IRSTI 31.21.15

[1]Zh.Zh. Akzhigitova, [1]A.M. Baiseitova, [2]Yang Ye, [1]J. Jenis

[1]Division of Applied Life Science (BK21 plus), Gyeongsang National University, Jinju, Korea
[2]Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai, China
*e-mail: janarjenis@mail.ru

Investigation of chemical constituents of *Artemisia absinthium*

Abstract: The aerial part of *Artemisia absinthium,* collected in Almaty region of Kazakhstan, investigated for chemical constituent with sufficiently completed quantitative and qualitative analysis. Biological active constituents such as organic acid (1.08%) and flavonoids (0.52%) together with the moisture content (7.14%), total ash (6.4%), and extractives (12.82%) of plant *A. absinthium* were determined. In the ash of the plant was found 8 macro-micro elements, main of them was K (417.930 μg/ml), Ca (116.0225 μg/ml), Mg (26.2250 μg/ml), Na (15.9825 μg/ml) by using the method of multi-element atomic emission spectral analysis at the Institute of Combustion Problems. Additionally, twenty amino acids and eight fatty acids were identified from *A. absinthium.* As the results showed that the major contents of amino acids were glutamate (2380 mg/100g), aspartate (1200 mg/100g) and alanine (712 mg/100g), and main contents of fatty acids were linoleic (44.8%) and oleic (30%) acids, relatively.
Key words: *Artemisia absinthium,* bioactive constituents, macro-micro elements, amino and fatty acids.

Introduction

The tribe Anthemideae of the family Compositae includes about sixty genera and over nine hundred species. The largest and most widely spread through the world of these is the genus *Artemisia*, also known as wormwood, comprising four hundred species [1]. *Artemisia* species is described in pharmacopoeia books in many countries around the world [2]. *A. absinthium* L. is used for the treatment of various inflammatory diseases, including chronic bronchitis, asthma, gastroenteritis and pruritus [3]. The aerial part of *A. absinthium* L. has shown to possess anti snake venom activity [4]. Antimalarial and anticancer activities are among the prominent biological effects reported for different species of the genus *Artemisia* [5]. For instance, a Chinese researcher, Tu Youyou, won her Nobel prize in Medicine for a drug, which she developed to treat malaria from herb plant *Artemisia annua*, or sweet wormwood [6].

Compounds of absinthin, artabsin and guainolides *are* main constituents of *A. absinthium* which is bitter tonic, aromatic, anthelmintic, stomachic, antiparasitic, antiseptic and choleretic, carminative medicinal plant and also possessing anti-inflammatory and mild antidepressant activities. The effectiveness of wormwood as an aromatic bitter and its antimicrobial properties come from the bitter compounds and its essential oil. The oil of the plant can be used as a cardiac stimulant to improve blood circulation. Pure wormwood oil is very poisonous, but with proper dosage poses little or no danger [7].

In the present study, the quantitative and qualitative analysis of phytochemical constituents of medicinal plant *A. absinthium* which grown in Almaty region of Kazakhstan have been made for the first time.

Materials and methods

Plant material

The aerial part of plant material *A. absinthium* was collected in Almaty region Kazakhstan in September, 2017. The botanical identification was made by Dr. Alibek Ydyrys, The Herbarium of Laboratory Plant Biomorphology, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan. The air dried aerial part of *A. absinthium* was cutted into small pieces and stored at room temperature.

Quantitative and qualitative analysis

The quantitative and qualitative analysis of biologically active constituents of the plant were made

© 2018 al-Farabi Kazakh National University

according to methods reported in the State Pharmacopeia XI edition techniques of RK.

Macro-micro elemental composition

In The Institute of Combustion Problems using the method of multi-element atomic emission spectral analysis in the ash of *A. absinthium* was analyzed elemental constituents. To determine the mineral composition of ashes was used Shimadzu 6200 series spectrometer.

Fatty acid composition

Determination of fatty acid composition of the plant *A. absinthium* extracted with a chloroform-methanol mixture (2:1) for 5 minutes, the extract is filtered through a paper filter and concentrated to dryness. Then, to taked extract add 10 ml of methanol and 2-3 drops of acetyl chloride and further methylation at 60-70° C in a special system for 30 minutes. The methanol is removed by rotary evaporation and the samples are extracted with 5 ml of hexane and analyzed using a gas chromatograph "CARLO-ERBA-420" allocated the Kazakh Academy of Nutrition for 1 hour. As a result, chromatograms of methyl esters of fatty acids were obtained. By comparison with reliable samples by the time of exit from the column, eight fatty acids were identified.

Amino acid composition

To determine the amino acids composition was made erenow [14] of the raw material used GS/MS device. GS/MS analysis: of *A. absinthium* were analyzed by Gas Chromatograph coupled to Mass Spectrometer using polar mixture of 0.31% carbowax 20 m, 0.28% silar 5 CP and 0.06% lexan in chromosorb WA-W-120-140 mesh, column (400x3 mm). The column temperature was programmed from 110°C (held for 20 min), at 6°C/min from 110°C to 180°C, at 32°C/min from 185°C to 290°C. When it reaches to 250°C, it should stay constant till fishing of exit of all amino acids. The chromatogram is counted according to an external standard.

Results and discussion

Quantitative and qualitative analysis

The quantitative and qualitative analysis of biologically active constituents itemizing moisture content, total ash, extractives contents were determined from aerial part of *A. absinthium.*The results shown in Table-1.

The amount of ash in plant raw materials varies within certain limits and depends on the specific nature of the raw material itself, the way it is collected and the drying conditions. Significant deviations usually indicate the contamination of raw materials with a mineral admixture or the untimely collection of raw materials and etc.

The complex of organic and inorganic substances extracted from plant raw materials by an appropriate solvent and quantitatively determined as a dry residue is conventionally called the extractive substances of medicinal plant matter. The content of extractive substances in medicinal plant raw materials is an important numerical indicator that determines its good quality for the content of biological metabolites. Typically, a solvent is used for a tincture or extract from this raw material that is more advantageous in preparation.

Organic acids play an important role in maintaining the acid-base balance of the human body. Organic substances increase the pH level of the medium, which improves the absorption of nutrients by internal organs and the excretion of slags.

Flavonoids – a group of useful plant substances, when ingested in human body with food, has an effect on the activity of enzymes. As medicines are very widespread in both traditional and traditional medicine. The therapeutic value of these unique biologically active substances is difficult to assess. They have an antioxidant effect, due to this, the body recovers. It has been scientifically proven that there is a direct correlation between cardiovascular diseases and daily consumption of flavonoids – the mortality rate is 5 times less.

Table 1 – Quantitative analysis of bioactive constituents of aerial part of *A. absinthium*

Content, %				
Moisture content	Ash	Extractives	Organic acids	Flavonoids
7.14	6.4	12.82	1.08	0.52

Macro-micro elemental composition

In the ash of *A. absinthium* were determined eight macro- and microelements, showed in Table 2 and Figure 1.

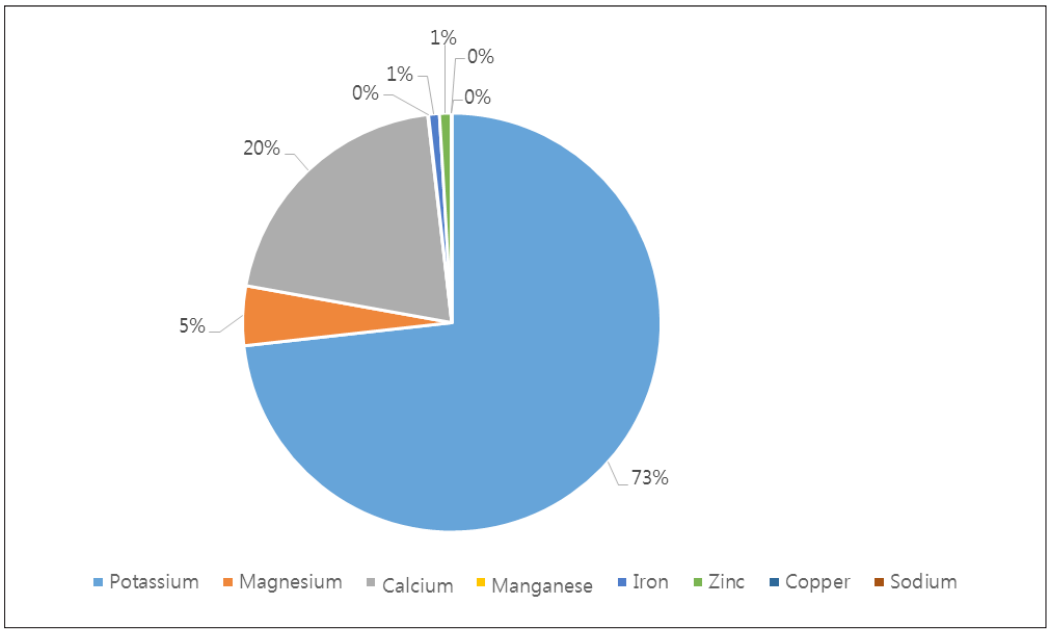


Figure 1 – Percentage contents of macro- and micro elements in ash of *A. absinthium*

Table 2 – Composition of macro-micro elements in the ash of plant *A. absinthium*

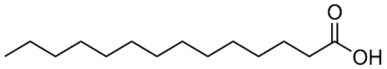
Element	K	Mg	Ca	Mn	Fe	Zn	Cu	Na
µg/ml	417.930	26.2250	116.0225	0.3317	4.7662	5.2564	0.1471	15.825

Trace elements are necessary for living organisms to ensure normal life activity. With a lack of potassium, there are disruptions in the work of the heart and skeletal musculature. Prolonged potassium deficiency can cause acute neuralgia. Prolonged deficiency of calcium and vitamin D in the diet leads to an increased risk of osteoporosis, and in infancy causes rickets. In living organisms, iron is an important microelement that catalyzes the processes of oxygen exchange (respiration). The main intracellular depot of iron is the globular protein complex – ferritin. Lack of iron is manifested as a disease of the body: chlorosis in plants and anemia in animals. Zinc is essential for the production of sperm and male hormones, is essential for the metabolism of vitamin E, is important for the normal functioning of the prostate, is involved in the synthesis of various anabolic hormones in the body, including insulin, testosterone and growth hormone, is necessary for the breakdown of alcohol in the body, alcohol dehydrogenase. Magnesium is necessary in maintaining the normal function of the nervous system and heart muscle, has a vasodilating effect, stimulates bile secretion, and increases the motor activity of the intestines, which helps to eliminate cholesterol from the body.

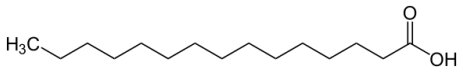
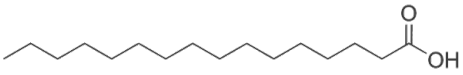
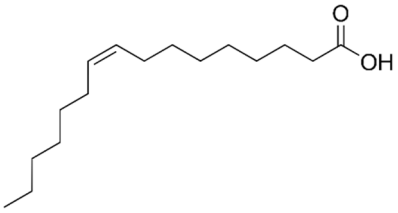
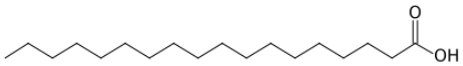
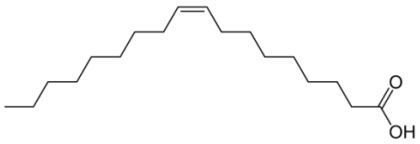
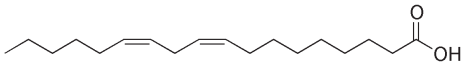
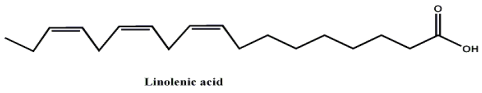
Fatty acid composition

The major fatty acids which is contained in *A. absinthium* were linoleic acid (44.8%) and Oleic acid (30%).

Table 3 – Fatty acids from *A. absinthium*

№	Fatty acids	Molecular formula	Structure	MW	Amount in plant, %
1	Meristic acid $C_{14:0}$	$C_{14}H_{28}O_2$		228	2.3

Continuation of table 3

№	Fatty acids	Molecular formula	Structure	MW	Amount in plant, %
2	Pentadecanoic acid $C_{15:0}$	$C_{15}H_{30}O_2$		242	2.1
3	Palmitic acid $C_{16:0}$	$C_{16}H_{32}O_2$		256	14.3
4	Palmitoleic acid $C_{16:1}$	$C_{16}H_{30}O_2$		254	1.2
5	Stearin acid $C_{18:0}$	$C_{18}H_{36}O_2$		284	4.6
6	Oleic acid $C_{18:1}$	$C_{18}H_{34}O_2$		282	30
7	Linoleic acid $C_{18:2}$	$C_{18}H_{32}O_2$		280	44.8
8	Linolenic acid $C_{18:}3$	$C_{18}H_{30}O_2$	 Linolenic acid	278	0.7

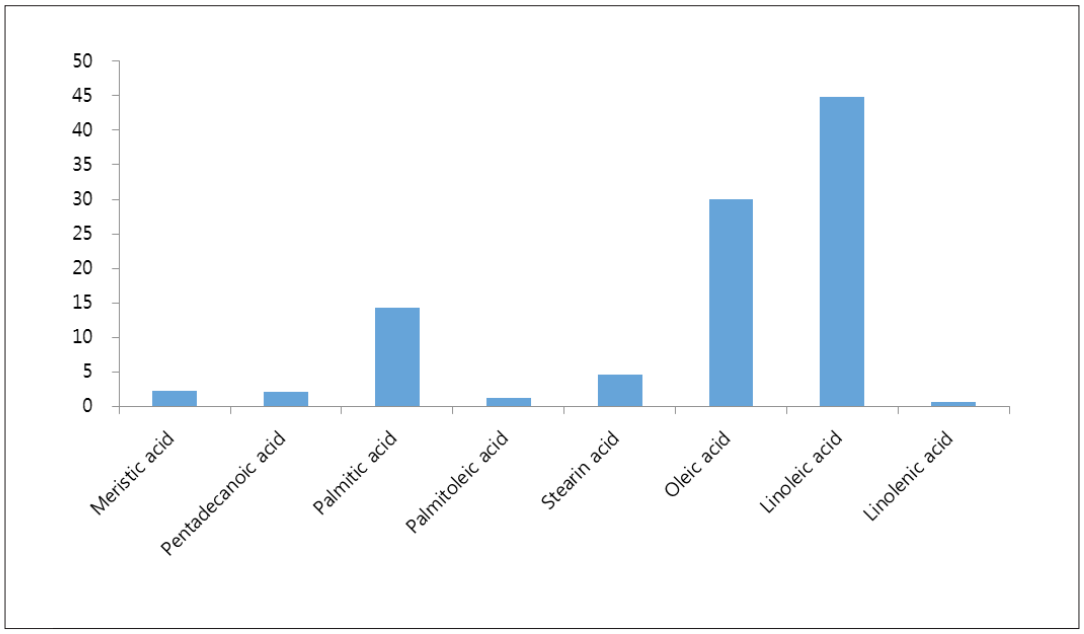


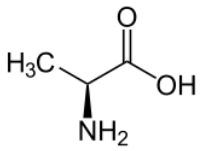
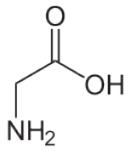
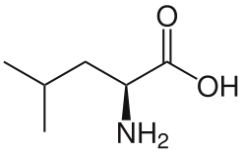
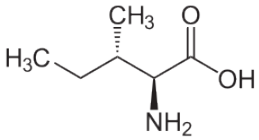
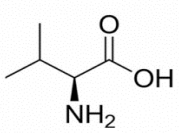
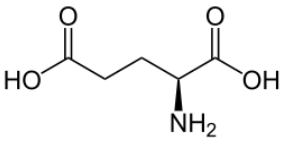
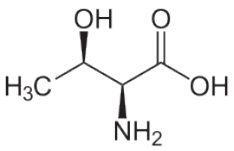
Figure 2 – Fatty acid contents of *A. absinthium*

Linoleic acid has received much attention in recent years because of its interesting biological benefits. The main health effects described for linoleic acid include reduction of carcinogenesis, atherosclerosis, inflammation, obesity, diabetes, as well as growth promoting and bone formation-promoting properties [9]. Oleic acid can inhibit the progression of diseases affecting the brain and adrenal glands, as well as improve memory and reduce blood pressure, but there is evidence that the substance can provoke cancer, in particular breast cancer [10].

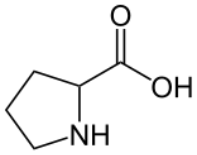
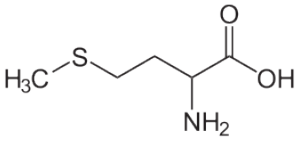
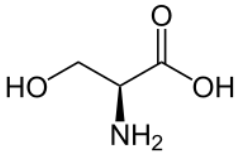
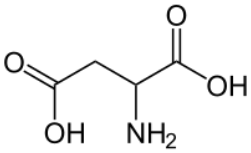
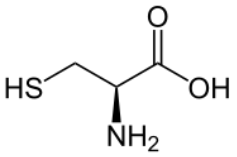
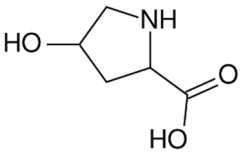
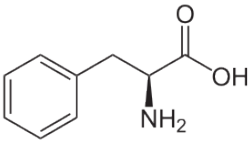
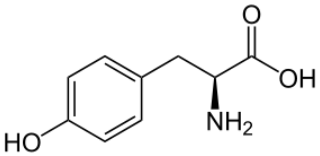
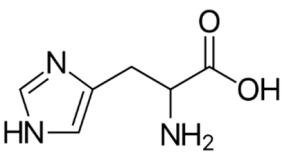
Amino acid composition

In the composition of amino acids mainly were glutamate (2380 mg/100g), aspartate (12 mg/100g) and alanine (712 mg/100g).

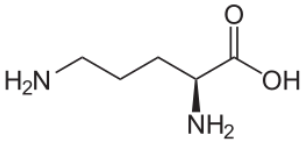
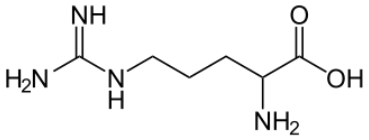
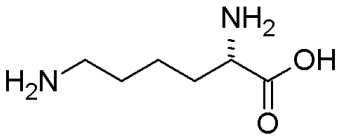
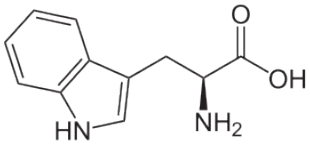
Table 4 – Amino acid contents of from *A. absinthium*

№	Amino acids	Molecular formula	Structure	MW	Amount in plant, mg/100g
1	Alanine	$C_3H_7NO_2$		89	712
2	Glycine	$C_2H_5NO_2$		75	340
3	Leucine	$C_6H_{13}NO_2$		131	326
4	Isoleucine	$C_6H_{13}NO_2$		131	315
5	Valine	$C_5H_{11}NO_2$		117	286
6	Glutamate	$C_5H_9NO_4$		147	2380
7	Threonine	$C_4H_9NO_3$		119	280

Continuation of table 3

№	Amino acids	Molecular formula	Structure	MW	Amount in plant, mg/100g
8	Proline	$C_5H_9NO_2$		115	596
9	Methionine	$C_5H_{11}NO_2S$		149	60
10	Serine	$C_3H_7NO_3$		105	406
11	Aspartate	$C_4H_7NO_4$		133	1200
12	Cysteine	$C_3H_7NO_2S$		121	1
13	Oxyproline	$C_5H_9NO_3$		131	1
14	Phenylalanine	$C_9H_{11}NO_2$		165	328
15	Tyrosine	$C_9H_{11}NO_3$		181	364
16	Histidine	$C_6H_9N_3O_2$		155	188

Continuation of table 3

№	Amino acids	Molecular formula	Structure	MW	Amount in plant, mg/100g
17	Ornithine	$C_5H_{12}N_2O_2$		132	1
18	Arginine	$C_6H_{14}N_4O_2$		174	505
19	Lysine	$C_6H_{14}N_2O_2$		146	140
20	Tryptophan	$C_{11}H_{12}N_2O_2$		204	80

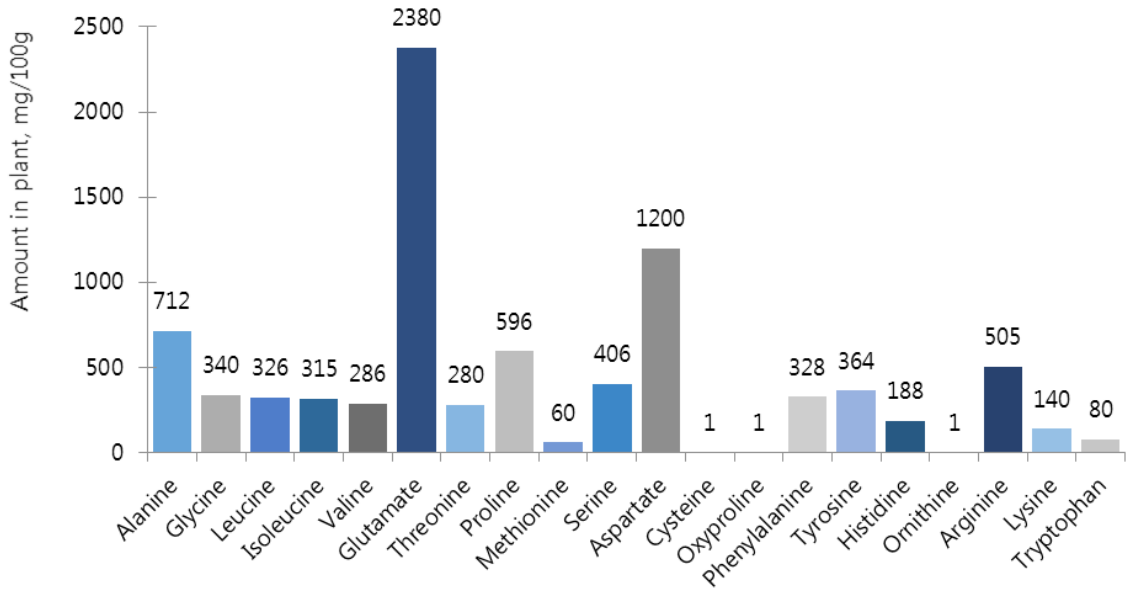


Figure 3 – Amino acid contents of plant *A. absinthium*

Glutamate is a nonessential amino acid, a major bioenergetic substrate for proliferating normal and neoplastic cells, and an excitatory neurotransmitter that is actively involved in biosynthetic, bioenergetic, metabolic, and oncogenic signaling pathways [11]. Aspartic acid increases immunity, metabolism, deactivates ammonia, participates in the formation of ribonucleic acids, promotes the removal of chemicals, including drugs, restores working capacity. Studies conducted by scientists have proved the effectiveness of taking asparaginic acid preparations for increasing testosterone levels. Aspartic acid is taken as an additive by bodybuilding athletes to improve strength, increase libido and testosterone in the blood [12]. Alanine plays a significant role in metabolic processes, as well as to regulate the level of sugar in the bloodstream. This amino acid protects against the development of cancer of the pancreas and prostate gland, it is an important part of sports nutrition, increases physical endurance and allows to build muscle mass [13].

Conclusion

In conclusion, quantitative and qualitative analysis of total bioactive constituents and the moisture, total ash, and extractives contents of *A. absinthium* were determined. Besides, macro-micro elements in the ash of the medicinal plant were investigated, and total eight macro-micro elements were identified by the method of multi-element atomic emission spectral analysis. Meanwhile, twenty amino and eight fatty acids were determined from *A. absinthium*. The results showed that the major contents of amino acids were glutamate (2380 mg/100g), aspartate (1200 mg/100g) and alanine (712 mg/100g), and main contents of fatty acids were linoleic (44.8%) and oleic (30%) acids, relatively.

A. absinthium has lots of medicinal properties and main of them are killing cancer cells, getting rid of parasites and treating Crohn's disease and SIBO (Small intestinal bacterial overgrowth). In addition, it has antimicrobial and antifungal abilities. Absinthin, an extract isolated from wormwood, is the most powerful antimalarial available and by this activity was invented as a drug.

Acknowledgements

The work was supported by grants from Ministry of Education and Science of the Republic of Kazakhstan (0118PK00458).

References

1 Geissman T. A., lrwin M. A. (2000) Chemical contributions to taxonomy and phylogeny in the genus Artemisia, pp.167-180.

2 Omera B., Krebsb S., Omerc H., Noord T.O. (2007) Steroid-sparing effect of wormwood (Artemisia absinthium) in Crohn's disease:A double-blind placebo-controlled study. Phytomedicine, pp.87-95.

3 Ke W. Z., Li X. L., Xiao M. S., Hai N. L., Fang J. S., Qian Y., Xin D., Yong J., Peng F. (2015) Caruifolin D from artemisia absinthium L. inhibits neuroinflammation via reactive oxygen species-dependent c-jun N-terminal kinase and protein kinase c/NF-κB signaling pathways. European Journal of Pharmacology, pp.82-93.

4 Nalbantsoy A., Erel S.B., Köksal C., Göcmen B., Yıldız M.Z., Karabay Y.N. (2013) Viper Venom induced inflammation with Montivipera xanthina (Gray, 1849) and theanti-snake venom activities of Artemisia absinthium L. in Rat. Toxicon, vol. 65, pp. 34–40

5 Mohammad A., Bilal H. A., Ihsan-ul-haq. (2013) Department. Production of commercially important secondary metabolitesand antioxidant activity in cell suspension cultures of Artemisia absinthium L. Industrial Crops and Products, pp. 400-406

6 "The Nobel Prize in Physiology or Medicine 2015" (PDF). Nobel Foundation. Retrieved 7 October 2015.

7 Mahboubeh T. (2014) Mutagenic and antimutagenic activities of Artemisia absinthium volatile oil by the bacterial reverse mutation assay in Salmonella typhimurium strains TA98 and TA100. Asian Pacific Journal of Tropical Disease, pp. S840-S844

8 Szymczycha-Madeja A, Welna M, Pohl P. (2012) Elemental analysis of teas and their infusions by spectrometric methods. TrAC Trends in Analytical Chemistry, vol. 5, pp. 165-181.

9 Yang B, Chen H, Stanton C, Ross RP, Zhang H, Chen YQ, Chen W. (2015) Review of the roles of conjugated linoleic acid in health and disease. Journal of Functional Foods, vol. 15, p. 314-325.

10 Bowen K.J., Kris-Ethertona P.M., Shearera G.S., Westa Sh.G., Reddivaric L., Jones P.J. (2017) Oleic acid-derived oleoylethanolamide: A nutritional science perspective. Progress in Lipid Research, vol. 67, pp. 1-15.

11 Stacey S. W., Shahriar K. G. (2013) Glutamate Receptors, and Downstream Signaling Path-

ways. International Journal of Biological Sciences, pp .948-959

12 Katane M, Kanazawa R, Kobayashi R, Oishi M, Nakayama K, Saitoh Y, Miyamoto T, Sekine M, Homma H. (2017) Structure–function relationships in human D-aspartate oxidase: characterisation of variants corresponding to known single nucleotide polymorphisms . BBA - Proteins and Proteomics, vol. 1865, pp. 1129-1140.

13 Liu L, Chen Y, Yang L. (2014) Inhibition study of alanine aminotransferase enzyme using sequential online capillary electrophoresis analysis. Analytical Biochemistry, vol. 467, pp. 28-30.