Eksakta : Berkala Ilmiah Bidang MIPA
VOLUME 24 NO 02 2023, pp 207-215
ISSN : Print 1411-3724 — Online 2549-7464
DOI : https://doi.org/10.24036/eksakta/vol24-iss02/389

Eksakta
Berkala Ilmiah Bidang MIPA
http://www.eksakta.ppj.unp.ac.id/index.php/eksakta

Article

Nutraceutical Product Ice Cream from Dry Flower (*Echinacea purpurea* Lour) As an Immunomodulator in Pandemic Era: Ekino Ice

Article Info

Article history :

Received January 18, 2023
Revised March 27, 2023
Accepted April 16, 2023
Published June 30, 2023

Keywords :

Ekinase flower, covid-19, ekino ice, immunomodulator, nutraseutical

Reza Putri Oktavia[1], Novia Vivin Safitri[1*], Nurus Samsiyah[1], Salsabila Tri Rahmi[2], Devi Purnamasari[3]

[1]Department of Medicine and Health Sciences, Faculty of Pharmacy, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Indonesia
[2]Department of Civil Engineering, Faculty of Engineering, Universitas Negeri Padang, Indonesia
[3]Department of Radiology Engineering, Universitas Awal Bros, Pekanbaru, Indonesia

Abstract. *Echinacea purpurea* is a flower that is rich in its main antioxidant content comes from flavonoids, phenols, and cystic acids can be utilized as an immunomodulator during the Covid-19 pandemic. Ekinase flowers are processed into new innovations in nutraceutical products with the Ekino Ice product label. Goal to innovate nutraceutical products made from dry flower *Echinacea purpurea* as immunomodulators in the era of the Covid-19 pandemic. This research uses laboratory experimental methods with a qualitative approach. Microscopic test results are known that the sample has a plant characteristic of *Echinacea purpurea*. KLT test results using UV 366 light is positive flavonoid blue color Rf 0.5 nm with ammonia stain appearance, positive phenol stain blue-black color Rf 0.75 nm with $FeCl_3$ stain appearance, positive cystic acid stain blue color Rf 0.75 nm. Brown organoleptic test results, characteristic smell of *Echinacea purpurea*, and taste sweet. pH test result 6.42 and melting time test result 16.22 minutes. Ekino Ice has the potential to be a new innovation nutraceutical product as immunomodulators in the era of the Covid-19 pandemic.

Corresponding Author :
Novia Vivin Safitri
Department of Medicine and Health Sciences, Faculty of Pharmacy, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Indonesia
Email : noviavivin28@gmail.com

1. Introduction

The Covid-19 pandemic is an outbreak of pneumonia characterized by symptoms of fever, dry cough, fatigue, and gastrointestinal. The virus was first discovered in the Wuhan wet market in late December 2019 [1-2]. Then, on January 30, 2020, WHO declared that Covid-19 included a Public Health Emergency of International Concern (PHEIC) due to a very significant increase in Covid-19 [3]. Corona virus is an infectious disease that has mild to severe symptoms [4-5]. An insufficiently strong immune response can lead to viral replication and tissue damage. Although it has been developed, various types of vaccines have not yet found a cure that can overcome the coronavirus, so the efforts that can be made are to maintain the body's immune system and strengthen it.

Ekinase or purple coneflower is a medicinal plant that has benefits as an immunomodulator. Ekinase belongs to the family Asteraceae. The type of ekinase plant with the highest level of cultivation as a medicinal plant commercially is the type of *Echinacea purpurea* [6-7]. The plant has a high immunomodulatory, anti-inflammatory, and antioxidant [8-9]. Ekinase contains chemical compounds that can function as immunomodulators including flavonoids, phenols, cystic acid, polysaccharides, polyacetin, alkyl amides, caffeine acids, and caffeine acid derivatives [6].

Ekinase flowers have a dry weight of 24.79 grams with the highest phenol content in flowers, which is 0.88% [6][10]. In addition to phenols, ekinase flowers contain cystic acid which is a polyphenol compound that has cytotoxic properties and is able to inhibit cell growth by the mechanism of antioxidants, apoptosis and cell cycle inhibition [11-12]. Phenols act as a supporter of antioxidant activity in the body to suppress free radicals [13]. In addition to these compounds, ekinase flowers contain flavonoid compounds. The plant family that has high levels of flavonoids is the Asteraceae family [14-15].

Flavonoids have the ability as immunomodulators, the mechanism of flavonoids as immunomodulators is to increase the activity of the enzyme Interleukin-2 (IL-2) and through cell division of lymphocytes, which will later affect the increase in phagocytosis to be faster in killing antigens that enter the body. Then based on the analysis that Burick et al. have done, (1997) it is known that the dry extract of Echinacea purpurea (L.) contains polyphenols including polyphenols as much as 4%; cystic acid 1.75%; caftaric acid 1.5%; and polysaccharides 23% [16-17].

The content of compounds contained in ekinase flowers requires innovation as a processed nutraceutical product that has a role in the Covid 19 pandemic. Ice innovation from the main ingredient *Echinacea purpurea* (L.) will become an interesting food product and in demand by the public so that it is expected to be able to have an impact as an innovation of food products that are efficacious as immunomodulators. Ice is a snack that can be consumed by various groups, ranging from young to old. Ice making goes through a homogenization to freezing process and is nutritionally valuable, cheap, and delicious [18-19]. This study aims to test the content of compounds that are useful as immunomodulators. This research can be a healthy food innovation to face the era of the Covid-19 Pandemic. Ekino ice nutraceutical products besides being delicious are able to provide health benefits, especially for improving the body's immune system.

2. Experimental Section

This study used a laboratory research design. In this study, researchers used a qualitative approach. The free variable used in the study was ekinase flower extract. While the bound variables are flavonoids, phenols, cystic acid, ammonia vapor stain viewers, and $FeCl_3$ stain holders. This research was carried out at the researcher's house and the Pharmacy Laboratory of the Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University Malang on March 29-April 22, 2022.

2.1. Microscopic Test

This test is carried out to find out the correctness of the type of simplicia used. Microscopic tests are performed by slashing the flowers, leaves, and stems of the plant ximplicia *Echinacea purpurea*, with

longitudinal incisions as thin as possible. Then an incision is placed on the glass of the object and then dripped with a solution of chloralhydrate and then observed under a microscope. The material needed is dry simplicia of *Echinacea purpurea* plant parts, namely stems, leaves, and flowers as well as the solvent used, namely chloralhydrate solution. While the tools used for microscopic tests are, light microscopes (Nikon), glass preparations, cutters or razor blades, and also tweezers. After the simplicia of the ekinase plant is carried out microscopic tests, then the selection of samples to be used is carried out, namely simplicia on the flower.

2.2. The Simplicia of the Ekinase Flower

The simplicia of the ekinase flower is mashed weighed by 35 grams and blended until smooth and sifted using a mesh sieve number 40, 1000 ml of water is added and extraction is carried out using the infundation method. The tools used to extract the ekinase flower simplicia are as follows, an infusion pan, digital scales (Ohaus NV212), filter paper, gas stove, stirring rod, 500 mL measuring cup, beaker glass, thermometer, glass funnel, mesh sieve number 40, and blender.

2.3. Make Ekino Ice

The process of making products begins with the preparation of tools and materials to be used, then mixing *Echinacea purpurea* flos extract, honey, and water is then stirred until homogeneous. Once the ingredients are mixed add Na CMC and stir until dissolved. After all the ingredients are homogeneously mixed, pour the Ekino Ice mixture of ingredients into the mold and freeze. The formulation of Ekino Ice consists of 5.25 grams of ekinase flower extract, 1.5 grams of Na CMC, 45 ml of honey, and 98.25 ml of water.

The ingredients used to make Ekino ice, such as honey are bought at convenience stores and selected natural honey, for Na CMC purchased in tools and chemicals stores with food grade standards, and for ekinase extract obtained from the extraction results of ekinase flower simplicia obtained from Tawangmangu, Central Java. For phytochemical screening tests using KLT (thin layer chromatography) the materials needed are KLT plate G60F254, acetic acid, n-butanol, aquadest, ammonia vapor spray reagent, n hexane, ethyl acetate, FeCl3 stain remover, and formic acid. The tools used are TLC Visualizer (CAMAG), chamber, filter paper, stain penotol, beaker glass (pyrex), drip pipette, measuring cup (pyrex) 100 mL, tweezers, stirring rod, hot plate (Thermometer), UV lamp 254 nm and 366 nm.

2.4. Qualitative Analysis of Flavonoids, Phenols, and Cystic Acid Compound

Qualitative analysis using the Thin Layer Chromatography (KLT) method aims to identify flavonoid, phenol, and cystic acid compounds. In the flavonoid test, the mobile phase used was glacial acetic acid: butanol: water (1: 4: 5) and the stain image used was ammonia vapor. The extract was subjected to 0.8 – 1µl in the previously saturated stationary phase. Then, it was observed at UV rays of 254 nm and 366 nm. The positive results of flavonoids are characterized by the formation of yellow-brown stains on visible rays, black stains on 254 nm UV rays and blue stains on 366 nm UV rays [20-21].

In the phenol test, the motion phase used was n-hexane: ethyl acetate (3:7) and the stain imposter used was $FeCl_3$. The extract was subjected to 0.8 – 1µl in the previously saturated stationary phase. Then, it was observed at UV rays of 254 nm and 366 nm. Positive phenol results are characterized by the formation of blue-black stains on 366 nm UV rays [20-21]. Then, in the cystic acid test, the mobile phase used was ethyl acetate: n-butanol: formic acid: aquadest (5:3:1:1). The extract was calculated as much as 0.8 – 1µl in the previously saturated stationary phase. Then, it was observed at UV rays of 254 nm and 366 nm. The positive result of cystic acid is characterized by the formation of bright blue fluorescent stains on UV rays of 366 nm [22].

2.5. Organoleptic Test

Organoleptic test is a preliminary test carried out to determine the physical characteristics of the product, including the aroma, color, and taste of the product. This test is carried out by physically observing Ekino Ice products using the five senses [23]. The senses used in this test include the senses of sight, touch, smell, and taste. Panelists are some of the people who can give assessments related to organoleptic tests. They were asked to explain the organoleptic (texture, smell, color, and taste) of Ekino Ice products.

2.6. pH Test

A pH test is a test carried out to determine the pH produced from a product declared safe for consumption and meets the requirements of a safe pH in the body. The pH test is carried out using a digital pH meter by dipping the pH meter in a melted Ekino Ice preparation. Then the pH meter will show the pH value of the preparation on the screen where there is a tool pad. A good pH value is around the pH value of 7.8 [24]. The tools used for the pH test are a stirring rod glass, a 100 mL beaker glass (pyrex), and a pH meter.

2.7. The Melting Time Test

The melting time test is carried out by placing frozen Ekino Ice in a beaker glass and then left until the ice melts with a temperature of 25 °C. The melting time gauge in this test used a stopwatch that started when the Ekino Ice was removed from the refrigerator and placed in a beaker glass. The melting time test was performed on 6 Ekino Ice samples from each mold. The standard value of a good melting time of ice cream according to what is on the market is 15-20 minutes [25].

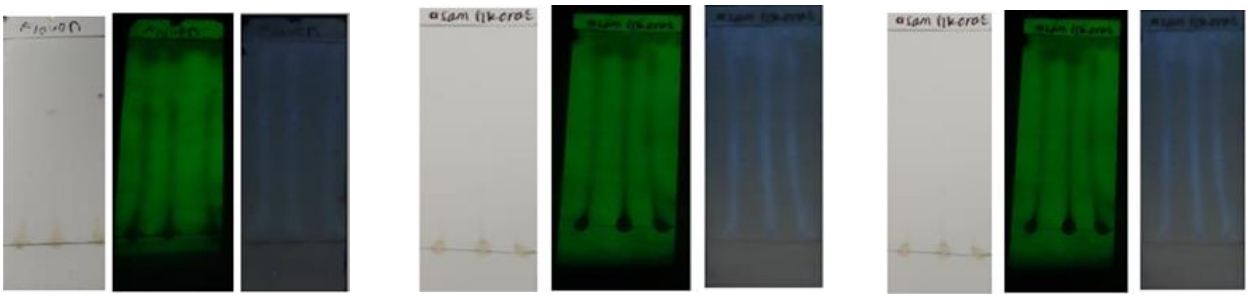
2.8. The Data Analysis

The data analysis used in this study is a descriptive analysis method. The analyzed data is presented in the form of tables, graphs, or images according to the results that have been obtained. Descriptive analysis is analyzing information from data by describing the data collected. This analysis is carried out by inferring the results of the study and compared with predetermined parameters [26].

3. Results and Discussion

3.1 Thin Layer Chromatography Test

A Thin Layer Chromatography test was carried out to determine the secondary metabolite compounds present in the ekinase flower. Secondary metabolite compounds that will be tested for their presence in ekinase flowers are flavonoids, phenols, and cystic acid. The sample tested was ekinase flower extract. The results of the Thin Layer Chromatography test are shown in the figure below.



(a) **(b)** **(c)**

Figure 1. Thin layer chromatography identification test results (a) flavonoid compounds, (b) cystic acid compounds, (c) phenol compounds

In the third Thin Layer Chromatography test, this secondary metabolite compound was also measured by the Rf value to find out that the compounds attracted by the solvent are indeed flavonoid, phenol, and cystic acid compounds. The results of the calculation of the Rf value and the color produced during the Thin Layer Chromatography test can be seen in Table 1.

Table 1. Phytochemical screening results using thin layer chromatography and Rf values

No	Test Name	Rf	Visible Rays	UV 254	UV 366
1	Flavonoid	0.50	Yellow Brown	Black	Blue
2	Phenol	0.75	Yellow Brown	Dark Brown	Dark Blue
3	Cystic Acid	0.75	Yellow	Black	Bright Blue

The presence of secondary metabolite compounds contained in *Echinacea purpurea* flowers was identified through phytochemical screening tests using Thin Layer Chromatography (KLT). This study used qualitative type phytochemical screening with the use of the KLT method. The presence of secondary metabolite compounds can be indicated by a color reaction formed by a specific reagent.The extraction method chosen in this study is the infundation extraction method. The reason for choosing the infundation method compared to other extraction methods is because this method is considered the safest if used for food products and can be done using aquadest solvents. Aquadest is a solvent with a high degree of polarity. While the infundation method itself is a method of extraction by hot means. The advantages of this method are that the process is easy, the time required is shorter, and the equipment used is simpler [27-29]. Thus, the above treatment will provide results in the form of extracts derived from *Echinaceae purpurea* simplicia as a material for working on the KLT method and can be known to prove what compounds are contained in it.

Phytochemical screening tests were performed on three secondary metabolite compounds that were shown to have immunomodulatory effects. These compounds include flavonoids, phenols, and cystic acid. The results of the flavonoid screening test showed positive results with the formation of yellow-brown in visible light, black at UV 254 nm, and blue at UV 366 nm after being given ammonia vapor reagent with an Rf value obtained of 0.5. Flavonoids have an immunomodulatory effect through their activity through the enzyme Interleukin-2 (IL-2) and lymphocyte cell division so that 9 affects the increase in phagocytosis to be faster and more efficient in killing antigens. The results of the phytochemical screening test on phenol also showed positive results with the formation of a blue-black stain color on UV light of 366 nm after spraying $FeCl_3$ reagent and the resulting Rf value of 0.75. The blue-black color (hesasianoferat iron) is formed from the result of reducing phenol to Fe^{3+} to Fe^{2+} [30-33].

Phenols have an immunomodulatory effect through their activity. Phenol compounds are able to increase the activity of macrophages in the phagocytosis of carbon particles entering the bloodstream. The third compound tested for presence is cystic acid. The results of the phytochemical screening test showed positive results with the formation of a bright blue stain color on 366 nm UV light with an Rf value of 0.75. Cystic acid has a high antioxidant activity and has benefits as an immunomodulator [22][34]. All of the above treatments or laboratory tests are a form of validation of compounds in *Echinacea purpurea* simplicia which have pharmacological effects in the form of immunomodulators are flavonoids, phenols, and cystic acid.

3.2 Ekino Ice Evaluation Test

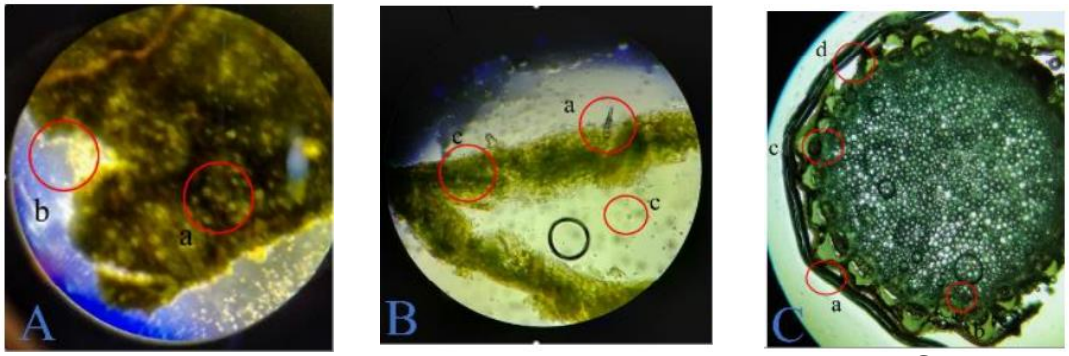
Organoleptic test is a preliminary test carried out to determine the physical characteristics of the product, including the aroma, color, and taste of the product. This test is carried out by physically observing Ekino Ice products using the five senses [23][35]. Organoleptic tests are used to test the physical appearance of the resulting Ekino Ice product. In the organoleptic test, the color results of Ekino Ice products are brown, have a distinctive aroma that is honey and ekinase flowers, and have a sweet taste.

A pH test is a test carried out to determine the pH produced from a product declared safe for consumption and meets the requirements of a safe pH in the body. In the pH test with three repeat test replications using a digital pH meter, the pH result of Ekino Ice products was 7,86. The pH value produced is in accordance with the established pH ice standard of 7.8 [24][36].

The melting time test is carried out by placing frozen Ekino Ice in a beaker glass and then left until the ice melts with a temperature of 25°C. The melting time gauge in this test used a stopwatch that started when the Ekino Ice was removed from the refrigerator and placed in a beaker glass. The melting time test is carried out to determine the durability of ice if it is to be marketed. In the melting time test with calculations using a stopwatch, the result of the melting time of Ekino Ice products was 20 minutes 18 seconds. The results of this melting time test are in accordance with existing standards, the standard of the melting time of ice in general or commercial ice cream is 15-20 minutes [25].

3.3 Microscopic Test

Microscopic tests are carried out to determine the characteristics and correctness of the *Echinaceae purpurea* simplicial to be used. Simplicia samples of *Echinacea purpurea* to be observed include stems, leaves, and flowers. The Thin Layer Chromatography test results are shown in the figure below.



A B C

Figure 2. (A) Flower part microscope results, a) loose pollen, b) oli glands (B) leaf part microscope results, a) hair cover, b) vascular beam, c) sotamata (C) stem part microscope results, a) epidermis, b) xylem, c) floem, d) cortex

Based on the results of microscopic observations on the sample part of the *Echinacea purpurea* flower simplicia, two crown identifying fragments of the flower were found, namely loose pollen and oil glands. The important role of the pollen part is not only as a support for smoothness in terms of crossing, but also to increase flowering. Other identifying fragments in the form of oil glands are proven to exist and correspond to secondary metabolites possessed by ekinase and have pharmacological effects in the form of essential oils [37]. Then in the fragments from the simplicia sample of the leaves of *Echinacea purpurea*, several cells or tissues were found in it, including covering hairs, vascular sheaves, and stomata. In figure B, the covering hair seen on the microscope is called trichomas as protection of leaf tissue from mechanical damage [38-39].

The network of leaf veins consisting of xylem and phloem has its own role. Xylem or commonly referred to as wood vessels have a role as tissues, which transports water and minerals. Meanwhile, phloem (tapis vessels) plays a role in transporting photosynthesis results [40]. In addition, there is one

simplicia of the leaf that has effectiveness in terms of interacting with the environment, namely stomata. This is because in stomata there is a pair of guard cells that have a gap to use as a way of exchange between water vapor and gas [41-42].

In the simplicia sample of the stem of *Echinacea purpurea* after receiving microscopic test treatment, images of several cells or tissues were obtained, including the epidermis, xylem, phloem, and cortex. Before undergoing secondary thickening, epidermal cells are the outermost layer of the stem [43]. Not only that, on the stem there are also two vessels, namely xylem and phloem. For the cortex (basic tissue) can form intercellular spaces as a way for water to enter before it reaches xylem. The cortex may thicken at the size of its cells. This can improve the course of water and the efficiency of water use [44]. The above review is a form of microscopic testing with the aim of knowing the truth of Echinacea purpurea simplicia used in the manufacture of Ekino Ice products.

4. Conclusion

Based on the research that has been done, it can be known that ekinase flowers can be used as a processed nutraceutical product in the form of ice cream. With the name "Ekino Ice" which has the physical characteristics of a product that meets commercial ice cream standards and contains secondary metabolite compounds such as flavonoids, phenols, and cystic acid which are useful as immunomodulators in the era of the Covid-19 pandemic. Thus, people can apply it to produce their own at home or be developed so that it becomes a nutraceutical product that has a high selling value as a substitute for health supplements.

References

[1] Wu, Y. C., Chen, C. S., & Chan, Y. J. (2020). The outbreak of COVID-19: An overview. *Journal of the Chinese Medical Association*, 83(3): 217–220.

[2] Torales, J., O'Higgins, M., Castaldelli-Maia, J. M., & Ventriglio, A. (2020). The outbreak of COVID-19 coronavirus and its impact on global mental health. *International journal of social psychiatry*, *66*(4), 317-320.

[3] Susanna, D. (2020). When will the COVID-19 pandemic in indonesia end. *Kesehatan Masyarakat*, 15(4): 160–162.

[4] Wicaksono, A., & Nurfianti, A. (2021). Increasing Covid-19 Prevention and Control. *Journal of Public Health,* 4(2):112-117.

[5] Young, S. D., & Goldstein, N. J. (2021). Applying social norms interventions to increase adherence to COVID-19 prevention and control guidelines. *Preventive Medicine*, *145*, 106424.

[6] Subositi, D., & Fauzi, F. (2016). Keragaman instar spesifikasi ekinase (Echinaceapurpurea L.) Moench hasil seleksi massa tahap 1 berdasarkan massa tahap 1 berdasarkan analisis SR. *Jurnal Tumbuhan Obat Indonesia*, 9(1): 11–18.

[7] Cui, Y., Hu, C., Zhu, Y., Cheng, K., Li, X., Wei, Z., ... & Gou, X. (2018). CIK receptor kinases determine cell fate specification during early anther development in Arabidopsis. *The Plant Cell*, *30*(10), 2383-2401.

[8] Megha, G., Sharma, D., Sharma, A., Kumari, V., & Goshain, O. P. (2012). A review on purple cone flower (Echinacea purpurea L. Moench). *Journal of pharmacy research*, *5*(8), 4076-4081.

[9] Chaiyana, W., Charoensup, W., Sriyab, S., Punyoyai, C., & Neimkhum, W. (2021). Herbal extracts as potential antioxidant, anti-aging, anti-inflammatory, and whitening cosmeceutical ingredients. *Chemistry & Biodiversity*, *18*(7), e2100245.

[10] Sharifi-Rad, M., Mnayer, D., Morais-Braga, M. F. B., Carneiro, J. N. P., Bezerra, C. F., Coutinho, H. D. M., ... & Sharifi-Rad, J. (2018). Echinacea plants as antioxidant and antibacterial agents: From traditional medicine to biotechnological applications. *Phytotherapy Research*, *32*(9), 1653-1663.

[11] Weng J.-R., Bai L.-Y., Lin W.-Y., Chiu C.-F., Chen Y.-C., Chao S. and Feng C. (2017). A Flavone Constituent from Myoporum bontioides Induces M-Phase Cell Cycle Arrest of MCF-7 Breast Cancer Cells, Molecules, 22 (472): 1–13.
[12] Chen, P., Yang, Z., Mai, Z., Huang, Z., Bian, Y., Wu, S., ... & Zhou, W. (2022). Electrospun nanofibrous membrane with antibacterial and antiviral properties decorated with Myoporum bontioides extract and silver-doped carbon nitride nanoparticles for medical masks application. *Separation and Purification Technology*, *298*, 121565.
[13] Badriyah, B., Achmadi, J., & Nuswantara, L.K. (2017). Kelarutan senyawa fenolik dan aktivitas antioksidan daun kelor (Moringaoleifera) di dalam rumen secara in vitro. *Jurnal Peternakan Indonesia (Indonesian Journal of Animal Science),* 19(3): 116-121.
[14] Bohm, B. A., & Stuessy, T. F. (2001). *Flavonoids of the sunflower family (Asteraceae)*. Springer Science & Business Media.
[15] Rolnik, A., & Olas, B. (2021). The plants of the Asteraceae family as agents in the protection of human health. *International journal of molecular sciences*, *22*(6), 3009.
[16] Putra, B., Azizah, R.N., & Nopriyanti, E. M. (2020). Efek imunomodulator ekstrak etanol herba krokot (Portulacaoleracea L.) terhadap tikus putih (Rattusnorvegicus) jantan dengan parameter delayed type hypersensitivity (DTH). *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal),* 6(1): 20–25.
[17] Catap, E. S., Kho, M. J. L., & Jimenez, M. R. R. (2018). In vivo nonspecific immunomodulatory and antispasmodic effects of common purslane (Portulaca oleracea Linn.) leaf extracts in ICR mice. *Journal of ethnopharmacology*, *215*, 191-198.
[18] Abbas Syed, Q. (2018). Effects of different ingredients on texture of ice cream. *Journal of Nutritional Health & Food Engineering*, *8*(6), 422–435.
[19] Marshall, R. T., Goff, H. D., Hartel, R. W., Marshall, R. T., Goff, H. D., & Hartel, R. W. (2003). Ice cream ingredients. *Ice cream*, 55-87.
[20] Yuda, P. E. S. K., Cahyaningsih, E., & Winariyanthi, N. P. Y. (2017). Skrining fitokimia dan analisis kromatografi lapis tipis ekstrak tanaman patikan kebo (Euphorbia hirta L.). *Jurnal Ilmiah Medicamento*, *3*(2).
[21] Alebiosu, C. O., & Yusuf, A. J. (2015). Phytochemical screening, thin-layer chromatographic studies and UV analysis of extracts of Citrullus lanatus. *J Pharm Chem Biol Sci*, *3*(2), 214-220.
[22] Sobolev, A.P., Brosio, E., Gianferri R., & Segre, A.L. (2005). Metabolic profile of lettuce leaves by high field NMR spectra. *Magnetic Resonance in Chemistry*, 43: 625–638.
[23] Salfo, O., Sidiki, T., Jules, Y., Aristide, T., Kadiatou, T., Marius, L., Felix, K., & Sylvin, O. (2018). Formulation and Evaluation of a Syrup Based on Balanites aegyptiaca L. Delile. *Journal of Pharmaceutical Research International*, 23(3): 1–9.
[24] Fiol, C., Prado, D., Romero, C., Laburu, N., Mora, M., & Iñaki Alava, J. (2017). Introduction of a new family of ice creams. *International Journal of Gastronomy and Food Science*, 7: 5-10.
[25] Agustin, C., Teknologi, D., Ternak, H., Peternakan, F., & Mada, U. G. (2021). Ice cream characteristic with cured egg yolk. *Jurnal Ilmu dan Teknologi Hasil Ternak*, 16(1): 11–20.
[26] Sugiyono. (2017). Buku Metode Penelitian. Bandung: Alfabeta.
[27] Sutomo, S., Kamali, D. N., Arnida, A., Normaidah, N., & Sriyono, A. (2020). Pharmacognostic Study and Antioxidant Activity of Mundar (Garcinia forbesii King.) leaves from Banua Botanical Gardens of South Kalimantan. *Borneo Journal of Pharmacy*, *3*(4), 209-215.
[28] Rusita, Y. D. (2016). Flavonoids content in extracts secang (Caesalpinia Sappan L.) maceration method infundation analysis and visible ultraviolet spectrophotometer. *International Journal of Medical Research & Health Sciences*, *5*(4), 176-181.
[29] Shobib, A., Kusumo, P., & Millah, N. (2022). Characterization Test of Binahong (Anredera Cordifolia (TEN.) STEENIS.) Leaves and Aloe Vera (Aloe Vera) Leaves Extracts using Infudation Method in Making Liquid for External Wound Healing. *Journal of Chemical Process and Material Technology*, *1*(1), 28-38.

[30] Setyaningrum, R. (2018). Skrining fitokimia dan analisis kromatografi lapis tipis dari ekstrak etanol daun bitangur (Calophyllum soulattri Burm. F.). *Jurnal Pendidikan Informatika dan Sains,* 7(1): 54-67.
[31] Marliana, S. D., Suryanti, V., & Suyono, S. (2005). The phytochemical screenings and thin layer chromatography analysis of chemical compounds in ethanol extract of labu siam fruit (Sechium edule Jacq. Swartz.). *Asian Journal of Natural Product Biochemistry*, *3*(1), 26-31.
[32] Sharma, M., Abid, R., & Sajgotra, M. (2017). Phytochemical screening and thin layer chromatography of Ficus carica leaves extract. *Pharmaceutical and Biosciences Journal*, 18-23.
[33] Tiwari, S., Nepal, S., Sigdel, S., Bhattarai, S., Rokaya, R. K., Pandey, J., ... & Bhandari, R. (2020). Phytochemical Screening, Antibacterial-Guided Fractionation, and Thin-Layer Chromatographic Pattern of the Extract Obtained from Diploknema butyracea. *Pharmacognosy Research*, *12*(4).
[34] Lucini, L., Rouphael, Y., Cardarelli, M., Canaguier, R., Kumar, P., & Colla, G. (2015). The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. *Scientia Horticulturae*, *182*, 124-133.
[35] Bhatta, S., Stevanovic, T., & Ratti, C. (2020). Freeze-drying of maple syrup: Efficient protocol formulation and evaluation of powder physicochemical properties. *Drying Technology*, *38*(9), 1138-1150.
[36] Clarke, C. (2015). *The science of ice cream*. Royal Society of Chemistry.
[37] Ulfah, S. M., Dorly., & Rahayu, S. (2016). Perkembangan buang dan uji viabilitas serbuk sari bunga lipstik aeschynanthus radicans var. "Monalisa" di Kebun Raya Bogor. *Botanic Gardens Bulletin*, 19(1): 21–32.
[38] Tambaru, E., Paembonan, S., Ura, R., & Tuwo, M. (2019). Analisis Anatomi dan Trikoma Tanaman Obat Dandang Gendis Clinacanthus nutans (Burm. f.) Lindau. *Jurnal Ilmu Alam dan Lingkungan*, 10(1): 35–41.
[39] Atalay, Z., Celep, F., Bara, F., & Doğan, M. (2016). Systematic significance of anatomy and trichome morphology in Lamium (Lamioideae; Lamiaceae). *Flora-Morphology, Distribution, Functional Ecology of Plants*, *225*, 60-75.
[40] Kurniawati, F., Zaenab, S., & Wahyuni, S. (2016). Analisis perbandingan bentuk jaringan pembuluh trakea pada preparat maserasi berbagai genus piper sebagai sumber belajar biologi. *JPBI (Jurnal Pendidikan Biologi Indonesia)*, 1(2): 148–157.
[41] Anu, O., Rampe, H. L., & Pelealu, J. J. (2017). Struktur Sel Epidermis dan Stomata Daun Beberapa Tumbuhan Suku Euphorbiaceae. *Jurnal MIPA*, *6*(1), 69.
[42] Ullah, F., Ayaz, A., Saqib, S., Parmar, G., Bahadur, S., & Zaman, W. (2021). Taxonomic implication of leaf epidermal anatomy of selected taxa of Scrophulariaceae from Pakistan. *Microscopy Research and Technique*, *84*(3), 521-530.
[43] Sabandar, A., Hiariej, A., & Sahertian, D. E. (2021). Struktur sel epidermis dan stomata aegiceras corniculatum d dan rhizophora apiculata pada muara sungai desa poka dan desa leahari. *Biosel: Biology Science and Education*, 10(1): 81.
[44] Ninilouw, J. P., Mukarlina., & Linda, R. (2015). Struktur Anatomi Akar, Batang, dan Daun Jabon Putih (Anthocephalus cadamba (Roxb.) Miq) yang Mengalami Cekaman Kekeringan dan Genangan. *Jurnal Protobiont,* 4(2): 113–120.