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Investigation of chemical activity, SCHIFF base reactions and staining effects of some amino acids by spectrophotometric and theorical methods

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ABSTRACT

In this study, the staining properties of selected amino acids with Brassica oleraceae extract in alum and alum-free media were investigated. Basic, acidic and neutral amino acids (arginine, glutamic acid and glycine) were used to investigate the effect of staining. It was determined that all amino acids were stained in alum media. In the second step, the R group of amino acids found in the proteins of the cell nucleus was reacted with salicyl aldehyde. This reaction was successful only with Arginine. The staining properties of the newly formed compound were also investigated in alum and alum-free environments. Evaluation of the results was done using FT-IR and ^{1}H NMR methods. All compounds were optimized with the Gaussian G09 program (DFT/B3LYP/6.311 G(d.p) basic set. HOMO, LUMO and HOMO-LUMO gap values were determined. Chemical reaction capabilities of amino acids were discussed with the help of HOMO-LUMO gap values.

1. Introduction

Protein molecules, which work as structural elements, transporters, regulators, catalysts etc. in all living things, are one of the four most basic molecules within the cell. Although there are about 300 kinds of amino acids in nature, which are the constituents of proteins, the proteins found in living systems and cells are composed of different combinations of only 22 different amino acids [1–8]. DNA packaging is an important step in the control of gene expression, with histone proteins playing a role in the first step of packaging. In eukaryotic cells, DNA is packaged in a chromatin structure with 5 types of histone proteins (H2A, H2B, H3, H4, and H1) to form the structure of the nucleosome. The nucleosome is a structure formed by wrapping 146 base-pairs of DNA with 1.65 rounds of turns on histone octamer and exists as repetitive units in DNA. Various posttranslational modifications such as acetylation, methylation, phosphorylation, ubiquitination, and sumolysis occur because the histone proteins forming the nucleosome remain outside the nucleus of the amino tail regions [9,10]. This tail region is a center where many cellular activities are carried out. Therefore, the chemical reactions and dyeing studies that can be performed here are particularly important.

There are various histological techniques involving phenolic compounds in the anthocyanin structure for staining of the cell nucleus. Carminic acid, hematoxylin, and anthocyanin from red cabbage, dahlia, and anthocyanins have been used as dye sources in histological staining and this is called carmine staining [10].

In this study, red cabbage extract was used in the dyeing process. Panagiotis et al. investigated the anthocyanins in red cabbage with High Performance Liquid Chromatography (HPLC/DAD-ESI/Qtrap MS) with mass spectroscopy and found that they were all composed of cyanides such as monoclonal aglycones, which are represented as mono and diglycosides. Based on this information, the red cabbage extract used in this study was considered to contain more cyanide molecules. In alum media, the spectral analysis of the chelate structures formed by this molecule in the cell was conducted and the staining and binding conditions were interpreted. Glycine is the simplest of amino acids. But it has very important benefits. It helps to treat low pituitary function and is effective in the treatment of progressive muscle meltdown. Excessive amounts of this amino acid lead to exhaustion, while the intake of appropriate amounts produces more energy. This small molecule is also necessary for the function of the central nervous system, used in the treatment of manic depression and hyperactivity, and can help prevent epileptic seizures [11].

Arginine has many benefits, including immune function, fertility, detoxification and improvement of brain power. Another important role of arginine is that it stimulates the body for the production of beneficial growth hormones and insulin, which helps deliver glucose to cells for

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growth and energy production. Arginine also combats inflammation, reduces arteriosclerosis and heart attack risk, repairs blood vessels, repairs congestive heart failure and combats coronary artery disease, helps to lower blood pressure, reduces muscle pain**, and** improves mental capacity and kidney function [12,13].

In addition to protein synthesis, glutamic acid has several key functions in the body. It acts as an important neurotransmitter and the body uses it to generate other neurotransmitters, such as GABA. Therefore, this amino acid is critical to healthy brain development and function [14,15].

For this purpose, the amino acids used (glycine, arginine, and glutamic acid) were stained with red cabbage extract. The results were interpreted using FT-IR spectroscopy. In the second stage, the -NH_2 groups of these molecules were reacted with Salicylic Aldehyde to obtain new Schiff bases [16–19]. The aim was to increase the reaction ability of the tail portion of the amino acids.

Compounds containing the C=N group and frequently encountered as ligands in coordination chemistry were synthesized by Hugo Schiff in 1864 for the first time and there after they began to be known as Schiff bases. Schiff bases can be represented by the general formula RCH = NR'. Compounds in this group are obtained from the reaction between carbonyl compounds and amines. Scheme 1 shows the general mechanism of Schiff Bases.

In the third and final stage, the reaction results were evaluated with the help of FT-IR and ^{1}H NMR spectroscopy and the new molecules were stained with red cabbage extract. The results obtained in the third stage and the results from the first stage were compared and discussed.

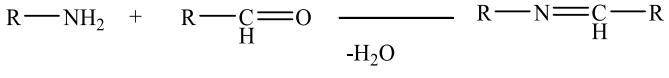
The questions, why are we trying to convert amino acids into Schiff bases? and how will it benefit us? may come to mind. why are we trying to convert amino acids into Schiff bases? How will it benefit us? We have stated that amino acids participate in reactions with tail portions outside the helix. When active groups are added to this external tail, the amino acid can be made more chemically active. This feature can be used in drug research. Cancer cells can be traced by staining with plant dyes under optimum conditions, or the cancer cell can be monitored by performing complex studies with transition metals over Schiff base. Even further, complexes with low chemical activity can be synthesized to block cancer cells.

It is possible to determine the chemical activity of these synthesized substances by making some theoretical calculations. Here, we see the term HOMO-LUMO gap.

HOMO is the highest occupied molecular orbital and LUMO is the lowest unoccupied molecular orbital [20,21].

The HOMO-LUMO gap is where the most likely electronic stimuli can most likely occur. Especially, when you have a large aromatic system, this leads to a small HOMO-LUMO gap. In this case, since it is easy for the π electrons to jump to a higher energy level that is close in energy, electronic mobility is greater, as is the case with large aromatic systems or transition metal complexes (and therefore tends to be colored). It is the most important energy deficit to consider.

Therefore, smaller HOMO-LUMO gap means low stability and high chemical activity. For this, we calculate calculated HOMO LUMO energy levels of all amino acids and new compound.



Scheme 1. General Reaction scheme for Schiff Bases.

2. Experimental methods

2.1. Materials

All used chemicals were supplied from the manufacturer. The red cabbage was obtained from Bafra, Samsun, Turkey.

2.2. Instrumentation

The FT-IR spectra of the synthesized compounds were recorded with a Schimadzu 8400 FT-IR spectrometer and 1H, 13C NMR spectra were recorded with a Bruker topspin 400 MHz spectrometer.

2.3. Preparation of compounds

2.3.1. Preparation of the red cabbage extract

The red cabbage, obtained from Bafra, Samsun was homogenized using an IKA T-18 homogenizer. 20 g of this material was taken and a 20% (w/v) solution was prepared by adding distilled water. The mixture was boiled at low heat for 30 min and filtered. The filtrate was stored in the refrigerator for use when needed.

2.3.2. Staining of amino acids for FT-IR analysis

Each Eppendorf tube contained the following:

1. 1 ml red cabbage extract
2. 0.05 g glycine (Merck) dissolved in 1 mL distilled water,
3. The two solutions are were mixed together.
4. The second tube was prepared as 1 ml red cabbage extract, 0.05 g Glycine and 0.05 g alum solution.

Using this method, two series of four tubes were prepared. Four of them were placed in a bath at 50 °C while the other four were placed in a bath at 80 °C in a bain-marie. After 90 min, the tubes were cooled down to room temperature and FT-IR analysis was performed. The same procedures were repeated for Arginine and Glutamic acid.

2.3.3. Recovering a Schiff base from amino acids

First, 0.01 mol amino acid was dissolved in distilled water. After solubilization in alcohol, 0.01 mol salicylic aldehyde was added to this mixture. The reaction was performed at room temperature to prevent degradation of the amino acids and then at 50 °C and 80 °C, respectively. Although the reaction of Arginine with salicylic aldehyde was successful, Glycine and Glutamic acid did not react with salicylic aldehyde. FT-IR and ^{1}H -NMR analysis of arginine was performed before and after its conversion into a Schiff base. The chemical formulas of the amino acids are shown in Fig. 1.

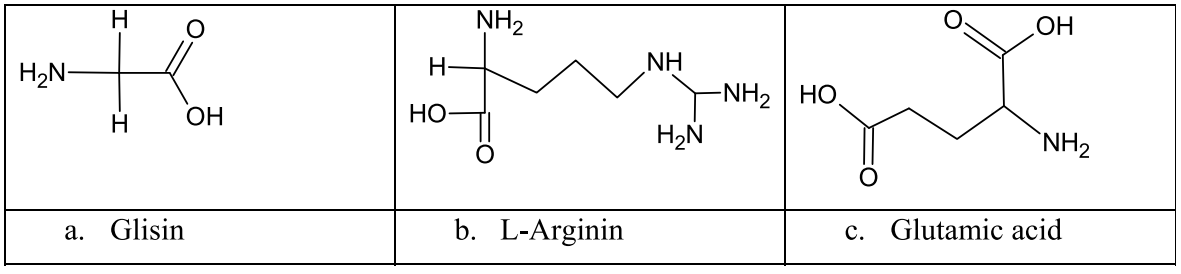


Fig. 1. The chemical formulas of the amino acids.

3. Results and discussion

Red cabbage extract contains cyanidin. A chelate complex is formed between the -OH groups of the cyanide and the metal atoms. The resulting chelate complexes were dyed in the visible region. Likewise, amino acids contain both -OH and -NH_2 groups and can create chelate complexes.

In a previous study [22] by the authors, onion roots were obtained and stained with red cabbage extract, these cells were reacted with salicylic aldehyde and this staining procedure was repeated two times. After the reaction was completed, it was seen that the seeds of the onion root cells were not stained. In the continuation of this study, to explain the above results, staining and closure studies were performed with amino acids, which are the constituents of histone proteins in the cell nucleus. This study used glycine, arginine, and glutamic acid as amino acids. In this study, glycine, arginine, and glutamic acid were used as amino acids. The staining studies were performed at 50 °C–80 °C. The pH was in the range of 3 and 11. This study determined the optimum conditions for the amino acids to be pH 6–8, 50 °C in aqueous media. The optimum conditions for the amino acids were determined to be pH 6–8, 50 °C in aqueous media.

Study results were examined under a separate title for each of the amino acids. FT-IR and ^{1}H NMR analyses of the amino acids were taken to evaluate the study results.

3.1. Glycine

According to the evaluated results of the FT-IR diagrams, while the determination of -NH_2 stretching vibrations were seen at 2901 cm^{-1} in the aqueous solution of Glycine at 50 °C and pH 6 without alum, this same vibration was observed toward 3008 cm^{-1} with alum. This result shows that Glycine interacted with the cabbage extract when alum was used [23]. In other words, it was stained. Thus, the intense bands at 1505 and 1410 cm^{-1} may be confidently assigned to the asymmetric and symmetric stretches of the CO_2 group [24], the bands at 1333 cm^{-1} to CH_2 deformations [25,26], and the bands at 1110 cm^{-1} to CN stretches. The same stretching vibrations were observed at 1480,1407,1438,1103 cm^{-1} in alum and *Brassica oleracea* extract environments, respectively [27,28]. Fig. 2 shows FT-IR diagrams of Glycine. a: Before staining, b: After staining with *Brassica oleracea* extract in alum media.

In the second step, the reaction of Glycine with salicylic aldehyde was investigated. Due to the small size of Glycine, it has a limited ability to react. It was seen that the reaction could not occur. Therefore, the staining properties of the new Schiff base were not investigated.

3.2. HOMO-LUMO studies

In Table 1, HOMO LUMO energies are given as a.u. In this case 1 au = 27.211 eV conversion Glycine HOMO = -7.23 eV and Glycine LUMO = -8.13 eV. From here Glycine HOMO-LUMO gap is 5.35 eV. HOMO and LUMO appear to be spread throughout the molecule.

3.3. Arginine

This study was repeated with Arginine, which has a larger size and contains three groups of -NH_2. In the FT-IR studies of Arginine, -NH_2 groups were detected at 2968, 3161, and 3344 cm^{-1} [29], and the -OH stretching vibration in the carboxyl group was identified as a broad band

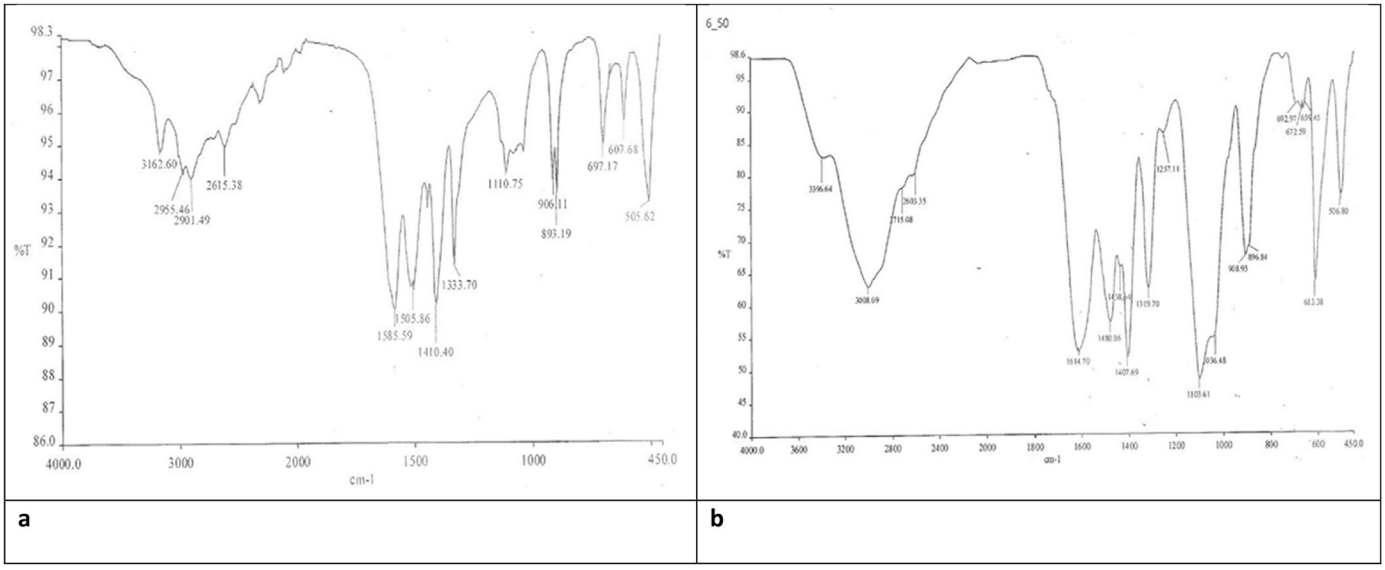
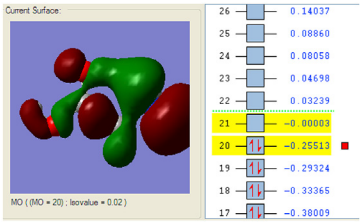
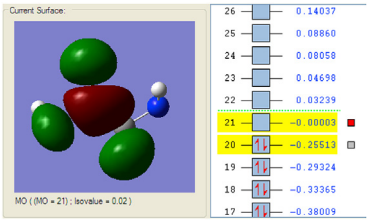


Fig. 2. FT-IR results of Glycine a: Before staining, b: After staining with *Brassica oleracea* extract in alum media.

Table 1
HOMO-LUMO energy level for Glycine.

Glycine HOMO	Glycine LUMO
	

under the -NH_2 groups. The C=N stretching vibrations was were detected at 1632, 1549, 1550 cm^{-1}. The performed FT-IR studies with Arginine amino acid by different investigators showed that the stretching vibration of the Arginine -NH_2 group was marked at 3261 cm^{-1}, the stretching vibration of the -NH group was marked at 3297 and 3324 cm^{-1}, and the -OH stretching vibration was marked at 3404 cm^{-1}. Literature [30] was compared with the obtained data from this study.

In the second phase of the experimental studies, it was observed that the samples were not stained at pH 8.3 and 50 °C without using alum. Shifts were not observed in the stretching vibrations of the -NH_2 groups and therefore, no staining was seen. However, the -NH_2 stretching vibration was observed at 3161 cm^{-1} for Arginine, which was shifted to 3173 cm^{-1}. The peak became broad in the alum media. In other words, arginine was stained with Brassica Oleraceae. Other -NH_2 stretching vibrations were observed at 2980 and 3345 cm^{-1} in alum and brassica environments. -CH_2 stretching vibrations were detected in alum and brassica environments at 1399, 1319 and 1416 cm^{-1}, respectively. The result of FT-IR diagrams are given in Fig. 3.

Arginine amino acid was converted to a Schiff base at the reaction temperature of 50 °C with salicylic aldehyde for 3 h. At the end of the reaction, the C=N stretching vibration was observed at 1633 cm^{-1}, intensified and shifted to 1645 cm-1 -NH_2 stretching vibrations were observed at 2876, 3161, and 3337 cm^{-1}. After reaction, the 2876 cm^{-1} -NH_2 stretching vibrations disappeared. Using only FT-IR is not sufficient to explain in which -NH_2 group the binding occurs. Hence, the ^{1}H-NMR diagram of a new compound was taken. Then, this new compound was called 2-amino-5-(amino(2-hydroxybenzilideneamino) methyleneamino) pentanoic acid. The salicylic aldehyde was thought to bind over the -NH_2 group at the tail end of the Arginine helix. The corresponding reaction is shown in Scheme 2.

The ^{1}H NMR spectrum of the Arginine molecule was taken from the human metabolism database and compared with the data in this study. The data shifts from the database are shown in the chain between the -CH_2 groups of 1.637 and 1.651 ppm, and the shifts of the-NH and -NH_2 groups are shown between 3.20 and 3.76 ppm. In the study's obtained spectrum of the Arginine molecule, the shift of -CH_2 groups are is marked

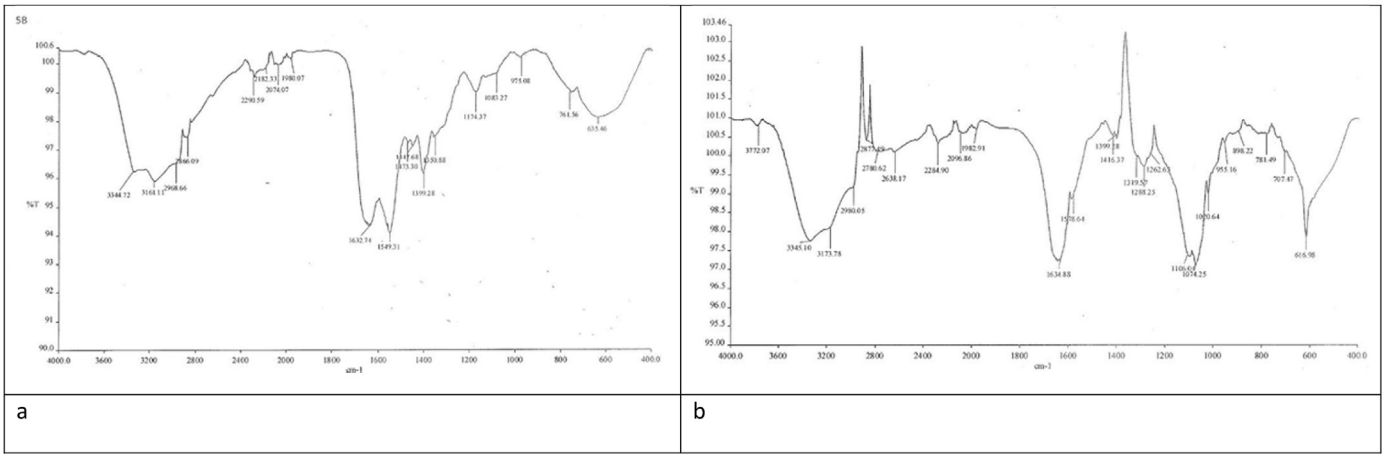
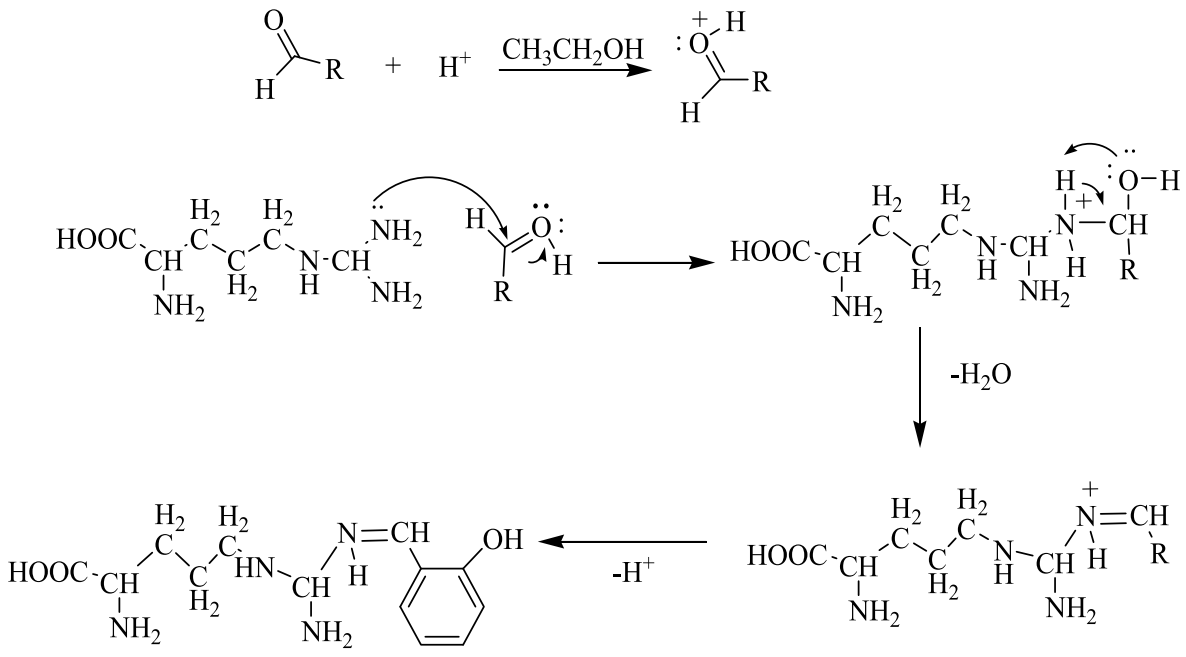


Fig. 3. The FT-IR diagrams of arginine: a: In aqueous media, b: With alum and Brassica oleraceae extract.



Scheme 2. The proposed reaction mechanism for 2-amino-5-(amino(2-hydroxybenzilideneamino)methyleneamino) pentanoic acid.

between 1.40 and 1.53 ppm. The shift values for the -NH and -NH_2 groups are marked between 3.07 and 4.71 ppm. The ^{1}H NMR spectrum of the Arginine used in the study is given in Fig. 4.

After the conversion of the amino acid molecule to the Schiff base (Scheme 2), the -CH_2 groups were marked at about 1.69 ppm. The-NH and -NH_2 groups were marked at 3.11 and 3.30 ppm. Then, the protons of the aromatic ring were marked between 6.94 and 7.57 ppm. The -N = CH bond signal of the new compound was detected at about 8.2–8.3 ppm. The ^{1} H NMR spectrum of the compound is given in Fig. 5.

These results show that this study is consistent with literature and that the arginine molecule in the cell was successfully converted to the Schiff base.

In the last section, an FT-IR diagram of 2-amino-5-(amino(2-hydroxybenzilideneamino)methyleneamino) pentanoic acid was given. The new compound was reacted with *Brassica oleraceae* extract with and without alum. When the results were examined, no change was observed in the FT-IR spectra. In other words, the new compound was not stained. This result proves that amino acids interact with a metal atom through their -NH_2 groups. FT-IR diagrams of the before and after staining are shown in Fig. 6.

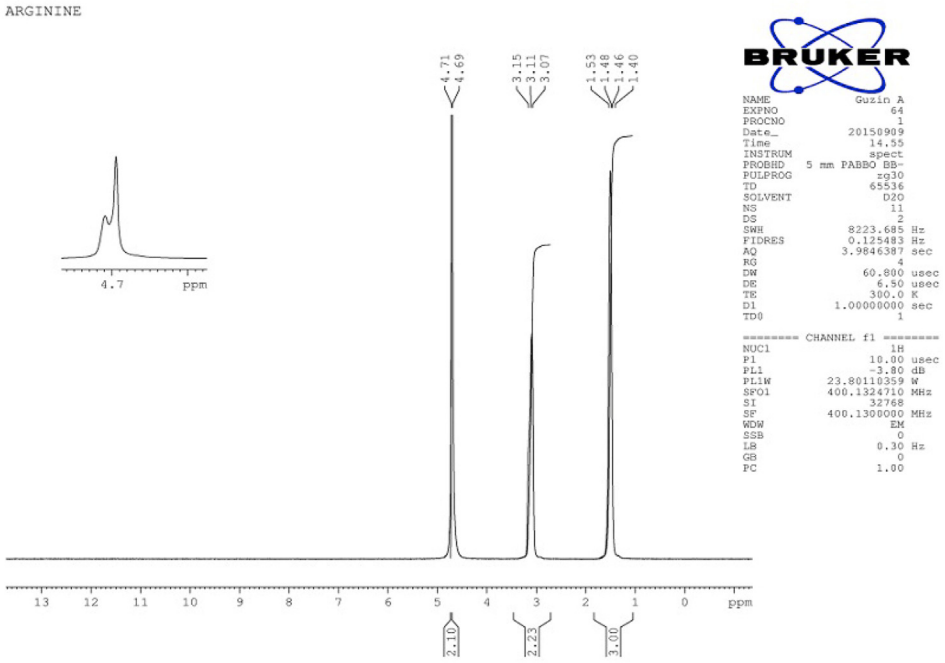


Fig. 4. The ^{1}H NMR spectrum of an Arginine molecule.

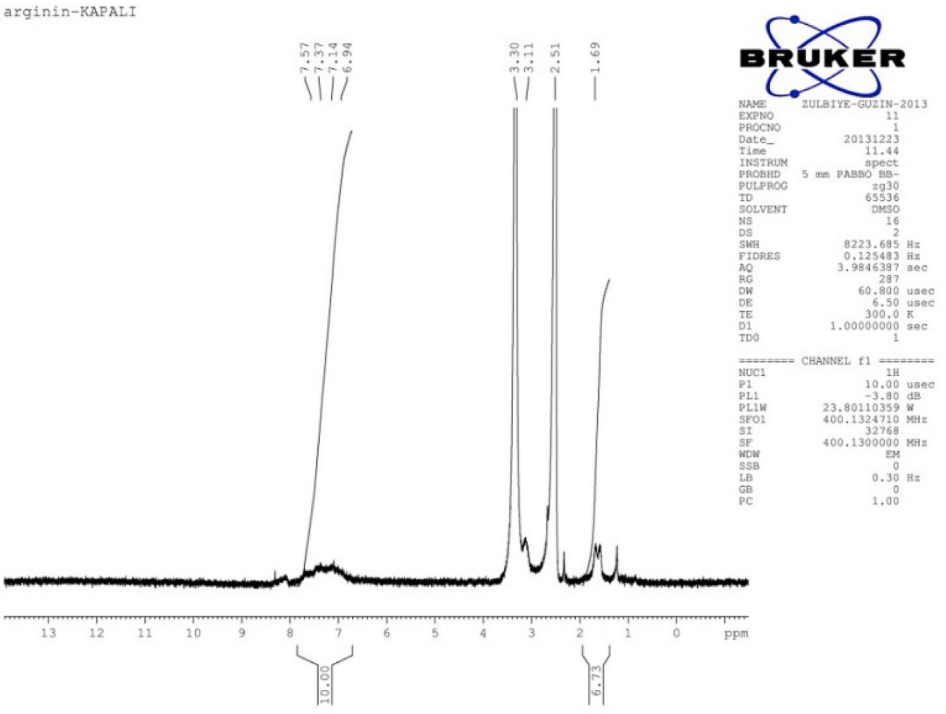


Fig. 5. ^{1}H NMR diagram of 2-amino-5-(amino(2-hydroxybenzilideneamino)methyleneamino)pentanoic acid molecule.

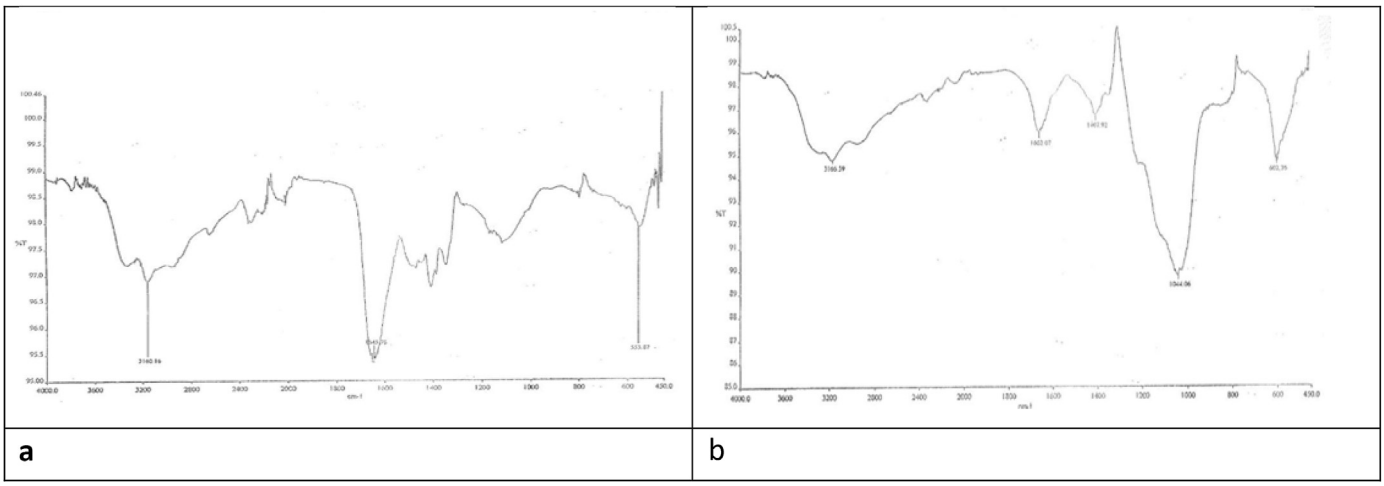


Fig. 6. The FT-IR diagrams of 2-amino-5-(amino(2-hydroxybenzilideneamino)methyleneamino)pentanoic acid. a: New compound, b: New compound stained with *Brassica oleraceae* extract in alum media.

3.4. HOMO-LUMO studies

The HOMO-LUMO results for arginine and 2-amino-5-(amino(2-hydroxybenzylideneamino)methyleneamino) pentanoic acid are given in Table 2.

In general, low value (1,5 eV) is indicating indicates that the compound is chemically active [31,32].

The results show that the HOMO-LUMO gap value of 2-amino-5-(amino(2-hydroxybenzilideneamino)methyleneamino) pentanoic acid is lower. It can be concluded that this compound is chemically more active than Arginine.

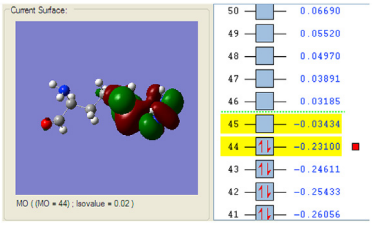
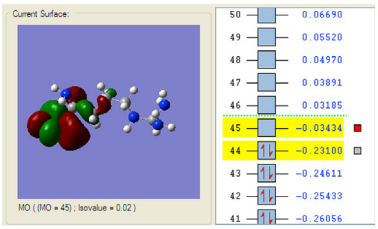
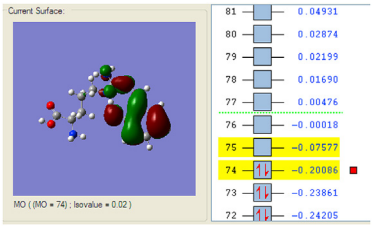
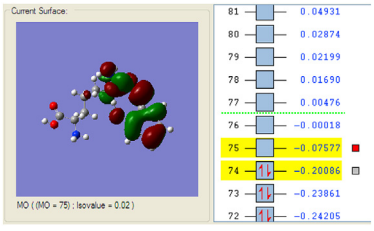
In addition, when the HOMO and LUMO maps created by Arginine Gaussian program are examined, it is seen that LUMO is on (1) group and HOMO is on (2) group. In other words, the molecule receives the electron through the first group (LUMO) and gives through the second group (HOMO). But in 2-amino-5- (amino (2-hydroxybenzylideneamino) methyleneamino) pentanoic acid compound, HOMO and LUMO belong to the same (1) group. That is, all chemical exchanges of the molecule are carried out through the same group. Arginine and 2-amino-5- (amino (2-hydroxybenzylideneamino) methyleneamino) pentanoic acid groups 1 and 2 are indicated in Fig. 7.

3.5. Glutamic acid

An FT-IR study of Glutamic acid was performed. An FT-IR diagram of the compound is shown in Fig. 8a. In the FT-IR studies of Glutamic acid, an -NH_2 stretching vibration was detected at around 3666 cm^{-1}. Other detected important vibrations are listed as follows: $\nu(CH_2)$ at 2970, ν_a (CO_2^-) at 1640, ν_s (CO_2^-) at 1417, ν (C-O) at 1211 and ν (NH_3^+) at 1124 cm^{-1}. Literature data [33,34] and obtained data from our study were compared with each other.

In the study of Glutamic acid under the same reaction conditions with alum, the -NH_2 group was observed around 3659 cm^{-1} as a broad and weak band. By looking at this significant amount of slipping, it can be said that Glutamic acid was stained with the *Brassica oleraceae* extract in the alum media. Other vibration frequencies were detected in the same

Table 2
Arginin and 2-amino-5-(amino(2-hydroxybenzilideneamino)methyleneamino) pentanoic acid HOMO-LUMO Gap diagram.

Arginin HOMO	Arginin LUMO
	
2-amino-5-(amino(2-hydroxybenzilideneamino)methyleneamino) pentanoic acid HOMO	2-amino-5-(amino(2-hydroxybenzilideneamino)methyleneamino) pentanoic acid LUMO
	

$Arginin_{HOMO}$ = -6.28 eV and $Arginin_{LUMO}$ = -0.93 eV. From here Arginin's HOMO-LUMO gap is 5.35 eV.2-amino-5-(amino(2-hydroxybenzilideneamino) methyleneamino) pentanoic acid's HOMO: -5.44 eV and LUMO: -2.06 eV. HOMO-LUMO gap value is 3.38 eV.

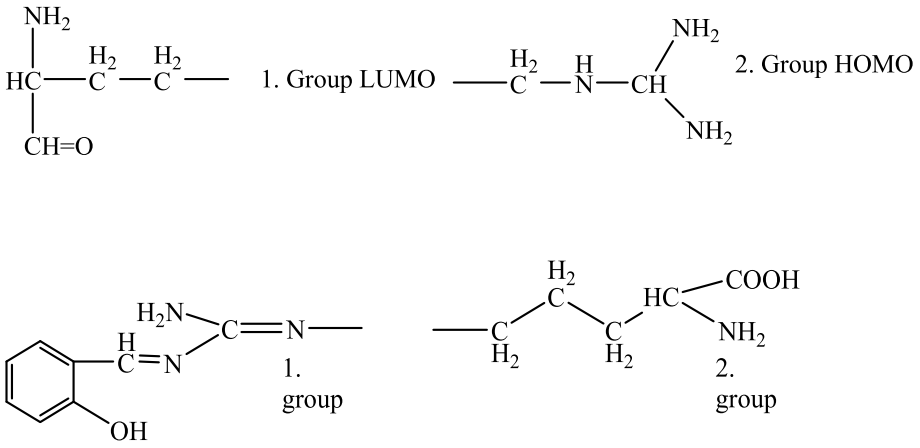


Fig. 7. Arginine and 2-amino-5- (amino (2-hydroxybenzylideneamino) methyleneamino) pentanoic acid's 1 and 2 groups.

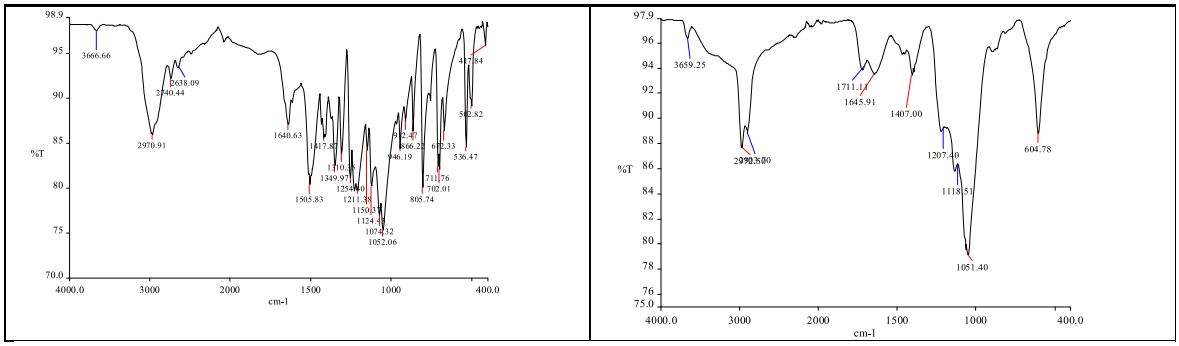


Fig. 8. a. FT-IR diagram of Glutamic acid a. with no alum b. Stained Glutamic acid with alum.

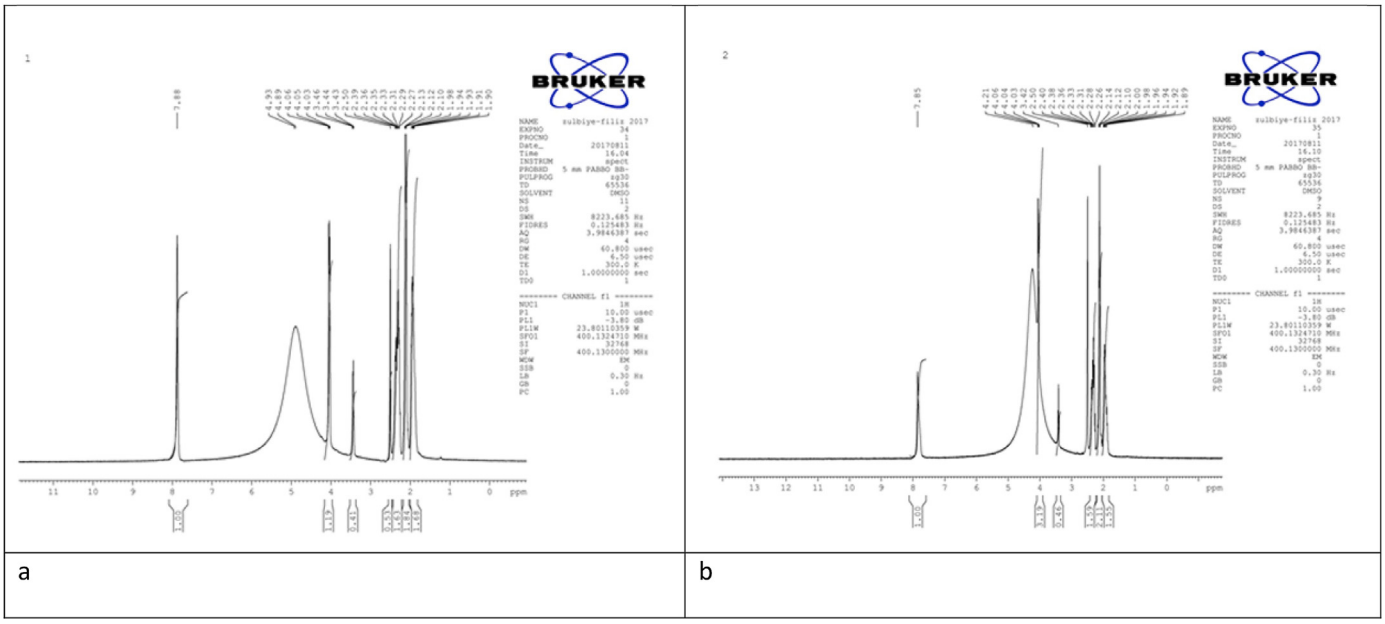
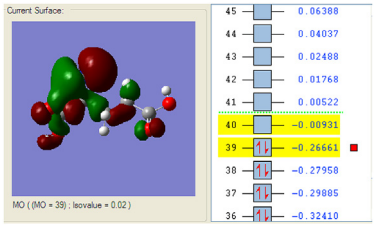
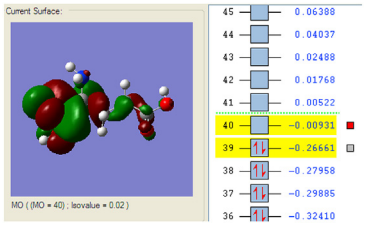


Fig. 9. [1]H NMR diagrams of Glutamic acid. a. Before the reaction with Salicylic Aldehyde, b. After the reaction with Salicylic Aldehyde.

Table 3
HOMO and LUMO energy level for glutamic acid.

Glutamic Acid HOMO	Glutamic Acid LUMO
	

Glutamic $Acid_{HOMO}$ = -7.23 eV and Glutamic $Acid_{LUMO}$ = -0.25 eV. From here, Glutamic Acid's HOMO-LUMO gap is 6.97 eV. HOMO and LUMO belong to the same group.

order at 2990, 1645, 1407, 1207, 1118 cm^{-1} in alum environment. significant shifts were not observed without alum. The FT-IR spectrum of stained Glutamic acid in alum media is given in Fig. 8 b.

In the second phase of this section, glutamic acid was interacted with salicylic aldehyde. The acid did not react with aldehyde at 50 °C and pH 6. This is understood from the fact that the ^{1}H NMR diagrams are the same before and after the reaction. Glutamic acid, like Glycine has only one $–NH_2$ group. Therefore, the reaction capability is limited and the reaction did not occur. The ^{1}H NMR spectra before and after the reaction with glutamic acid and salicylic aldehyde are shown in Fig. 9a and b.

3.6. HOMO-LUMO studies

The HOMO-LUMO results for Glutamic Acid are given in Table 3.

4. Conclusion

As the research has demonstrated, glycine, arginine and glutamic acids were stained with red cabbage extract in alum at pH 6–8 and 50 °C. Reaction studies with salicylic aldehyde were successful with Arginine. But Glycine and Glutamic acid did not react. It was determined that (2-amino-5-(amino (2-hydroxybenzylideneamino)methyleneamino)pentanoic acid) compound did not stain with and without alum. This result was interpreted as as such that the staining process did not occur because the active end of the amino acid was closed by salicylic aldehyde. In theoretical studies, the chemical activities of amino acids were found to be low. The activity of 2-amino-5-(amino(2-hydroxybenzylideneamino) methyleneamino) pentanoic acid compound is higher than amino acids. Amino acids can carry out many reactions through $-NH_2$ groups. From these results, it can be said that additional $-NH_2$ groups to be added to amino acids will significantly increase the activity. The increasing chemical activity value shows that 2-amino-5- (amino (2-hydroxybenzylideneamino) methyleneamino) pentanoic acid can be evaluated for drug and anticancer.

Notes

Compliance with ethical standards.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jics.2021.100315.

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