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CRUDE PLANT EXTRACTS' POTENTIAL ANTI-DIABETIC EFFECTS

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Abstract

Background: The aim of this study was to evaluate the anti-diabetic effect of the aqueous and alcoholic extracts on alloxan-induced diabetic mice.

Methods: Following an acute toxicity test, alloxan was used to cause diabetes in Swiss albino mice for scientific purposes. The study examined the two-week fasting mean blood glucose level in normal, diabetic mice that were not treated, and diabetic mice that were treated with alcoholic and aqueous extracts. Version 20 of the Statistical Package for the Social Sciences was used to statistically analyse the data. A P-value of less than 0.05 was deemed statistically noteworthy.

Results: Steroids, phenolic compounds, flavonoids, and terpenes were found in the extracts after phytochemical screening, and these substances may have an impact on the extracts' anti-diabetic properties. However, anthraquinones and alkaloids were absent from the extracts.

Conclusion: The aqueous extract (200 mg/kg) showed the highest percentage reduction in blood glucose levels and the ability of *Hibiscus syriacus* extracts in reducing blood glucose levels presumably due to the presence ofantioxidant constituents such as flavonoids.

Keyword- Hibiscus syriacus, Diabetes

1. Introduction

The importance of medicinal plant in drug development is known to us and humans have used them for different diseases from the beginning of human history [1]. Traditional folk treatment from wild plants has always guided researchers to search for novel medications to develop healthy life for humans and animals. In addition, some medicinal plants are still obscured within the plant which needs to be scientifically evaluated.

Hibiscus syriacus, also called the Korean rose, is a striking flowering shrub from the Malvaceae family. Originating in Asia, it is extensively grown for its decorative and therapeutic uses. The plant displays vibrant large flowers in an array of hues such as pink, purple, white, and blue. Apart from its visual allure, *Hibiscus syriacus* holds significance for its healing attributes. In traditional herbal medicine, both the flowers and leaves are employed to address different health ailments like fever, inflammation, and digestive problems.[2]

Studies have indicated that *Hibiscus syriacus* is rich in bioactive components like flavonoids and phenolic acids, which play a role in its healing properties. These elements possess both antioxidant and anti-inflammatory characteristics, establishing the plant as a significant asset in herbal medicine.[3]

Plants have been utilized for their medicinal qualities since ancient times. Different parts of plants, including leaves, stems, roots, flowers, and fruits, contain a wide range of active compounds. [4]These active compounds are also known as phytochemicals and have recently received significant attention due to their potential health benefits. Phytochemicals are natural plant substances that demonstrate antioxidant, anti-inflammatory, anti-cancerous properties as well as antimicrobial and immunomodulatory effects.

To effectively utilize the therapeutic potential of these phytochemicals it is essential to employ suitable extraction methods for obtaining them from plant materials. The process of extraction plays a crucial role in harnessing the valuable healing properties present in bioactive compounds obtained from plants.[5]

Soxhlet extraction involves a repeated cycle of solvent extraction and evaporation. This method involves placing the plant material in a permeable extraction thimble and constantly circulating a solvent through it. The evaporated solvent condenses in a separate flask and then returns to the extraction chamber, ensuring efficient extraction of bioactive compounds.[6, 7]Phytochemical analysis is the study of plant chemistry aimed at identifying and measuring bioactive compounds present in plants. These bioactive compounds, referred to as phytoconstituents, may encompass alkaloids, flavonoids, terpenes, saponins, steroids, glycosides, and other secondary metabolites.[8]

Diabetes mellitus is a long-term disorder that impacts the body's ability to convert food into energy. It encompasses various forms, such as type 1, type 2, and gestational diabetes. In these different types of diabetes, there is either insufficient production of insulin or ineffective utilization of the produced insulin by the body [9] .Studies have indicated that specific natural compounds and botanical extracts might possess properties that can combat diabetes. For instance, substances such as berberine, curcumin, and resveratrol have demonstrated promise in regulating blood glucose levels and enhancing insulin sensitivity [10].Preclinical research on the effectiveness of natural compounds and plant extracts in regulating blood sugar levels and enhancing insulin sensitivity employs various methods. Animal models with induced diabetes, like streptozotocin or alloxan-induced diabetic mice or mice, are commonly used to assess the safety and efficacy of these substances before advancing to human clinical trials. Researchers can then observe the impact of administering natural compounds or plant extracts on glucose metabolism, insulin production, and other pertinent factors using these models [11].

2. Materials and Methods

Animals

Adult male Swiss albino miceweighing 25–35 g were acquired from the animal house. Water and normal rodent feed were available to the mice ad libitum. All animals received human care in compliance with guidelines of Institutional Animal Ethical Committee (IAEC) constituted under The Prevention of Cruelty to Animals Act, 1960. Before the trial started, the animals were quarantined for seven days to get acclimated to the laboratory setting. Throughout the experiment, the animals were kept in polypropylene cages (each cage

housing three animals). The temperature (25 ± 1°C), humidity (50 ± 15%), and photoperiod (12 to 12 h light-dark cycles) of the animals were kept under strict supervision.

Acute toxicity studies (LD50)

Acute toxicity tests were performed on both plant extracts (alcoholic and aqueous extracts) after the animals had fasted overnight and consumed only water [11]. Each mouse's weight was recorded prior to administering the extract. The animals were randomly assigned to one control group and three treatment groups (separate for both extracts), each with five mice.

The control group received only the vehicle (1% Tween 80), whereas each treatment group received alcholic and aqueous extracts of *Hibiscus syriacus* orally at doses of 1000, 2000, and 3000 mg/kg [40]. Animals were closely monitored for explicit toxicities and/or behavioural changes such as restlessness, tremor, diarrhoea, sluggishness, weight loss, and paralysis at regular intervals for the first 4 hours after receiving the extract [12], and then daily for two weeks for any change in general behaviour and/or other physical activities. Food was available after four hours following extract administration.

Anti-dabetic Study

Induction of diabetes

After an overnight fast, mice were given an intraperitoneal injection of freshly produced alloxan (80mg/kg body weight; B.W.) dissolved in 0.1M sodium citrate buffer (pH 4.5).Control mice were administered an equivalent volume of citrate buffer alone. The development of hyperglycemia in mice was confirmed by fasting blood glucose measurement from tail vein samples using fine test strips and glucometer (Accu-Chek, Roche, Germany) 48 h post-alloxan administration. Mice exhibiting fasting blood glucose levels exceeding 11.0mmol/L were categorized as diabetic.

Experimental design

The animals were placed into seven groups to evaluate their fasting blood glucose levels and oral glucose tolerance tests, with five animals in each group.

Group 1: Control mice were injected with 1% Tween 80 intraperitoneally;

Group 2: Mice were made diabetic by a single intraperitoneal injection of alloxan (80 mg/kg b.w.);

Group 3: Diabetic mice treated with glibenclamide (10 mg/kg b.w.) daily by oral administration for 2 weeks. [12].

Group 4: Diabetic mice treated with Alcoholic extract of *Hibiscus syriacus*(100 mg/kg b.w.) daily by oral administration for 2 weeks;

Group 5: Diabetic mice treated with Alcoholic extract of *Hibiscus syriacus* (200 mg/kg b.w.) daily by oral administration for 2 weeks;

Group 6: Diabetic mice treated with Aqueous extract of *Hibiscus syriacus* (100 mg/kg b.w.) daily by oral administration for 2 weeks;

Group 7: Diabetic mice treated with Aqueous extract of *Hibiscus syriacus* (200 mg/kg b.w.) daily by oral administration for 2 weeks;

Alcoholic extract of the plant was dissolved in one drop of Tween(80%, Prolabo, France) and distilled water.

Samples

They were treated with plant extracts two days after receiving alloxan injections, with the exception of diabetic control groups. Blood samples were obtained from each group on days 1, 7, and 14 of the study period to measure blood glucose levels [12]. Changes in body weight were also documented.

Oral glucose tolerance test

After two weeks of treatment with plant extracts, the animals were fasted for 12-14 hours while having unrestricted access to water, and their fasting blood glucose levels were assessed four times. Glucose solution (2 g/kg bodyweight) was given orally in a volume of 1 mL/kg.Blood samples were taken 30, 60, and 120 minutes after glucose injection [13].

Statistical analysis

Data are presented as mean ± standard deviation. Two-way analysis of variance (ANOVA) was used to compare treatment group means, and P-values < 0.05 indicated significant differences. Data were statistically analysed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software. Bar and line charts were created with Excel2007.

3. Results and discussion

Acute toxicity test

The acute toxicity study found that administering graded doses of both aqueous and alcoholic extracts of *Hibiscus syriacus* did not produce any observable signs of toxicity up to a dose of 3000 mg/kg.

This was corroborated by the lack of substantial changes in behaviours such as alertness, motor activity, weight loss, sluggishness, paralysis, respiration, restlessness, diarrhoea, convulsions, and coma. In addition, no deaths were reported in two weeks, and they were physically active. The findings show that the plant extracts had no obvious deleterious effects at the tested levels, meaning that the medium lethal dose (LD50) in mice is larger than 3000 mg/kg body weight. The extract of *Hibiscus syriacusis* is nontoxic because its median lethal dose (LD50) is larger than 3000 mg/kg [14].

Effect of *Hibiscus syriacus*extracts on fasting blood glucose level

Hibiscus syriacus extracts, both aqueous and alcoholic, were given orally to Alloxan-induced diabetic mice once a day for a period of 14 days. Fig. 2 shows the impact of various dosages of *Hibiscus syriacus* extracts on fasting blood glucose levels.

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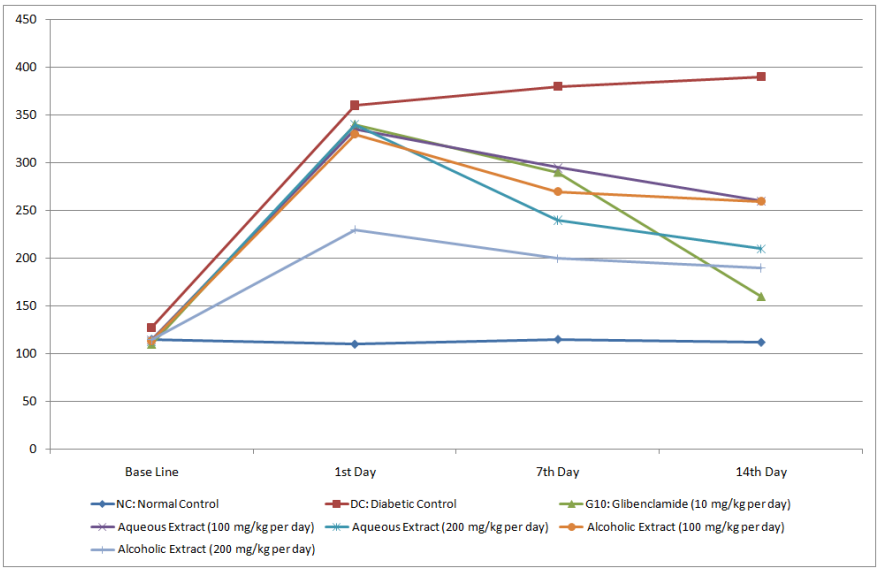

Fig. 2 Effect of extracts of Hibiscus syriacus on fasting blood glucose level in normal control and alloxan-induced diabetes mice.

The goal of this study was to investigate the potential anti-diabetic properties of *Hibiscus syriacus* leaf and flower extracts. *Hibiscus syriacus* dosages of 100 and 200 mg/kg body weight were chosen in accordance with earlier research on the Hibiscus species [15].

In experimental mice, diabetes mellitus has been induced using alloxan monohydrate [46, 47]. Mice were successfully given a single intraperitoneal injection of a 20 mg/kg body weight alloxan monohydrate solution to produce diabetes mellitus. The mice's higher fasting blood glucose level, which was measured 48 hours after injection, was used to validate this. Alloxan causes diabetes by selectively destroying pancreatic β-cells that secrete insulin as a result of building up through the glucose transporter 2 (GLUT2), which reduces the amount of glucose absorbed by peripheral tissues. It is well known that alloxan triggers the production of free radicals through redox processes that damage tissue, cause ß-cells to degranulate, and ultimately cause degeneracy[16].

The mean blood glucose level in the diabetic control group increased by 9.1% ± 1.5, as anticipated, and there was a significant difference ($p < 0.0001$) when compared to the normal control group of mice. Diabetic mice had their blood glucose levels measured prior to and during the first, seventh, and fourteen days of treatment. With regard to normal and diabetic control mice, there is a statistically significant difference ($p < 0.0001$) between the treatment groups receiving aqueous and alcoholic *Hibiscus syriacus* extracts. There is also a statistical significant difference between each dose of aqueous extracts ($P < 0.005$) and the alcoholic extract (100 mg/kg) ($P < 0.0001$). Nonetheless, no statistically significant variations ($P > 0.05$) were seen between the 200 mg/kg alcoholic extract, aqueous extracts, and Glibenclamide.

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Table 2 Average Percentage Decrease of Blood Glucose Level

Group of Treatments	DBGL (%)
Diabetic + Aqueous Extract of *Hibiscus syriacus* (100 mg/kg body weight)	28.43 ± 1.96
Diabetic + Aqueous Extract of *Hibiscus syriacus* (200 mg/kg body weight)	35.96 ± 0.57
Diabetic + Alcoholic Extract of *Hibiscus syriacus* (100 mg/kg body weight)	27.26 ± 1.52
Diabetic + Alcoholic Extract of *Hibiscus syriacus* (200 mg/kg body weight)	28.94 ± 1.91
Diabetic + Glibenclamide (10 mg/kg body weight)	50.10 ± 1.95

The average percentage of blood glucose level reduction (Table 2) increased as the relative dose of *Hibiscus syriacus* extract administration increased. After 14 days of treatment administration, the *Hibiscus syriacus* aqueous extract with a dose of 200 mg/kg body weight exhibited a bigger percentage decrease (35.96 ± 0.57) than any other extract. As a positive control, diabetic mice treated with glibenclamide (10 mg/kg body weight) demonstrated a reduction of 50.10 ± 1.95 percentages.

Hibiscus syriacus leaf and flower extracts lowered high fasting blood glucose levels. The inclusion of well-known antioxidant phytochemicals including flavonoids, polyphenols, and terpenes, which function as free radical scavengers, may be the mechanism behind the anti-diabetic benefits of the extracts of *Hibiscus syriacus* leaves and flowers [17].

The antioxidants were thought to work by mimicking the effects of insulin on the peripheral tissues, either by promoting the process of regeneration or by releasing insulin from the pancreas' existing β-cells. In addition to this method, other processes also contribute significantly to the lowering of blood glucose levels, making them promising anti-diabetic plants. Among the others include boosting the release of glucose by improved metabolism or integration into fat deposits, a process connected to the pancreas' production of insulin, and quickening the release of glucose from the circulation by speeding filtration and renal excretion [18].

The study's findings confirmed what Indian traditional healers had long said about the plant extract's usage in the treatment of diabetes. The mechanism underlying the extracts' anti-diabetic effect is unknown to us, nevertheless. Consequently, additional research must be done.

Oral glucose tolerance test

Figure 3 shows the mean blood glucose levels of diabetic mice treated with *Hibiscus syriacus* extracts, diabetic mice left untreated (positive control), and normal mice (negative control) after two weeks of the mice being tested for glucose tolerance.

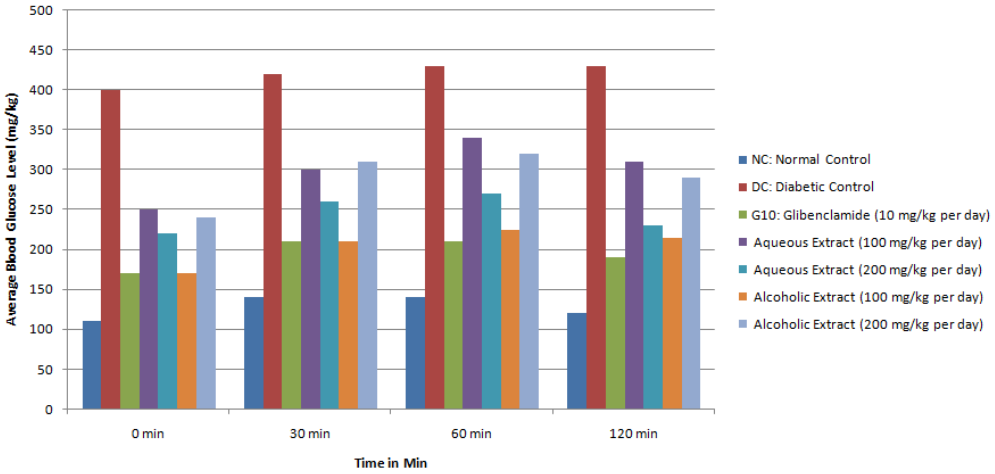

Figure. 3 Glucose tolerance test of A.remota extracts on alloxan-induced diabetic mice.

After the animals in each group (n = 5) fasted for 12–14 hours, oral glucose administration (2 g/kg body weight) was given to them as a baseline, and the fasting mean blood glucose level was assessed. After a 60-minute glucose load, the mean blood glucose level in the normal control mice increased to a peak value and then dropped to a level that was almost normal after 120 minutes. However, as was predicted, the value in diabetic control mice rose to a peak following a 60-minute glucose load and stayed high for the following 60 minutes. After 60 minutes of glucose loading, the animals who received the extracts demonstrated a decrease in their mean blood glucose levels. The blood glucose level in both aqueous extracts peaked at 60 minutes.

Table 1 presents a summary of the test findings and shows the presence of phenolic chemicals, flavonoids, and terpenes. This outcome was in line with earlier findings. Anthraquinones and alkaloids were absent from the extracts. Several chemicals have been identified from the plant that have the ability to lower blood glucose levels. [19-25].

Thus, the significant anti-diabetic effect of the extracts of *Hibiscus syriacus*could be due to the presence of the above-mentioned components in the extracts, which could act synergistically and/or independently to enhance the activity of glycolytic enzymes.

Conclusion

The extracts were given orally to mice at doses ranging from 1000 to 3000 mg/kg/day, no discernible behavioural alterations occurred, suggesting that the extracts are not harmful in the conditions that were observed. Flavonoids, phenolic compounds, and terpenes were found in *Hibiscus syriacus* leaf and flower extracts; some of these substances are thought to be bioactive components in the treatment of diabetes. On alloxan-induced Swiss albino mice, the *Hibiscus syriacus* extracts at doses of 100 and 200 mg/kg body weight demonstrated anti-diabetic benefits. At doses of 100 and 200 mg/kg body weight, the percentage reduction in blood glucose levels of Hibiscus syriacus aqueous and alcoholic extract is 28.43 ± 1.96, 35.96 ± 0.57, 27.26 ± 1.52 and 28.94 ± 1.91, respectively. Therefore, the chemical components of the plant extract may work as a preventative measure against diabetic problems and as a

substitute for the current generation of anti-diabetic medications. It is advised to conduct more research to support the plant's use as an anti-diabetic.

Reference

1. Pan SY, Litscher G, Gao SH, Zhou SF, Yu ZL, Chen HQ, et al. Historical Perspective of Traditional Indigenous Medical Practices: The Current Renaissance and Conservation of Herbal Resources. Evidence-Based Complementary and Alternative Medicine [Internet]. 2014;2014(525340):1–20.
2. Balkrishna A, Mishra S, Singh A, Srivastava D, Singh S, Arya V. *Hibiscus syriacus* L.: A Critical Review of Medicinal Utility &Phytopharmacology with Mechanistic Approach. J Phyto pharmacol. 2022 Jun 25;11(3):204-10.
3. Maganha EG, HalmenschlagerRdC, Rosa RM, Henriques JAP, Ramos ALLdP, Saffi J. Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. Food Chemistry. 2010 Jan;118(1):1-10.
4. Maganha EG, HalmenschlagerRdC, Rosa RM, Henriques JAP, Ramos ALLdP, Saffi J. Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. Food Chemistry. 2010 Jan;118(1):1-10.
5. Carocho M, Ferreira ICFR, Morales P, Soković M. Antioxidants and Prooxidants: Effects on Health and Aging. Oxidative Medicine and Cellular Longevity. 2018;2018:1-2.
6. Chemat F, Vian MA, Cravotto G. Green Extraction of Natural Products: Concept and Principles. IJMS. 2012 Jul 11;13(7):8615-27.
7. Ameer K, Shahbaz HM, Kwon J. Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review. Comp Rev Food Sci Food Safe. 2017 Mar;16(2):295-315.
8. Adedapo,A.A.,Mogbojuri,O.M.,andEmikpe, B.O.(2009).Safety evaluations oftheaqueousextractoftheleavesofMoringaoleifera in rats. J.Med.Plant 3, 586–591.
9. Porras G, Chassagne F, Lyles JT, Marquez L, Dettweiler M, Salam AM, et al. Ethnobotany and the Role of Plant Natural Products in Antibiotic Drug Discovery. Chem Rev. 2021 Mar 24;121(6):3495-560.
10. Kalpna D. Rakholiya, Mital J. Kaneria, Sumitra V. Chanda,Chapter 11 - Medicinal Plants as Alternative Sources of Therapeutics against Multidrug-Resistant Pathogenic Microorganisms Based on Their Antimicrobial Potential and Synergistic Properties, Editor(s): Mahendra Kumar Rai, Kateryna Volodymyrivna Kon, Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and Their Components,Academic Press,2013,Pages 165-179,ISBN 9780123985392,
11. Ragavan B, Krishnakumari S. Antidiabetic effect of T. arjuna bark extract in alloxan induced diabetic rats. Indian Journal of Clinical Biochemistry. 2006 Sep;21:123-8.
12. OECD (Organization for Economic Cooperation and Development)guidelines for testing of chemicals 425. Acute Oral Toxicity-Up and Downprocedure, first adoption, Octoberv2008, Paris, France (https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg425.pdf).

13. Burger C, Fischer DR, Cordenunzzi DA, Batschauer APD, Filho VC, SoaresARD. Acute & sub-acute toxicity of the hydroalcoholic extract from WedeliaPaludosa (Acmelabrasiliensis) (Asteraceae) in mice. J Pharm Sci. 2005;8(2):370–3.
14. Nagappa AN, Thakurdesai PA, Rao NV, Singh J. Antidiabetic activity ofTerminaliacatappa Linn fruits. J Ethnopharmacol. 2003;88(1):45–50.
15. Kumar GPS, Arulselvan P, Kumar DS, Subramanian SP. Antidiabetic activity offruits of Terminaliachebulaonstreptozotocin induced diabetic rats. J HealthSci. 2006;52(2):283–91.
16. Loomis TA, Hayes AW. Loomis's essentials of toxicology. 4th ed. San Diego,California, USA: Academic Press; 1996.
17. Akter S, Ali H, Shati AA, Alfaifi MY, Elbehairi SE, Sayyed RZ, Yeasmin T. Antidiabetic activity of methanolic extract of Hibiscus sabdariffa Linn. fruit in alloxan-induced Swiss albino diabetic mice. Open Agriculture. 2024 Feb 15;9(1):20220243.
18. Carvalho EN, Carvalho NAS, Ferreira LM. Experimental model of induction ofdiabetes mellitus in rats. Acta Cir Bras. 2003;18:60–4.
19. Kamalakkanan N, Prince PSM. Hypoglycemic effect of water extracts of Aegle marmelos fruits in streptozotocin-diabetic rats. J Ethnopharmacol. 2003;87(2–3):207–10.
20. Muhtadi, Primarianti AU, Sujono TA. Antidiabetic Activity of Durian (Durio zibethinus Murr.) and Rambutan (Nephelium lappaceum L.) Fruit Peels inAlloxan Diabetic Rats. Procedia Food Science 2015; 3: 255–261.
21. Sharma B, Balomajumder C, Roy P. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from Eugenia jambolana seeds on streptozotocininduced diabetic rats. Food Chem Toxicol. 2008;46:2376–83.
22. Yang JE, Ngo HT, Hwang E, Seo SA, Park SW, Yi TH, et al. Dietary enzyme-treated Hibiscus syriacus L. protects skin against chronic UVB-induced photoaging via enhancement of skin hydration and collagen synthesis. Archives of Biochemistry and Biophysics. 2019;662:190-200.
23. Wei Q, Ji XY, Xu F, Li QR, Yin H. Chemical constituents from leaves of Hibiscus syriacus and their α-glucosidase inhibitory activities. Zhong yaocai. 2015;38(5):975-79.
24. Shi LS, Wu CH, Yang TC, Yao CW, Lin HC, Chang WL, et al. Cytotoxic effect of triterpenoids from the root bark of Hibiscus syriacus. Fitoterapia. 2014;97:184-91.
25. Hsu RJ, Hsu YC, Chen SP, Fu CL, Yu JC, Chang FW, et al. The triterpenoids of Hibiscus syriacus induce apoptosis and inhibit cell migration in breast cancer cells. BMC Complementary and Alternative Medicine. 2015;15(65):1-9.