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Original Article

Biological Activities of Aloin-rich Extracts Obtained from *Aloe vera* (L.) Burm.f.

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ABSTRACT

Aloe vera (L.) Burm.f. is an important medicinal plant which belonging to the Liliaceae family. This plant has widely been applied in the pharmaceutical, food, and cosmetic industries. In this study, Aloin was extracted from *A. vera* leaves using ultrasonic and stirring methods. The extract was purified, and their Aloin A and B percentages were determined using HPLC. According to the results, in all samples, the amount of Aloin B was significantly higher than Aloin A. The ethyl acetate extract of dried latex obtained by ultrasonic method had the highest yield (24.50 %) and amount of total Aloin (84.22%) compared to other samples and methods. Also, the relative percentages of Aloin B and A were 86.48 and 13.52%, respectively. Finally, the antioxidant and cytotoxic properties of Aloin-containing extracts were investigated by DPPH and MTT methods, respectively. According to the results, the ethyl acetate extract of dried latex obtained by ultrasonic method exhibited the highest antioxidant and cytotoxic activities. There was a significant correlation between extracts containing more Aloin and higher biological activity, and extracts with more Aloin had higher biological properties. Therefore, the extract of the dried latex obtained by ultrasonic method was the best sample in terms of the amount of Aloin and biological properties. In conclusion, using the ultrasonic method together with a dried sample provides the most Aloin and biological properties.

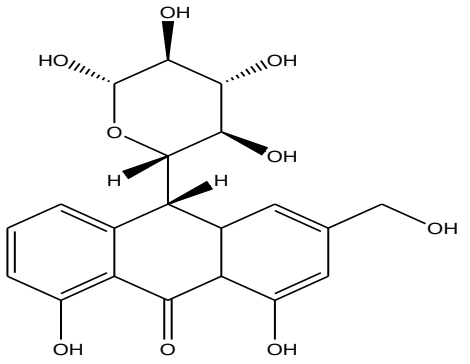
INTRODUCTION

The *Aloe* genus comprises more than 548 species of semitropical perennial flowering plants. The most common of these is *Aloe vera* L. This plant has been used in various industries such as pharmaceuticals, food, cosmetics, and its latex is usually recognized for its laxative properties [1]. Also, this plant was cultivated for the treatment of dermatitis, burns, and fungal infections [2,3]. The major active components existing in the exudates of *Aloe* leaves include the small molecular phenolic compounds, of which Aloin is the main component. This compound is a yellow-colored anthraquinone-C glycoside that consists of 0.1% to 6.6% of the leaves dry weight and represents 3% up to 35% of the total latex [4,5]. Aloin facilitates alcohol metabolism [6], and has antiinflammatory activities [7]. Also, it has also been reported to has bitter tonic, anti-oxidant [8], antimicrobial [9], antitumor properties [10,11], and is used in the treatment of ulcers cancer, and diabetes [12,13]. Furthermore, it is reported that Aloin is effective in elevation of the immune response, therefore, it could be used as potent immunostimulant in aquaculture [14].

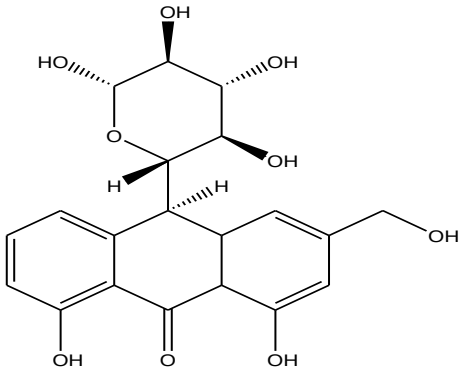
Aloin occurs naturally as a mixture of diastereoisomers, Aloin A (barbaloin) and Aloin B (isobarbaloin) (Fig. 1). Researches of Aloin's biosynthesis showed that Aloin B is preferentially made. Non-enzymatic conversion to Aloin A is understood to product in a mixture of Aloins A and B, as observed for naturally derived Aloin [15,16].

Pure natural products play a main role in human therapy, as many medicines are either natural products or derivatives thereof. Indeed, it is estimated that about 40% of all medicines are either natural products or their semisynthetic derivatives

[17]. Isolation and purification of these compounds play an important role in natural product research. Up to now, extraction of Aloin from dry leaves exudate of *A. vera* was done by different methods such as maceration, soxhlet, ultrasonic, and microwave, and they analyzed by some methods such as HPLC and UPLC, and their results showed that the percentage of Aloin in obtained extracts were low [18-20]. In view of the wide biologically activities, the preparation of Aloin with high purity has been of much interest to pharmaceutical chemists. Also, the preparation of Aloin as reference standard for quality control purposes is in urgent need in research as well as industrial communities. Therefore, in this work, the dried and liquid latex were selected and attempted to obtain Aloin with high purity and biological activity using ultrasonic and stirring extraction methods.



(A)



(B)

Fig. 1 Chemical structures of (A) Aloin, and (B) Aloin

MATERIALS AND METHODS

Chemicals

Solvents and chemicals used for the extraction and biological tests were purchased from Merck, German. Aloin standard was developed from Sigma Aldrich, which was 70% pure. Also, the solvents used in the HPLC were grade HPLC.

Aloe vera (L.) Burm.f. Latex Collection

Three years *A. vera* leaves were collected from the greenhouse located in Gugan-Tabriz, and identified by the Department of Medicinal Plants and Botany of Azerbaijan Shahid Madani University (Herbarium number: ASMU1402). Then, on September 11, 2019, the *A. vera* leaves were cut with a knife from the lower part, and the yellow latex was taken and stored in sterilized falcon that had been submerged in liquid N_2, and immediately transferred to a -80 °C freezer. Then, half of the latex in falcons were transferred to the freeze drier for 24 hours to dry completely.

Extraction and Purification of Aloin by Stirring

For the extraction and purification of Aloin, 2 liters of n-hexane were added to 200 g of latex and stirred for 2 hours to remove non-polar compounds. The west was then placed in a magnetic stirrer with 2 liters of ethyl acetate solvent and was stirred at high speed for 2 hours. The extract was filtered and concentrated in a rotary evaporator. Furthermore, isobutanol was added to extract with the ratio of one-to-twelve v/v, and stirred for 30 minutes. The solution was cooled at 5 °C for 4 hours until the Aloin crystallization. The Aloin crystals were separated, dissolved in methanol HPLC grade, and injected into HPLC for analysis.

Extraction and Purification of Aloin by Ultrasonic Assisted

Ultrasonic assisted extraction was used for the extraction of Aloin from *A. vera* latex. n-Hexane (200 mL) was added to latex (20g) in a cylindrical flat bottom glass vessel. The vessel was kept in an ultrasound bath (DSA100-XN2-4.OL, Max machine Co.), and then sonicated for 30 min with 100 kHz frequency, at 30 °C, and 100 W power . The extract was filtered and concentrated in a rotary evaporator. Moreover, isobutanol was added to extract, and stirred for 30 minutes. The solution was cooled at 5 °C for 4 hours until the Aloin crystallization. The Aloin crystals were separated, dissolved in methanol HPLC grade, and injected into HPLC for analysis.

Analysis of Aloins by HPLC

The analysis of Aloins (i.e., Aloin A and B) was done using an HPLC (Knauer, Berlin, Germany) system equipped with a 100 µl loop, a diode-array detector, and a C18 column (250 mm × 0.46 mm, 5 µm). The separation was performed with gradient elution solvent A and B, namely acetonitrile and water, respectively. Gradient conditions were: 15% A, in 0 min; 20% A, in 14 min; 20% A, in 18 min; 30% A, in 20 min; 40% A, in 40 min. The flow rate was adjusted at 0.5 mL/min and the wavelength was set at 365 nm.

Evaluation of Antioxidant Activity

The antioxidant activities of the obtained extracts were evaluated using the DPPH assay [21]. 1 mg of the extracts were dissolved in 1 ml of methanol, and then 150 µL of each of the produced solutions were mixed with 150 µL of DPPH solution (0.02 mM), and the mixture was left in the dark for 30 minutes. Then, the adsorption of the solutions and the control solution were measured using a microplate reader spectrophotometer (Epoch, Biotek instrument, Inc, USA) at a wavelength of 517 nm. Finally, the percentage of radical inhibition was calculated, and the IC_{50} value was evaluated.

Evaluation of Cytotoxic Activity

The cytotoxic activities of Aloin extracts against ovarian cancer cells (OVCAR-3) was determined using the 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay [22]. OVCAR-3 cancer cells were cultured in RPMI-1640 media with 10% FBS and antibiotics (100 U/ml penicillin G and 100 g/ml streptomycin) in a humid incubator at 37°C. After culture, the cells were incubated for 12 hours in 96-well microplate wells with a density of 2×10^6 cells. The different concentrations of extracts were incubated with the cells for 48 and 72 hours at 37 ° C. Then, 20 µL of MTT solution was added and incubated for 4 hours. The produced crystals were dissolved in DMSO. Finally, the absorbance of the samples was read using a microplate reader spectrophotometer (Epoch, Biotek instrument, Inc, USA) at 490 nm and the cell viability percentage was calculated, and finally the IC_{50} value was evaluated.

Statistical Analysis

Data were analyzed by the SAS 9.2 using a one-way analysis of variance (ANOVA) of a completely randomized design, and the mean comparisons were determined by Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

Aloin Purification

In order to obtain an Aloin extract with high purity, two samples (dried and liquid latex), as well as two extraction methods (ultrasonic and stirring) were employed. According to previous researches, the highest Aloin was extracted using methanol and ethyl acetate solvents [19]. Since methanol destroys the structure of Aloin and converts it to other substances such as Aloe-emodin anthrone [23,24], hence only ethyl acetate was selected and after extraction, purification, and crystallization, the amount of Aloin and the relative percentages of Aloins (A and B) were measured by HPLC.

The HPLC chromatogram of one of the samples are shown in Figure 2. Based on the results (Fig. 2), the amount of Aloin B in all samples was higher than Aloin A. The ethyl acetate extract of dried latex obtained by ultrasonic method had the highest yield (24.50%) and the amount of Aloin (84.22 %) compared to other samples and extraction methods (Fig. 3). In addition, the dried latex extract of stirring method had the second level, and the amount of Aloin was 71.56 % (Fig. 3). The results obtained from the analysis of liquid latex ethyl acetate extract showed that the ultrasonic extraction contained 41.96 %, and the extract obtained by the stirring method also had the lowest amount of Aloin (37.12 %) (Fig. 3).

The study on the relative percentages of Aloins A and B in the extracts indicated that the relative percentage of Aloin B in the ethyl acetate extract of dried latex obtained by ultrasonic method was 86.48%, whereas Aloin A accounted for 13.94% (Fig. 4). Also, in the extract of dried latex by stirring method, the content of Aloin B and A were 74.50 and 25.50%, respectively (Fig. 4). Moreover, the analysis of ethyl acetate extract of liquid latex obtained by ultrasonic method showed that the relative percentages of Aloins B and A were 65.32 and 34.68%, respectively (Fig. 4).

According to the results, it can be concluded that the type of sample has a significant effect on Aloin purification, and dry latex had a better performance than liquid latex in both extraction methods. According to previous study, the drying the latex inhibits the activity of its enzyme and thus the

amount of Aloin remains constant in compared to the liquid sample [25], which the results of this study confirmed our results.

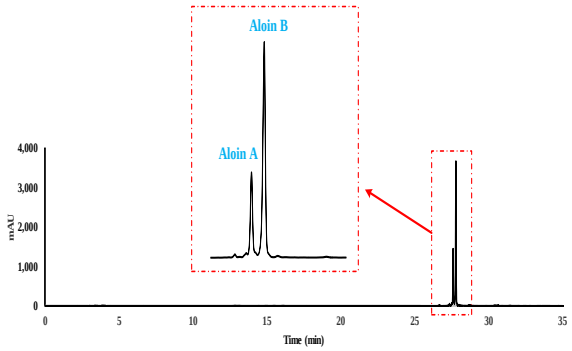


Fig. 2 HPLC chromatogram of the dried latex extract obtained by stirring method. Separation conditions involved Waters Spherisorb column (5 µm ODS 4.6 × 250 mm) and mobile phase acetonitrile (solvent A) and water (solvent B). The flow rate and wavelength were adjusted at 1.0 mL/min and 270 nm, respectively.

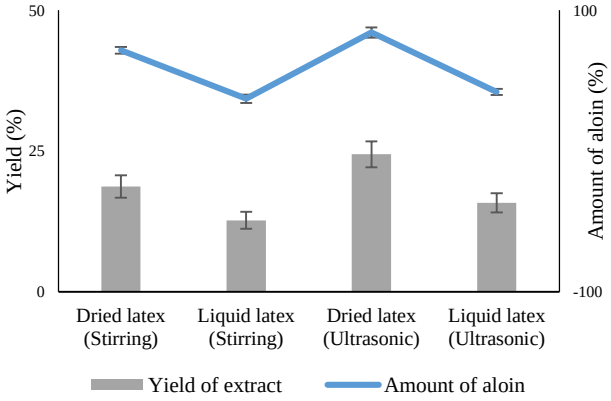


Fig. 3 Yield and amount of Aloin extracted from dried and liquid latex by stirring and ultrasonic methods.

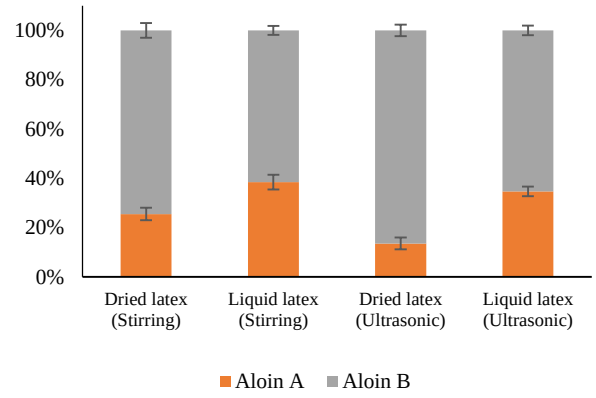


Fig. 4 The percentage of Aloin A and B extracted from dried and liquid latex by stirring and ultrasonic methods.

Regarding the comparison two extraction methods performance, it concluded that the amount of Aloin in the extract obtained from the ultrasonic method was higher than the stirring method, and this can be related to the extraction mechanism that the ultrasonic method improves the extraction process due to cavitation mechanism [26]. As a result, dried latex and ultrasonic method should be employed to extract the maximum amount of Aloin.

Antioxidant Activity

The extracts obtained from *A. vera* latexs have significant amounts of Aloin, so the antioxidant properties of the extracts were evaluated by DPPH assay (Fig. 5). According to the results (Fig. 5), the antioxidant activity of the ethyl acetate extract of dried latex obtained by ultrasonic method had the highest antioxidant activity with IC_{50} value of 35.45 µg/mL. The quantity of Aloin in this extract discovered that it contained more Aloin than other extracts. It also observed that extracts with lower amounts of Aloin had less antioxidant activities. Aloin is a secondary metabolite that belongs to the polyphenol, and since most polyphenol compounds have significant antioxidant activities, therefore, these results indicate that there is a direct relationship between antioxidant activity and the amount of Aloin, and it could explain the Aloin extracts have such significant antioxidant capabilities. In a study by Asamenew *et al.* [27] antioxidant and antimicrobial properties of Aloin and chromone isolated from *A. Harlana* latex were evaluated and was found that these substances had significant antioxidant properties due to their phenolic nature.

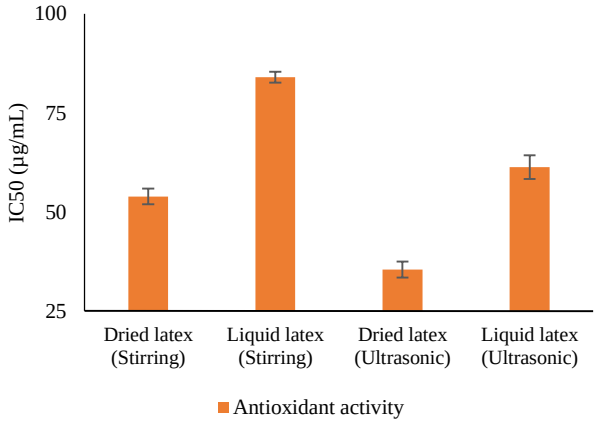


Fig. 5 Antioxidant activities of Aloin extracted from dried and liquid latex by stirring and ultrasonic methods.

The high antioxidant properties of ethyl acetate extract obtained by ultrasonography may be attributed to the high amount of Aloin in the samples.

In another research by Lee *et al*. [28] on the antioxidant properties and phenolic content of *A. vera*, the highest phenolic content was related to the ethyl acetate fraction, which was also the best in terms of antioxidant activity.

Cytotoxic Activity ResultS

The cytotoxic activities of the obtained extracts against cancer cells were evaluated by the MTT assay. According to the obtained data (Fig. 6), the amount of IC_{50} at ethyl acetate extract of dried latex obtained by the ultrasonic method at 24 and 48 hours were 21.35 and 16.15 µg/mL, respectively. The IC_{50} values of the extract of dried latex obtained by stirring method were 35.89 and 29.67 µg/mL at 24 and 48 hours, respectively. Moreover, the highest value of IC_{50} (47.00 µg/mL) was related to the extract of liquid latex by stirring method at 24 hour.

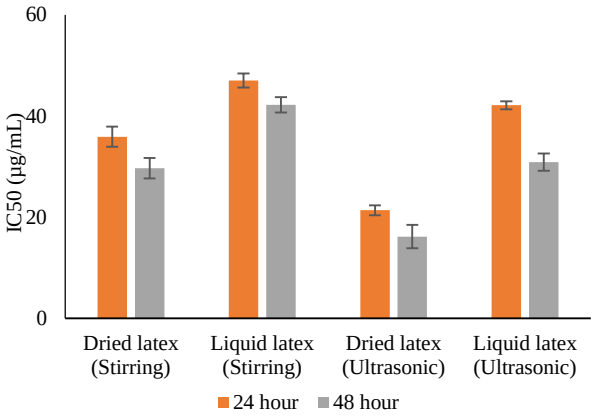


Fig. 6 Cytotoxic activities of Aloin extracted from dried and liquid latex by stirring and ultrasonic method

Despite the fact that Aloin is a phenolic compound, many studies have been conducted on its anti-cancer properties of A. vera. Aloin has shown chemical protective effects against 1,2-dimethylhydrazine-induced perineoplastic lesions in the large intestine of Wistar rats [29,30]. It also has been shown to suppress the secretion of vascular endothelial growth factor in cancer cells, which stops endothelial cells from proliferating and spreading [31]. This compound inhibits cancer cells by disrupting cell cycles through the mitochondrial pathway, resulting in loss of cell membrane integrity and eventually apoptosis [11]. A study has shown the protective effect of Aloin on the synthesis of iNOS nitric oxide and the nuclear factor synthesizing kappa B of HaCat cells induced by ultraviolet B radiation. Aloin inhibits the activity of kappa B nuclear factor P65 by down-regulating UVB-induced iNOS mRNA expression [32]. In another experiment, Aloin was tested on HeLaS3 human uterine cancer cells and exhibited an antiproliferative impact, stopping the cell cycle in phase S and significantly increasing HeLaS3 cell death. The radiosensitivity of HeLaS3 cells was also tested using Aloin, indicating that Aloin had a cytotoxic impact [33]. According to a study by Im *et al*. colon cancer associated with colitis in animal models reduced by oral administration of processed A. vera gel [34].

Comparing the cytotoxicity results with the amount of Aloin in the samples revealed a significant relationship between them, as extracts with more Aloin showed higher cytotoxic properties. Therefore, the extracts of dried latex obtained by the ultrasonic method had the best results for Aloin levels and cytotoxic activity. It is concluded that with the increase of Aloin content, the inhibitory power of cancer cells improves.

CONCLUSION

Today, it is well understood that a pure compound or extract containing more bioactive compounds are more efficient in the treatment of many diseases, therefore purifying bioactive compounds from medicinal plants is extremely crucial. In this study, the Aloin was purified from A. vera latex, and then their antioxidant and cytotoxic activities were investigated. The results revealed that the samples which were dried in a freeze dryer and extracted by ultrasonic method contained the highest amount of Aloin. Also, there was a direct correlation between the Aloin content, antioxidant capabilities, and the cytotoxicity of extracts.

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REFRENCES

1. Steenkamp V., Stewart M.J. Medicinal applications and toxicological activities of Aloe. Prod Pharm Boil. 2007;45(5):411-420.
2. Sahu PK, Giri DD, Singh R, Pandey P, Gupta S, Shrivastava AK, Pandey KD. Therapeutic and medicinal uses of *Aloe vera*: a review. Pharm Pharm. 2013;4(08):599.

3. Sánchez M., González-Burgos E., Iglesias I., Gómez-Serranillos M.P. Pharmacological update properties of *Aloe vera* and its major active constituents. Molecules. 2020;25(6):1324.
4. Reynolds T. The compounds in Aloe leaf exudates: a review. Botan J Linnean Soc. 1985;90(3):157-177.
5. Javed S. *Aloe vera* gel in food, health products, and cosmetics industry. In Studies in Natural Products Chemistry; Elsevier; 2014;41:261-285.
6. Chung J.H., Cheong J.C., Lee J.Y., Roh H.K., Cha Y.N. Acceleration of the alcohol oxidation rate in rats with Aloin, a quinone derivative of Aloe. Biochem Pharmacol. 1996;52 (9):1461–1468.
7. Park M., Kwon H., Sung M. Intestinal absorption of Aloin, aloe-emodin, and aloesin; a comparative study using two in vitro absorption models. Nutr Res Pract. 2009;3(1):9-14.
8. Tian B., Hua Y. Concentration-dependence of prooxidant and antioxidant effects of Aloin and aloe-emodin on DNA. Food Chem. 2005;91(3):413-418.
9. Gokulan K., Kolluru P., Cerniglia C.E., Khare S. Dose-dependent effects of Aloin on the intestinal bacterial community structure, short chain fatty acids metabolism and intestinal epithelial cell permeability. Frontiers Microb. 2019;10:474.
10. Esmat A.Y., Tomasetto C., Rio M-C. Cytotoxicity of a natural anthraquinone (Aloin) against human breast cancer cell lines with and without ErbB-2-topoisomerase IIαcoamplification. Cancer Biol Ther. 2006;5:97–103.
11. Buenz E.J. Aloin induces apoptosis in Jurkat cells. Toxicol In Vitro. 2008;22(2):422-429.
12. Pérez Y.Y., Jiménez-Ferrer E, Zamilpa A, Hernández-Valencia M, Alarcón-Aguilar FJ, Tortoriello J, et al. Effect of a polyphenol-rich extract from *Aloe vera* gel on experimentally induced insulin resistance in mice. Am J Chin Med. 2007;35(6):1037-1046.
13. Sun R., Zhai R., Ma C., Miao W. Combination of Aloin and metformin enhances the antitumor effect by inhibiting the growth and invasion and inducing apoptosis and autophagy in hepatocellular carcinoma through PI3K/AKT/mTOR pathway. Can Med. 2020;9(3):1141-1151.
14. Srivastava A., Nigam A.K., Mittal S., Mittal A.K. Role of Aloin in the modulation of certain immune parameters in skin mucus of an Indian major carp, Labeo rohita. Fish Shel Immunol. 2018;73:252-261.
15. Grün M., Franz G. Untersuchungen zur Biosynthese der Aloine in Aloe arborescens Mill. Archiv der Pharmazie. 1982;315(3):231-241.
16. Zonta F., Bogoni P., Masotti P., Micali G. High-performance liquid chromatographic profiles of Aloe constituents and determination of Aloin in beverages, with reference to the EEC regulation for flavouring substances. J Chrom A. 1995;718(1):99-106.
17. Jacob E.J. Natural Products-Based Drug Discovery: Some Bottlenecks and Considerations. Current Science, 2009;96(6):753-754.
18. Wang G., Su P., Zhang F., Hou X., Yang Y., Guo Z. Comparison of microwave-assisted extraction of aloe-emodin in Aloe with Soxhlet extraction and ultrasound-assisted extraction. Sci China Chem. 2011;54(1):231-236.
19. Jawade N.R., Chavan A.R. Ultrasonic-assisted extraction of Aloin from *Aloe vera* gel. Procedia Eng. 2013;51:487-493.
20. Logaranjan K., Devasena T., Pandian K. Quantitative detection of Aloin and related compounds present in herbal products and *Aloe vera* plant extract using HPLC method. American J. Anal Chem. 2013;2013.
21. Mollaei S., Hazrati S., Lotfizadeh V., Dastan D., Asgharian P. Phytochemical variation and biological activities of Zosima absinthifolia during various stages of growth. Inter J Food Prop. 2020;23(1):1556-1567.
22. Asnaashari S., Delazar A., Safarzadeh E., Tabibi H., Mollaei S., Rajabi A., Asgharian P. Phytochemical analysis and various biological activities of the aerial parts of Scrophularia atropatana growing in Iran. Iran J. Pharm. Res. 2019;18(3):1543.
23. Chang X.L., Wang C., Feng Y., Liu Z. Effects of heat treatments on the stabilities of polysaccharides substances and barbaloin in gel juice from *Aloe vera* Miller. J. Food Eng. 2006;75(2):245-251.
24. Ramachandra C.T., Rao P.S. Processing of *Aloe vera* leaf gel: a review. American J Agri Biol Sci. 2008;3(2):502-510.
25. Coats BC. Hypoallergenic stabilized *Aloe vera* gel. US patent. 1979;4:178, 372.
26. Syahir A., Sulaiman S., Mel M., Othman M., Sulaiman S.Z. An Overview: Analysis of ultrasonic-assisted extraction's parameters and its process. In IOP Conference Series: Material Sci Eng. 2020;778(1):012165, IOP Publishing.
27. Asamenew G., Bisrat D., Mazumder A., Asres K. In vitro antimicrobial and antioxidant activities of anthrone and chromone from the latex of Aloe harlana Reynolds. Phytoth Res. 2011;25(12):1756-1760.
28. Lee K.Y., Weintraub S.T., Yu B.P. Isolation and identification of a phenolic antioxidant from Aloe barbadensis. Free Rad Biol Med. 2000;28(2):261-265.
29. Boudreau M.D., Mellick P.W., Olson G.R., Felton R.P., Thorn B.T., Beland F.A. Clear evidence of carcinogenic activity by a whole-leaf extract of Aloe barbadensis Miller (*Aloe vera*) in F344/N rats. Toxicol Sci. 2013;131(1):26-39.
30. Hamiza O.O., Rehman M.U., Khan R., Tahir M., Khan A.Q., Lateef A., Sultana S. Chemopreventive effects of Aloin against 1, 2-dimethylhydrazine-induced preneoplastic lesions in the colon of Wistar rats. Human Exp Toxicol. 2014;33(2):148-163.

31. Suboj P., Babykutty S., Gopi D.R.V., Nair R.S., Srinivas P., Gopala S. Aloe emodin inhibits colon cancer cell migration/angiogenesis by downregulating MMP-2/9, RhoB and VEGF via reduced DNA binding activity of NF-κB. European J Pharm Sci. 2012;45(5):581-591.
32. Song X., Aie X.U., Zhigang B.I. Effect of Aloin on inducible nitric oxide synthase and nuclear factor Kappa B synthesis of hacat cells irradiated by Ultraviolet B. Chinese J. Dermatol. 2003;12:wpr-525625.
33. Niciforovic A., Adzic M., Spasic S.D., Radojcic M.B. Antitumor effects of a natural anthracycline analog (Aloin) involve altered activity of antioxidant enzymes in HeLaS3 cells. Cancer Biol Therap. 2007;6(8):1211-1216.
34. Im S.A., Kim J.W., Kim H.S., Park C.S., Shin E., Do S.G., Lee C.K. Prevention of azoxymethane/dextran sodium sulfate-induced mouse colon carcinogenesis by processed *Aloe vera* gel. International immunopharmacology. 2016;40:428-435.