26.39	391.67 ± 27.54
Arginine	94.25 ± 7.03 ^{a}	86.33 ± 7.51 ^{a}	41.00 ± 5.57 ^{b}
Threonine*	32.29 ± 4.52 ^{a}	13.33 ± 2.52 ^{c}	21.00 ± 2.65 ^{b}
Valine*	9.57 ± 0.85 ^{a}	9.57 ± 0.65 ^{a}	7.37 ± 0.71 ^{b}
Methionine*	382.33 ± 17.38 ^{c}	677.33 ± 41.97 ^{a}	554.67 ± 22.48 ^{b}
Leucine*	86.67 ± 3.58 ^{c}	169.67 ± 3.06 ^{a}	128.33 ± 6.11 ^{b}
Isoleucine *	41.17 ± 2.75 ^{c}	87.33 ± 4.04 ^{a}	68.33 ± 2.52 ^{b}
Phenylalanine *	374.47 ± 19.62 ^{c}	540.33 ± 37.29 ^{a}	421.67 ± 17.56 ^{b}
Tryptophan*	207.93 ± 24.91	208.33 ± 13.58	233.33 ± 15.28
Lysine*	51.08 ± 5.63 ^{b}	41.67 ± 6.51 ^{b}	83.00 ± 7.55 ^{a}
TE	1745.16 ± 90.58 ^{c}	2238.23 ± 55.51 ^{a}	1950.37 ± 7.27 ^{b}
TNE	3874.57 ± 64.96 ^{c}	4688.93 ± 108.35 ^{b}	5648.95 ± 42.41 ^{a}
TA	5619.73 ± 110.81 ^{c}	6927.16 ± 154.16 ^{b}	7599.32 ± 313.16 ^{a}
TE/TNE%	45	48	35
TE/TA%	31	32	26

Note: *: Essential amino acid, TE: Total essential amino acid, TNE: Total non-essential amino acid, TA: Total amino acid, Statistical difference indicated in table with the different letter the highest average value's expressed with the letter "a" and the lowest value's with the letter "c" in addition the same letter indicates no statistical difference. There are significant differences at the $p<0.05$ level between the values indicated with different letters on the same line. There is no significant difference at the $p>0.05$ level between values without lettering in the same line.

Aspartic acid plays a role in the tricarboxylic acid cycle, defence systems, and the production of signal amino acids (Sanchez et al., 1998). Glutamic acid, which is important for proline biosynthesis, is effective in the carbon and nitrogen cycle in metabolism (Forde and Lea, 2007). The highest aspartic acid and glutamic acid were observed in the samples grown in Iran and Iraq region, respectively while the lowest amount was found in Iraq and Iran (Table 1 and Figure 1).

Asparagine, which is an important in regulating the glucose balance in the cell, provides nitrogen accumulation (Haroun et al., 2010). While asparagine was found highest in the *Loranhtus europaeus* grown in Iraq region, the lowest was the samples from Türkiye ($p<0.05$).

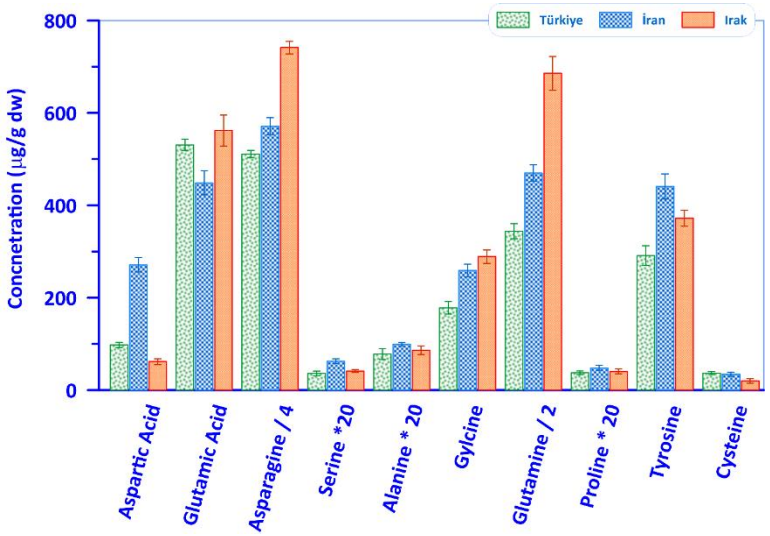

Figure 1. Non-essential amino acid amounts in *Loranthus europaeus* samples from different regions (asparagine value divided by 4, glutamine value divided by 2, serine, alanine, proline values multiplied by 20)

Serine has a fundamental role in signalling, while glycine is effective in reducing the effect of oxygen-dependent free radicals (Ros et al., 2014). The highest amount of glycine and serine were observed in the samples grown in Iran and Iraq, respectively.

While glutamine regulates nitrogen metabolism in the cell (Miflin and Habash, 2002), alanine plays a role in the regulation of the defence system and intracellular pH (Kalefetoğlu and Ekmekçi, 2005). The lowest glutamine and alanine were found in samples grown in Turkey (Table 1 and Figure 1).

Bakar et al. (2021) reported that the amount of serine in white myrtle fruit (2.75 mg/g dw) and glutamic acid (1.8 mg/g dw) in black myrtle fruit. Proline, which involves in protein synthesis and structure, has an important role in wound healing, antioxidant reactions and immune responses in addition to metabolism and nutrition (Wu et al., 2011). The difference between the proline content of *Loranthus europaeus* grown in all three regions is statistically insignificant when compared with each other ($p>0.05$). Duan et al. (2020) found the amounts of aspartic acid, glutamic acid, proline, serine and glycine in licorice 0.56, 0.37, 6.38, 4.86 and 0.14 mg/kg, respectively.

Tyrosine is required for the synthesis of a variety of natural compounds in plants, such as tocopherols, ubiquinone, betalains, and benzylisoquinoline alkaloids. Tyrosine-derived metabolites, tocopherols and ubiquinone are important for plant survival (Xu et al., 2019). Cysteine is very important in supporting protein folding and stability by forming disulfide bonds with other cysteine molecules. Cysteine is a precursor molecule for the synthesis of glutathione, which plays an important role in the response of plants to stress (Mendoza-Cozatl et al., 2010). The highest tyrosine was observed in the sample grown in Iran, while the lowest was observed in the sample grown in Türkiye ($p<0.05$). The lowest cysteine was observed in the sample grown in Iraq ($p<0.05$), the difference between the samples from Türkiye and Iran was statistically insignificant ($p>0.05$).

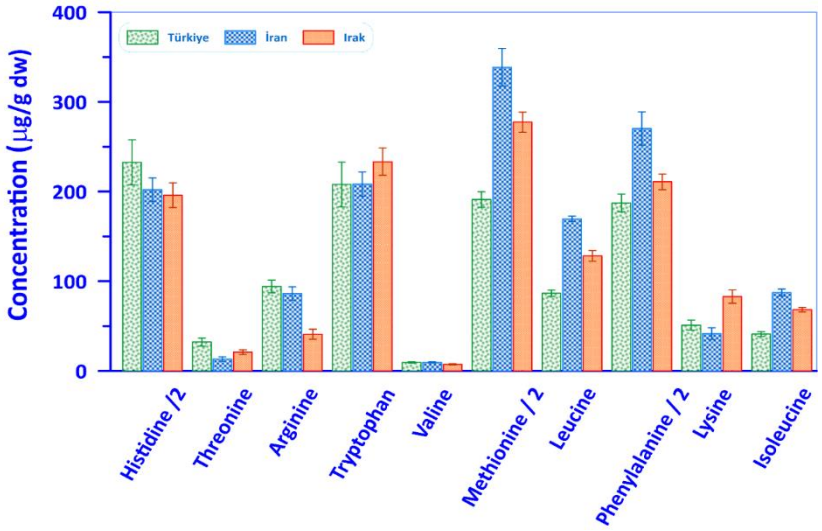

Figure 2. Essential amino acid amounts in *Loranthus europaeus* samples from different regions (Histidine, methionine and phenylalanine values divided by 2)

Histidine is necessary for the formation of red blood cells and the myelin sheath, arginine is an important for growth hormone synthesis and strengthening the immune system (Lee and Kim, 2019). The difference between the amount of histidine in the samples grown in Türkiye, Iraq and Iran was found to be statistically insignificant ($p>0.05$). Arginine content of the sample grown in the Iraq region is the lowest and Türkiye region is the highest, on the other hand the difference between samples from Iran and Türkiye regions were insignificant ($p>0.05$). Mukhtar et al. (2022) found the amounts of glycine, asparagine, glutamine, histidine and arginine in bitter tomato eggplant grown in Nigeria 4.58, 5.36, 1.22, 8.40 and 0.09 mg/g dw, respectively.

Threonine, which is an important in fat metabolism and immune system, is also found in the structure of proteins such as collagen and elastin (Olgun et al., 2016). While the highest amount of threonine was observed in the sample grown in Türkiye, the lowest amount was determined in the sample grown in Iran, and the difference between regions was significant ($p<0.05$).

Proteins rich in proline, glycine, leucine and methionine play an important role in cell wall growth, while leucine, isoleucine and valine have been reported to protect the cell against osmotic stress (Zemanova et al., 2017). Phenylalanine, tyrosine and tryptophan are aromatic amino acids derived from the shikimate pathway and are required for protein synthesis and production of aromatic secondary metabolites, e.g. anthocyanin. It is stated that tryptophan, which is important in cell development, is the precursor of neurotransmitter biomolecules (Zemanova et al., 2017). The lowest valine was found in the *Loranthus europaeus* sample grown in Iraq, the highest values found in the samples from Iran and Türkiye regions. While the highest amount of methionine, leucine, isoleucine, phenylalanine was observed in samples grown in Iran region, the lowest was determined in samples grown in Türkiye, and the difference between regions is statistically significant ($p<0.05$) (Table 1 and Figure 2). Mukhtar et al. (2022) found the amounts of methionine, tryptophan, lysine, cysteine and tyrosine in the dark eggplant sample grown in Turkey 3.71, 1.0, 6.80, 1.88 and 1.02 mg/g dw, respectively.

In the parsley leaf, alanine, proline, leucine, isoleucine, valine and threonine were found to be 2.79, 0.80, 0.35, 0.22, 0.79 and 0.79 g/kg, respectively (Duan et al., 2020). The difference between tryptophan content in samples grown in all three regions is insignificant ($p>0.05$). Lysine has a role in metabolism, such as producing glutamic acid and increasing resistance to stress (Azevedo and Lea, 2001). While the highest amount of lysine is found in the sample grown in the Iraq region ($p<0.05$), the difference between other regions is statistically insignificant ($p>0.05$). While total essential amino acid amounts 1745.16,

1950.37 and 2238.23 were found in *Loranthus europaeus* samples grown in Turkey, Iraq and Iran, the total amino acid values 5619.73, 7599.32 and 6927.16 µg/g dw were found respectively. The difference between the values of the three regions were significant ($p<0.05$) (Table 1).

Mukhtar et al. (2022) reported that the total amount of essential amino acids in five different eggplant samples ranged from 19.31 to 27.21 mg/g dw. Sadiq et al. (2013) found the amount of essential amino acids in the pulp and seed of the date palm (Phoenix dactylifera), respectively 12.78 and 7.11 g/100 g dw, while the total amino acid amount 28.22 and 15.28 g/100 g dw were reported.

The total amount of essential and non-essential amino acids in Nitraria tangutorum Bobr were 44.39 and 65.65 mg/g dw, respectively, and it has been reported that TE/TA, TE/TNE ratios should be over 40% and 60%, respectively, in a good protein source (Zhou et al. 2019).

As seen in Table 1, TE/TNE ratio in *Loranthus europaeus* samples were between 35-48%, while TE/TA values were between 26 and 32%. From these results, it can be said that the essential amino acid ratios are lower than the recommended amount in all samples studied (Table 1).

CONCLUSION

In terms of non-essential amino acids, it can be said that the samples grown in Iran and Iraq are richer than Türkiye. It can be said that the *Loranhtus europaeus* samples grown in Türkiye is rather poor in terms of asparagine, serine, glycine, glutamine, alanine, proline and tyrosine amino acids compared to other regions. *Loranhtus europaeus* grown in Iran is rich in aspartic acid, serine, alanine, proline and tyrosine, while the samples grown in Iraq is richer in glutamic acid, asparagine, glycine and glutamine then the other regions.

It can be said that *Loranthus europaeus*, which grows in the Iran region, is richer in total essential amino acids compared to other regions.

The difference between the regions in terms of total amino acid, essential amino acid and non-essential amino acid was statistically significant ($p<0.05$) when compared with each other.

Loranthus europaeus is thought to have the potential to be used in important sectors such as pharmaceuticals and food additives in terms of essential amino acids such as histidine, trypthophane, methionine and phenylalanine.

Conflict of Interest

The article authors declare that there is no conflict of interest between them**.**

Author's Contributions

The authors declare that they have contributed equally to the article**.**

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