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Amino acids profile of Two Chinese nuts (*Juglans sigillata* and *Castanea mollissima*) in local markets of Kurdistan region/Iraq using HPLC.

Yaseen Noori M. ALshekhany¹, Rajaa M. Hasbullah², Nazanin S. Salam³ and Kaban S. Hamadamin⁴.

^{1,3&4} Biology Department , Faculty of Science & Health , Koya University ,University Park , Daniel Miterrand Boulevard , Koysinjak KOY45, Koya City , Erbil , Kurdistan Region , Iraq.

² General Directorate of Education Salahuddin, Ministry of Education.

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Corresponding Author*:

yasin.nori@koyauniversity.org

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ABSTRACT

Aim: current study was aimed to study the amino acid contents of two Chinese nuts, walnuts (*Juglans sigillata* Dode.) and chestnuts (*Castanea mollissima* Blume.) from the local market of the Kurdistan region / Iraq.

Methodology: Walnut fruits (*Juglans sigillata*) and chestnut fruits (*Castanea mollissima*) were collected from local market in the Kurdistan region / Iraq, samples were collected, dried and ground. one milgram of each sample was weighed, then dissolved in 10 ml High Performance Liquid Chromatography (HPLC) methanol, the sample was shaken and agitated in an ultrasonic bath for 10 minutes , then concentrated by evaporating the solvent with a stream of liquid N₂ until it reached 1 ml, filtrated the liquid sample on deposable sep-pak cartridge filter (supleco Co, Ltd),then 20 ml were injected on HPLC column .

Results: firstly current study can identified thirteen amino acids from chestnuts but there are only eight amino acids were identified from walnuts. Secondly, there are big different between them in the percentage of amino acids where valine is appear in the highest percentage which record 69.43 % , while it recorded 6.32% in chestnut fruit , While threonine showed the highest percentage in chestnuts arrived to 54.09% compared to only 3.72% in walnuts fruits.

وصف الأحماض الأمينية لاثنتين من المكسرات الصينية الكستناء والجوز (*Juglans sigillata* و *Castanea mollissima*) في الأسواق المحلية في إقليم كردستان / العراق باستخدام HPLC.

ياسين نوري^١، رجاء منصور^٢، نازنين سامان^٢ و كابان صالح^٢.

^{١,٣&٤} قسم علوم حياة، كلية العلوم والصحة، جامعة كويه، أربيل، إقليم كردستان، العراق.

^٢ المديرية العامة لتربية صلاح الدين.

الملخص

الهدف: هدفت الدراسة الحالية إلى دراسة محتويات الأحماض الأمينية لأول مرة لاثنتين من المكسرات الصينية، الجوز (*Juglans sigillata*) والكستناء (*Castanea mollissima*) من السوق المحلي لإقليم كردستان / العراق.

المنهجية: تم جمع ثمار الجوز (*Juglans sigillata*) وثمار الكستناء (*Castanea mollissima*) من السوق المحلي لإقليم كردستان / العراق، وتم جمع العينات وتجفيفها وطحنها. تم وزن ١ ملغم من كل عينة، ثم إذابتها في ١٠ مل من الميثانول كروماتوغرافيا الغاز العالي، وتهتز العينة وتهيج في حمام بالموجات فوق الصوتية لمدة ١٠ دقائق، ثم تتركز عن طريق تبخير المذيب مع تيار من السائل N٢ حتى تصل إلى ١ مل، قم بترشيح العينة السائلة على مرشح خرطوشة sep-pak القابل للتصرف (supleco Co, Ltd)، ثم تم حقن ٢٠ مل على عمود HPLC.

النتائج: في الدراسة الحالية تم تحديد ثلاثة عشر حمضا أمينيا من الكستناء ولكن هناك ثمانية أحماض أمينية فقط تم تحديدها من الجوز، ثانياً، هناك اختلاف كبير بينهما في نسبة الأحماض الأمينية حيث يظهر الفالين في أعلى نسبة والتي سجلت ٦٩,٤٣٪، بينما سجل ٦,٣٢٪ فقط في فاكهة الكستناء، بينما أظهر ثريونين أعلى نسبة في الكستناء وصلت إلى ٥٤,٠٩٪ مقارنة ب ٣,٧٢٪ فقط في ثمار الجوز.

Introduction.

Proteins and protein complexes are the workhorses of our cells, and amino acids are their component parts. The breakdown of amino acids in cells yields a wide variety of useful byproducts, including purines, pyrimidines, neurotransmitters, and many more. Protein, unlike lipids (which are stored in lipid droplets in adipose tissue) and carbs (which are stored as glycogen in the liver and muscle), has no specific storage depot in the body. [1].

China is a home of two species of walnut trees (*J. regia* and *J. sigillata*). There are several types of walnuts, each with its own unique physical and chemical characteristics in the kernel and varying levels of production. At least some of them have shown promise in evaluations, thus they might be used as breeding germplasm [2]. Nutrients including quinones, oils, tannins, essential fatty acids, albumin, mucilage, mineral matter, amino acids, and more may be abundant in walnut kernels, which are widely recognized abundance in walnut kernels, which are widely recognized as a superfood. Scientists are looking at the effects of eating grown walnuts, which contain variousof nutrients, on human health. It was shown that eating walnut kernels regularly may help improve vascular function and reduce blood cholesterol levels [3]. Furthermore, phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine, and histidine are considered necessary human nutrientsfor humans. Critical amino acids for newborns and developing children include cysteine (or sulfur-containing amino acids), tyrosine (or aromatic amino acids), and arginine [4]. Since the human body cannotto synthesize the necessary amino acids, they play a crucial function in physical growth. Arginine, cysteine, glycine, glutamine, histidine, proline, serine, and tyrosine are considered essential amino acids, although the others are only needed under certain circumstances [5].

Chestnuts (*C. mollissima*) have been cultivated for chestnuts (*C. mollissima*) dates back more than two thousand years in China, making them a valuable and historically significant culinary fruit. The amounts of vital fatty acids, fiber, saccharides, vitamins, and minerals it provides are impressive. In a study [6], Additionally, chestnuts are a great way to get your daily dose of plant-based protein. It was calculated that 100 grams of fresh chestnut fruit had 3.5 grams of crude protein, which equates to around 9.2% of the Recommended Dietary Intake (RDI) for women and 7.6% for men. It is well known that plant-based proteins may replace animal-based proteins in the human diet; in fact, plants provide around 65% of the world's protein supply on a per capita basis. In addition, the amino acid requirements of the human body may be met by consuming plant protein as a full and balanced supply. Recent research has shown that aspartic acid, asparagine, and glutamic acid are the most abundant free amino acids in chestnut.6,7 As a result, chestnuts might be a useful addition to the diet. According to research [7].

Therefore, the present work aims to study for the first time the amino acid contents, also determine their proportions and importance in two Chinese nuts, namely walnut (*J. sigillata*) and chestnut (*C. mollissima*) from local markets of Kurdistan region / Iraq.

Plant materials.

Description of walnut (*J. sigillata*).

The iron walnut, or *J.sigillata*, is the second-most-popular kind of walnut tree in cultivation, after the Persian walnut, or *Juglans regia*. Widespread across the region including the Himalayas and western China. The tree has

been grown for its edible nuts, and following the successful use of grafting technique, at least 80 varieties have been generated.

Miner-Williams [8] study, the nuts are thick-shelled and round, with lumps and seal-like depressions (sigillatae) in the shell; the species is known as the "iron walnut" because of its hard exterior. The tree's wood is also put to good use. Yunnan is the most productive province in China for walnuts, both in terms of land area and crop output [9], although you may also find them in Guizhou, Sichuan, and Xizang. As a decorative plant, it is occasionally cultivated.

Scientific classification.

Kingdom: Plantae

Clade: Angiosperms

Order: Fagales

Family: Juglandaceae

Genus: *Juglans*

Species: *sigillata*

Binomial name: *Juglans sigillata*

Description of chestnut (*C. mollissima*) .

Native to China, Taiwan, and Korea, *Castanea mollissima*, or the Chinese chestnut, is a species of chestnut in the Fabaceae family [10]. It's a broad-crowned, deciduous tree that may reach heights of 20 metres. The simple, alternating leaves are 10-22 centimetres in length, 4.5-8 centimetres in width, and have a serrated border. Catkins 4–20 cm in length bear male and female flowers, respectively, with the females located near the catkin's base. The fruit, a spiny cupule 4-8 cm in diameter, contains two or three shiny brown nuts; in the wild, the nuts are smaller, measuring just 2-3 cm in diameter. The tender downiness of the young leaves and stems inspired the species name *mollissima* in botany. [11]

Scientific classification.

Kingdom: Plantae

Clade: Angiosperms

Order: Fagales

Family: Fagaceae

Genus: *Castanea*

Species: *mollissima*

Binomial name : *Castanea mollissima*

Methods and Materials.

Collection of walnuts and chestnuts.

From the Kurdistan region's local markets, we procured walnut (*J. sigillata*) and chestnut (*C. mollissima*) fruits, which we then dried and powdered. Powdered samples were stored in sterile containers until chemical analysis using the HPLC method could be performed.

Extraction and chemical analysis.

Each sample was weighed out at 1 mg, dissolved in 10 ml HPLC methanol, shaken and agitated in an Ultrasonic bath for 10 minutes, concentrated by evaporating the solvent with a stream of liquid N₂ until reaching 1 ml, and finally filtered through a disposable sep-pak cartridge filter (supelco Co., Ltd) before 20 ml were injected onto an HPLC column [12].

Separation conditions of amino acids.

-HPLC column: C18 (50×4.6mm I.D. 3µm).

-Mobile phase : Buffer : Mix

-Detection: UV set at 220 nm.

-Flow rate: 2 ml/min.

-Temperature: 30 C

-Sequences of the eluted material of the standard were as follow, each standard was 50µg /ml.

- Dilution : 1

The concentration for each Amino acid was quantitatively determined by comparing the peak area of the standard (Figure 1 and table 1) with that of the samples at same retention time / minute.

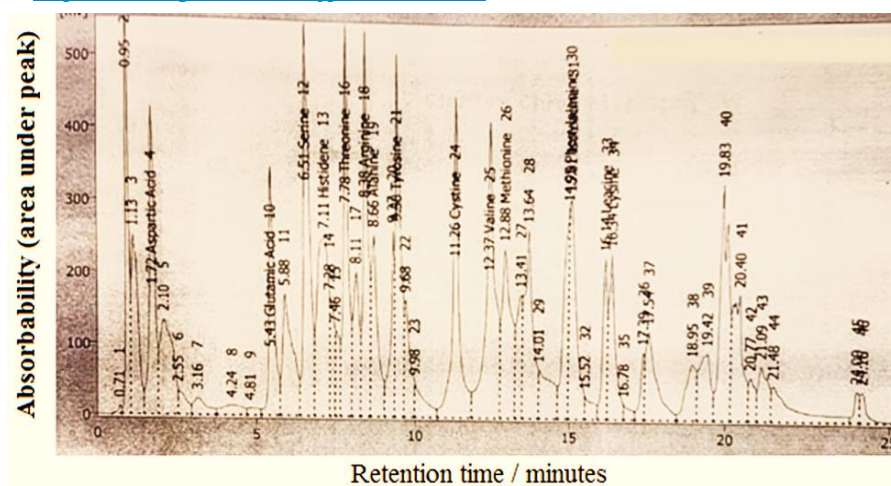


Figure 1. The standard Amino acids peaks by HPLC.

Table 1. The standard analysis of amino acids with retention time, area and concentration.

No.	Amino acids	Retention time /minute	Area	Concentration mg/ml
1	Aspartic acid	1.724	4953.428	10
2	Glutamic acid	5.432	3957.989	10
3	Serine	6.512	7238.555	10
4	Histidine	7.108	6132.243	10
5	Threonine	7.780	5705.922	10
6	Arginine	8.380	5466.186	10
7	Alanine	8.660	3771.473	10
8	Tyrosine	9.380	5527.861	10
9	Cysteine	11.260	6485.364	10
10	Valine	12.360	8283.545	10
11	Methionine	12.880	5108.140	10
12	Phenylalanine	14.952	3351.253	10
13	Isoleucine	15.008	5156.387	10
14	Lucien	16.136	2337.050	10
15	Lysine	16.344	3499.403	10

For calculation of unknown concentration of amino acids, the following equation is using [13, 14 and 15]:

$$\text{Unknown conc. of sample } \mu\text{g/ml} = \frac{\text{Area of sample under peak}}{\text{Area of standard under peak}} \times \text{conc. of standard} \times \text{dilution factor.}$$

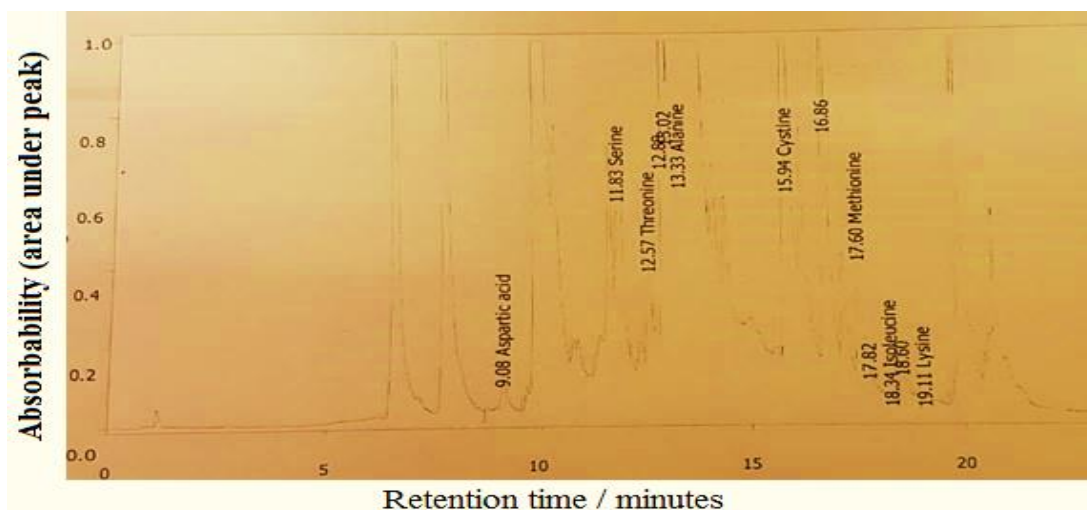
Result and discussion.

Amino acids content of Chinese walnut (*Juglans sigillata*).

By following Table 2 and Figure 2 it is clear that *J. sigillata* contain eight types of amino acids, which are (aspartic acid ,serine , threonine , alanine , cysteine ,methionine , isoleucine and lysine). These amino acids appeared in different concentrations and proportions after performing chemical analysis with the technique of HPLC , These amino acids give great importance to the Chinese nut that is abundant in the local markets of the Kurdistan Region / Iraq , where amino acids are considered the basic building block for building protein in living organisms, including humans [16]. It is clear that the amino acid Alanine is the highest concentrated, which gives it superiority over the rest of the amino acids and its ratio was (52.753mg/ml), while the lowest concentrated amino acid is (0.0123 mg / ml) and in which the Isoleucine appeared. It is worth mentioning that Threonine , Cysteine Methionine , Isoleucine and Lysine are an important amino acids that the body cannot produce and it is very important to take them through a daily diet, [3] [17].

Table 2. The concentration and percentage of amino acids which separated from *J. sigillata*.

Seq.	Amino acids	Area	Concentration mg/ml	Concentration %
1	Aspartic acid	982.124	1.991	2.62
2	Serine	3468.308	4.791	6.31
3	Threonine	1184.302	2.076	2.73
4	Alanine	30446.076	52.753	69.43
5	Cysteine	6284.047	9.690	12.75
6	Methionine	2231.464	3.910	5.15
7	Isoleucine	6.350	0.0123	0.015
8	Lysine	266.959	0.763	1.00

**Figure 2** Amino acids peaks of *J. sigillata* extract by HPLC.

Amino acids content of Chinese Chestnut (*C. mollissima*).

Figure 3 appears Thirteen Amino acids (aspartic acid ,glutamic acid ,serine , histidine , threonine , arginine , alanine , tyrosine , valine , phenylalanine , isoleucine , lucien , lysine) were identified in *C. mollissima* . Threonine showed the highest level (2.507 mg/ml) compared with other Amino acids identified while Lysine exhibited the lowest level (0.032 mg/ml) (table 3) . Threonine and Valine have been reported as being responsible for the biological function of the metabolism system because they are considered one of important amino acid which our body cant synthesis but must be taken from daily diet [18], [19] and [20] . This study was in line with previously conducted studies on amino acids of other plants [21] and [22].

Table 3. The concentration and percentage of Amino acids which separated from *Castanea mollissima*

Seq.	Amin acids	Area	Concentration mg/ml	Concentration %
1	Aspartic acid	34.172	0.069	1.49
2	Glutamic acid	73.342	0.185	3.99
3	Serine	235.226	0.325	7.03
4	Histidine	236.350	0.384	8.28
5	Threonine	1464.309	2.507	54.09
6	Arginine	37.498	0.069	1.49
7	Alanine	11.063	0.293	6.32
8	Tyrosine	0.339	0.0006	0.01
9	Valine	440.238	0.531	11.46
10	Phenylalanine	13.024	0.039	0.84
11	Isoleucine	400.354	0.077	1.66
12	Lucien	28.785	0.123	2.65
13	Lysine	86.648	0.032	0.69

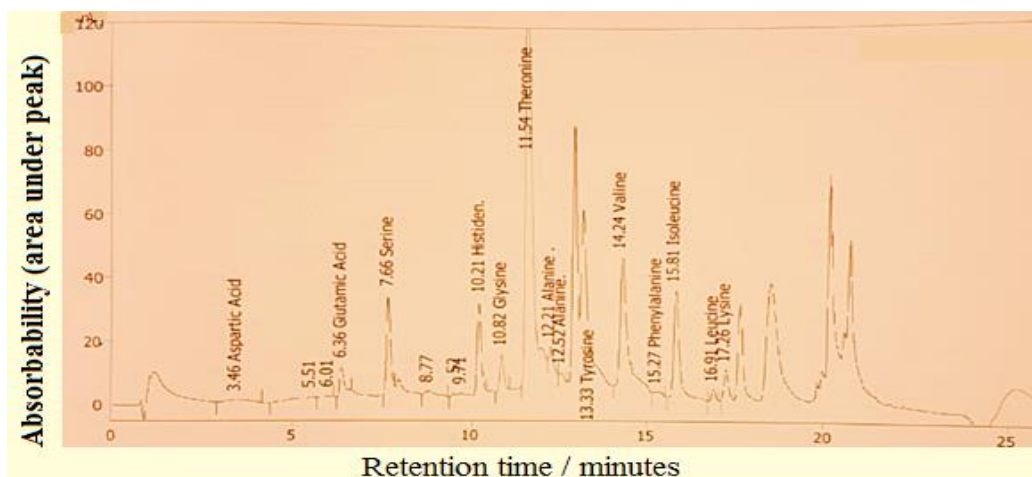


Figure 3. Amino acids peaks of *Castanea mollissima* extract by HPLC.

In order to more discuss the findings and to clarify the percentage of amino acids and make compare between chinees walnut and chains chestnut, figure 4 shows that there are clear differences between them. firstly our study identified thirteen amino acids from chestnuts (*C. mollissima*) but there are only eight amino acids were identified from walnuts (*J. sigillata*). The second point there is a big difference between them in the percentage of amino acids where alanine appears in the highest percentage which records 69.43 % , While it only scores 6.32% in chestnut fruit and threonine shows the highest percentage in chestnuts arriving at 54.09% compared to only 3.72% in walnut fruits.

If we compare it here to which one is better, we see that the two are important because they have a lot of important amino acids for our bodies, as far as the types of amino acids are concerned, chestnuts contain more amino acids, but at the same time, the percentages of these acids are few compared to nuts and at the same time there are high ratios of the amino acids in the nut but the number of amino acids is less compared to chestnut (figure 4). Begum [23] found that chestnuts and walnuts are an important source of protein, and this is consistent with what this study showed, because the presence of these amino acids, which are the primary ancestors of protein, is evidence of protein [24].

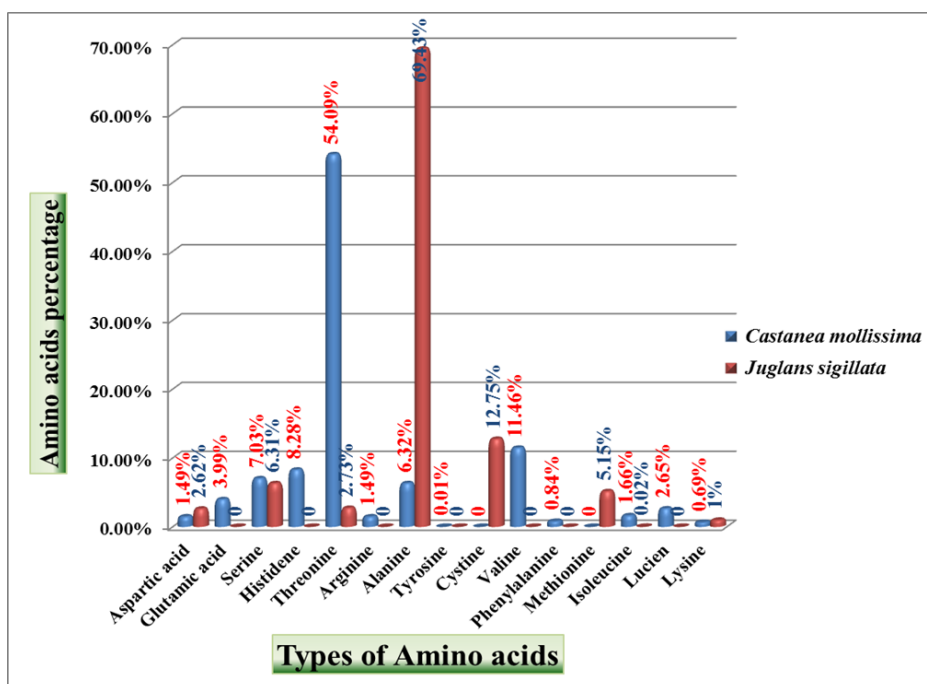


Figure 4. Percentage data of Amino acids content for *C. mollissima* and *J. sigillata*.

Conclusions:

1- Chinese walnuts which found in the local markets of the Kurdistan region / Iraq carry high levels of amino acids which important to the body and thus can be an important source of protein that the body need.

2- Chestnut is considered one of the important sources of many amino acids that the body cannot produce and since the chestnuts present in the Iraq Kurdistan region's markets its only source is China, therefore, Chinese chestnut is very important and may go into many treatments because the large number of amino acids in it may give it medicinal importance as well.

References

1. **Cahill**, G.F. (2006). Jr. Fuel metabolism in starvation. Annu. Rev. Nutr. 2006, 26, 1–22.
2. **Martinez**, M. L. ; Labuckas, D. O. ; Lamarque, A. L.. Maestri, D. M.(2010). Walnut (*Juglans regia* L.): genetic resources, chemistry, by-products. J. Sci. Food Agric., 90 (12): 1959–1967
3. **Zhang**, Y (2014). "Germplasm resource of walnut in Yunnan and its exploitation and utilization". J Northwest for Univ. 19 (2): 38–40.
4. **Zheng**, Y. J., Y. Li, Y. L. Zhang, R. G. Zhang, Q. A. Zhang, Y. F. Zhang and S. L. Zhao (2015). RSC Adv., 5, 12613.
5. **Suresh**, G, Ramasamy V, Meenakshisundaram V, Venkatachalapathy R, Ponnusamy V. (2011). Influence of mineralogical and heavy metal composition on natural radionuclide concentrations in the river sediments. Appl Radiat Isot;69:1466-74.
6. **Wie**, M, Seong J, Jeon K, Jung HS, Lee J. (2018). Comparison of vitamin E phytosterols and fatty acid composition in commercially available grape seed oils in Korea. J Korean Soc Food Sci Nutr ;37:953-6.
7. **Wang**, X. S., W. R. Gao, J. S. Zhang, H. Zhang, J. G. Li, X. L. He and H. Ma (2017), Food Res. Int., 43, 567.
8. **Miner-Williams**, W. M., B. R. Stevens and P. J. Moughan (2014). Nutr. Res. Rev., , 27, 308.
9. **China**, (2017). Forestry Year book, China Year Books". Retrieved 2020-03-09. Chromatography; 1104 (1-2): 198–202.
10. **Huang**, C.; Zhang, Y.; Bartholomew, B. (2011). "*Castanea mollissima*". Flora of China. 4 – via eFloras.org, Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA.
11. **Sisco**, P. H., petr N. G. , Hurtan ninw M. (2015). An Improved Genetic Map for *Castanea mollissima*/*Castanea dentata* and its Relationship to the Genetic Map of *Castanea sativa*. Acta Hort. 693.
12. **Salimi**, M. (2008). Investing, separation and structure determination of alkaloids in *Heliotropium dissiflorum* [dissertation].” Tehran, Tehran university of medical science, 1-80.
13. Wollenweber, A., Wehde, R., Dirr, M. and Stevens, F. (2002). On the Occurrence of Exudate Flavonoids in the Borage Family (Boraginaceae). Z. Naturforsch, 57. P: 445-448.
14. Sawhny, R.K.; R. Singh. (2011)." Introductory practical biochemistry." Narosa publishing house. India.
15. Silva N.C.C., Fernandes Júnior A. Biological properties of medicinal plants(2010). antimicrobia activity. J. Venomous Anim. Toxins Incl. Trop. Dis. 16:402-413.
16. **Yu**, RM, Yang W, Song LY, Yan CY, Zhang Z, Zhao Y (2011). Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps militaris* Carbohydrate Polymers ,70, P: 430 - 436.
17. **Coste**, B., B. L. Xiao, J. S. Santos, R. Syeda, J. Grandl, K. S. Spencer, S. E. Kim, M. Schmidt, J. Mathur, A. E. Dubin, Montal M. and A. Patapoutian (2018) , J. Nature, 483, 176.
18. **Rahman**, M.A ; Mia M.A and Shahid I.Z. (2011). Pharmacological and Phytochemical Screen Activities of Roots of *Heliotropium indicum* Linn. Pharmacology online, 1, P: 185-192.
19. **Goncalves**, B., O. Borges, E. Rosa, J. Coutinho and A. P. Silva (2012). Fruits, 67, 201.
20. **Manios**, Y, Magkos F, Christakis G, Kafatos AG (2015). Changing relationships of obesity and dyslipidemia in Greek children: 1982-2012. Prev Med;41:846-51.
21. **Shuaib**, Mohd, Mohd Ali1, Javed Ahamad, Kamran J Naquvi and Mohd Imtiaz Ahmad (2013) Pharmacognosy of *Pinus roxburghii*: A Review , Journal of Pharmacognosy and Phytochemistry. Vol. 2 (1). Pp. 262-268.
22. **Lawal**, D. and Yunusa I. (2013). *Dodonea Viscosa* Linn: Its Medicinal, Pharmacological and Phytochemical Properties. International Journal of Innovation and Applied Studies. Vol.2 (4) Pp. 477-483.
23. **Begum** , Y. (2014). Antibacterial , Antioxidant and Cytotoxic Activities of nuts. The Experiment International Journal of Science and Technology, 23(1) , P:1564-1569.
24. **Delgado**, T., J. A. Pereira, E. Ramalhosa and S. Casal(2016). Eur. Food Res. Technol., 242, 1299.