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LCMS ANALYSIS OF ROSELLA EXTRACTS (HIBISCUS SABDARIFFA LINN)

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ABSTRACT

Rosella *(Hibiscus sabdariffa Linn)* is a traditional medicinal plant with more than 300 species distributed in tropical and subtropical regions. The plant is grown for its fibre as a medicinal ingredient. Rosella has various benefits due to its diverse chemical compounds, such as alkaloids, anthocyanins, flavonoids, tannins, steroids, and sterols. This study discussed the potential of rosella as an anti-inflammatory, antioxidant, antibacterial, antihypertensive, antidiabetic, and anticholesterol. To observe the chemical compound content of rosella flower extract. Methods: LCMS (Liquid Chromatography-Mass Spectrometry) test to see the content of chemical compounds in rosella flower extract. LCMS test showed that rosella flower extract contains flavonoids, tannins, phenolic-quinic acid, phenolic, and alkaloid chemical compounds. Rosella flower extract contains chemical compounds such as flavonoids, tannins, phenolics, quinic acid, and alkaloids, which have antibacterial, antioxidant, anti-inflammatory properties, as well as diuretic, anticholesterol, antidiabetic, and antihypertensive effects. These compounds give rosella great potential as a natural ingredient with various pharmacological applications. Although the results showed that rosella has many pharmacological benefits, further research is still needed to understand the deeper mechanisms and potential clinical use of rosella for the treatment of various diseases, especially in dentistry.

INTRODUCTION

One of the plants used in traditional medicine in Indonesia is rosella (Hibiscus Sabdariffa Linn). Rosella (Hibiscus Sabdariffa Linn) has more than three hundred species spread in tropical and subtropical regions in the world. Rosella is grown or cultivated for its fibre for medicinal use (Khan, 2017). The plant is about 3.5 m tall and has dark green or red stems. The leaves are 7.5 - 12.5 cm in size, reddish-green coloured and have long petioles. The petals are red in colour and pointed around the base. The fruit is a velvety capsule, 1.25-2 cm long and has five valves containing 3-4 light brown seeds (Chaudhari & Chavan, 2022). All parts of the rosella plant have various benefits, especially the flower petals, which have been widely researched and studied both domestically and abroad. The great potential of the rosella plant as a herbal ingredient lies in the content of chemical compounds contained in it (Nurnasari & Khuluq, 2018).

Obouayeba et al. (2014) found the content of chemical compounds contained in rosella, namely alkaloids, anthocyanins, flavonoids, tannins, steroids and sterols (Obouayeba et al., 2014). Similar results were found by Lestari et al. (2022) that rosella extract contains alkaloid, flavonoid, tannin and saponin chemical compounds (G. A. D. Lestari, 2022). Some studies found rosella is useful as an anti-inflammatory, antioxidant, antibacterial, antihypertensive, antidiabetic and anticholesterol. Research conducted by Kusumastuti et al. (2018) on COX-2 expression and the number of inflammatory phase neutrophils in the wound healing process after systemic administration of rosella ethanolic extract in vivo study in wistar rats showed the results of rosella extract can inhibit COX-2 expression and reduce the number of inflammatory phase neutrophils in the wound healing process (Kusumastuti et al., 2014). Research conducted by Amriani (2021) through the antioxidant activity test of ethanol-water extract (1: 1) of Rosella flowers (Hibiscus sabdariffa L.) using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method showed the results of rosella flowers had very strong antioxidant activity with an IC50 value of 43µg/ml and IC50 Vitamin C 2,058 µg/ml (Amriani & Tuahatu, 2021). Research conducted by Ramadhani (2024) on flavonoids from rosella petal extracts against PBP2a as a basis for antibacterial activity against methicillin-resistant Staphylococcus aureus showed the results of kaempferol-3-rutinoside in the main flavonoid in rosella that has the potential as a PBP2a inhibitor, it is recommended to develop semisynthetically to produce drugs that are useful in overcoming resistant bacterial infections (Ramadhani et al., 2024).

Generally, the isolation of secondary metabolites from natural materials uses extraction, which is the process of separating chemical components from a mixture using a particular solvent. The extraction aims to extract the chemical components or secondary metabolites present in the sample. Factors that influence the extraction include extraction method, solvent type, particle size, and extraction duration. The basic principle of extraction is to separate the components contained in the material with the help of a particular solvent. Maceration is a simple extracting technique in which the simplisia powder is immersed in a solvent. This method was chosen because it can prevent damage to compounds that are sensitive to heat. The maceration method has the advantage of simple procedures and equipment. The working principle of maceration is based on the ability of the solvent to penetrate the cell wall and enter the cell cavity containing active components so that the active substances can dissolve in the solvent (Asworo & Widwiastuti, 2023).

Attempts to obtain more accurate data regarding the content of chemical compounds in rosella used the LCMS (Liquid Chromatography Mass Spectrometry) technique. LCMS combines liquid chromatography analysis techniques with mass spectrometry detection analysis. The principle of LCMS operates by separating sample components according to their polarity, followed by the detection of charged ions using a mass spectrometer. The result of the LC-MS data analysis test is to determine the molecular weight of the compounds contained in the extract, so that we can know the number of compounds in each sample. LC-MS data can provide information about the molecular weight, structure, identity, and quantity of certain components in the sample. Compounds are separated based on their relative interaction with the chemical layer of the particles (stationary phase) and solvent elution through the column (mobile phase). The eluted component from the chromatography column is then passed to the mass spectrometer through a special interface. The principle is to separate the analytes based on their polarity. The device consists of a column (stationary phase) and a specific solution as the mobile phase. High pressure is used to push the mobile phase. The mixture of analytes will separate based on their polarity, and their speed to reach the detector (retention time) will be different. This will be observed in the spectrum where the peaks are separated. With the help of a pump, the liquid mobile phase flows through the column to the detector. The sample is introduced into the mobile phase flow by injection. Separation of the mixture

components occurs in the column due to differences in the strength of interaction between the solution and the stationary phase. The solution that interacts less strongly with the stationary phase will come out of the column first. On the other hand, if a solution interacts more strongly with the stationary phase, it will take longer to leave the column, be detected by the detector, and recorded in the form of a chromatogram. The LCMS test has the advantage of analyzing a wider range of components, such as thermally labile compounds, high molecular mass or high polarity, and proteins (Mangurana et al., 2019).

Based on this background, this study aimed to determine the content of chemical compounds in rosella extract (Hibiscus sabdariffa Linn) using the maceration method through LCMS analysis. This research is expected to provide a clearer picture of the chemical compounds contained in rosella extract, as well as a reference for further studies in developing the potential of rosella as a natural ingredient for pharmacological applications.

METHOD

The design used in this research was laboratory experimental. The research stage started with the extraction of rosella flowers (Hibiscus sabdariffa Linn), then continued with the second stage, namely qualitative descriptive analysis of secondary metabolites in rosella flower extract using LC-MS test.

The population in this study were rosella flower petals (Hibiscus sabdariffa Linn) in the territory of Indonesia. The sample of this study was rosella flower extract (Hibiscus sabdariffa Linn).

The rosella plant (Hibiscus sabdariffa Linn) has a single leaf that is ovoid, rayed, blunt tip, serrated edges, and a notched base, a leaf length of 6-15 cm, and a width of 5-8 cm. The rounded petiole is green in color with a length of 4-7 cm. Rosella flowers (Hibiscus sabdariffa Linn) have brightly colored flowers, the petals of rosella flowers are dark red, bell-shaped, and do not fall.

The number of samples used for this research is dried rosella flowers as much as 2 kg, which will be extracted and produce a thick extract of 300.1 grams. The tool used in this research is an LCMS instrument, and the material used in this research was rosella flower extract.

Rosella flowers were baked at 50°C for 24 hours until they were marked as easily crushed when squeezed (moisture content ±8 percent). The dried rosella flowers were then crushed with a blender until smooth and then sieved using a 60-mesh sieve. Rosella flower extract (Hibiscus sabdariffa Linn) was made by maceration method by weighing 2 kg of rosella flower powder and put into an erlenmeyer flask then added 96% ethanol solvent and the ratio of powder to ethanol was 1:4, then macerated for 2 hours at 60°C. The solution was then filtered using a large cloth, the rosella extract was filtered using Whatman paper no. 1 and concentrated using a rotary evaporator at a temperature of 40-50oC with a pressure of 10 mBar so that a thick extract of rosella petals of 300.1gram was obtained. The termination of the evaporation process was determined by the dripping of the solvent.

The LCMS test is an analytical test that combines the physical separation capabilities of LC (Liquid Chromatography) with the mass analysis capabilities (Mass Spectometry). LC-MS data can be used to provide information on the molecular weight, structure, identity and quantity of specific sample components. Compounds are separated based on relative interactions with a chemical layer of particles (stationary phase) and elution of solvent through the column (mobile phase).

The LC-MS/MS conditions in this study are:

Detector	:	UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific
Column	:	Accucore C18, 100 x 2.1 mm, 1.5 µm (Thermo Scientific)
Eluent	:	A: H2O + 0.1% asam format B: asetronitril + 0.1% asam format

Gradient	:	0-1 minutes (5%B), 1-25 minutes (5-95%B), 25-28 minutes (95%B), 28-33 minutes (5%B)
Injection volume	:	2 µL
Column temperature	:	30 °C
Flow rate	:	0,2 mL/min
Mass Range	:	100 – 1500 m/z
Ionization Mode	:	negative

The column used in this study was Accucore C18, 100 x 2.1 mm, 1.5 µm (Thermo Scientific), and a ThermoScientific Vanquish Tandem Q Exactive Plus Orbitrap HRMS UHPLC mass spectometer detector. The pump used was a non-fixed flow mode or gradient elution to obtain the optimum mobile phase composition during analysis. The mobile phase composition was set automatically with a gradient of 5%-95% acetonitrile solution with timing. Meanwhile, the column temperature was set at room temperature of 30 C.

In the beginning, the LC instrument was prepared by purging the LC column to remove the remnants of eluent that were still on the column. After purging, it was continued by pumping the eluent or mobile phase for approximately 5 minutes and equilibrating for 5 minutes. This aimed to stabilize the column so that the LC system is ready to be used for analysis.

LCMS (Liquid Chromatography-Mass Spectrometry) works by separating the components of analytes in a mixture through a liquid chromatography process. The eluted components from the chromatography column are passed to the mass spectrometer via a special interface. The basic principle of the system is the separation of analytes based on their polarity, where the column acts as the stationary phase and a specific solution serves as the mobile phase, which is driven under high pressure. The mixture of analytes injected into the column will separate based on the difference in polarity and the speed of the analytes in reaching the detector, which is reflected in the difference in the retention time of each component. This will be seen in the chromatogram spectrum, where the peaks are separated according to the analyte exit time. The pump moves the liquid mobile phase through the column to the detector, and separation occurs within the column due to differences in the strength of interaction between the analyte and the stationary phase. Solutions that interact less strongly with the stationary phase will exit the column faster, while solutions that interact more strongly with the stationary phase will exit more slowly. All separated components are then detected by the detector and the results are recorded in the form of a chromatogram, which gives an idea of the composition of the analytes in the sample.

RESULT AND DISCUSSION

RESULT

The LCMS test aims to strengthen the suspicion of the presence or absence of chemical compounds. Sample preparation is done by dissolving 10 mg of sample in 1 mL MeOH, then filtered with PTFE membrane 0.2 µm, and then injected into the LCMS instrument using a micro syringe. The first result obtained is a chromatogram. The chromatogram is obtained after the sample enters the column and the process of separating the chemical compounds contained in the isolate occurs until the compounds pass through the detector. The stationary phase or column used in this instrument is Accucore C18, 100 x 2.1 mm, 1.5 µm (Thermo Scientific), while the mobile phase/eluent used in this instrument is a combination of eluent A (water: 0.1% formic acid) and eluent B (acetonitrile: 0.1% formic acid). Chromatography in this study uses a reversed-phase system, namely a stationary phase that is non-polar and a mobile phase that is polar so that the compounds that appear in the chromatogram at the beginning of the retention time are polar compounds and the longer the retention time, the compounds that appear will be increasinglynon-polar.

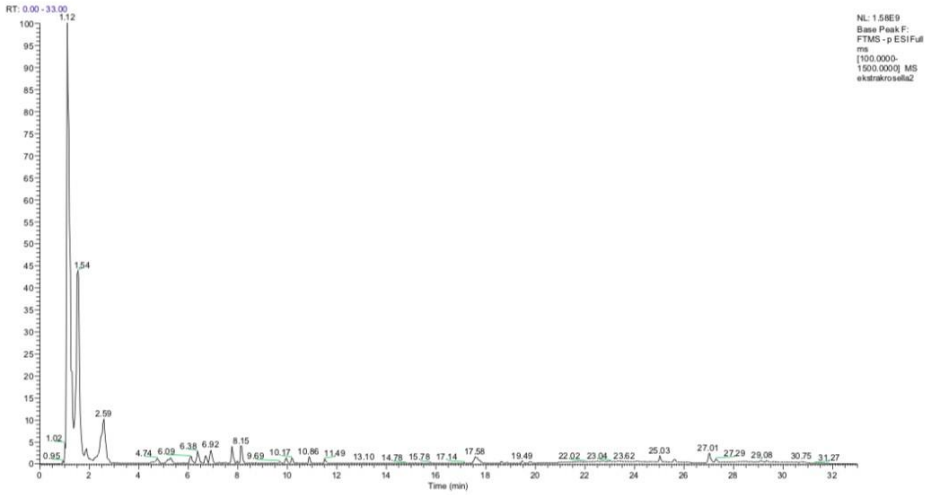

Figure 1. Chromatogram Results of Rosella Extract

The results of the rosella extract chromatogram can be seen in Figure 1. Samples that have been separated in UPLC will enter the MS system by being ionized first using the negative ESI method. Furthermore, the molecules that have been ionized will be selected and separated using a Quadrupole and time-of-flight type mass analyzer. The results of the sample separation will be displayed in the form of spectra on each detected peak. Identification results contain chemical compounds including flavonoids and tannins. The retention time in the mass spectra results shows the polarity of a compound, the stationary phase on the LC column is nonpolar while the mobile phase is polar. The larger the molecular size of a compound and is a long chain, it has more nonpolar properties. LCMS results can be seen in Table 1.

Table 1. LCMS Screening Test Results

NO	Compound	Classification of Compounds	Retention Time (Minutes)	Molecular Formula	Mass (m/z)	
					Target Mass	Calculated Mass
1.	Rutin	Flavonoid	8,45	$C_{27}H_{30}O_{16}$	609.14801	610.1534
2.	Quercetin	Flavonoid	11,51	$C_{15}H_{10}O_7$	301.03595	302.0427
3.	Quercetin-3β-D-glucoside	Flavonoid	8,75	$C_{21}H_{20}O_{12}$	463.08957	464.0955
4.	Fraxetin	Flavonoid	7,51	$C_{10}H_8O_5$	207.02997	208.0372
5.	Isoferulic Acid	Flavonoid				
6.	1,5-Anhydro-2-O-(3,4,5-trihydroxybenzoyl) -D-glucitol	Tannin	2,87	$C_{13}H_{16}O_9$	315.07306	316.0794
7.	Caffeic acid	Tannin	6,71	$C_9H_8O_4$	179.03470	180.0423
8.	3-O-feruloyl-D-quinic acid	Phenolic-Quinic acid	7,78	$C_{17}H_{20}O_9$	367.10406	368.1107
9.	Chlorogenic acid	Phenolic-Quinic acid	6,39	$C_{16}H_{18}O_9$	353.08859	354.0951
10.	Neochlorogenic acid	Phenolic-Quinic acid	4,76	$C_{16}H_{18}O_9$	353.08875	354.0951
11.	2,2-Methylenebis(4-methyl-6-tert butylphenol)	Phenolic	25,03	$C_{23}H_{32}O_2$	339.23334	340.2402
12.	Salicylic acid	Phenolic	9,95	$C_7H_6O_3$	137.02377	138.0317
13.	2,5Dihydroxybenzaldehyde	Phenolic	5,38	$C_7H_6O_3$	137.02393	138.0317
14.	Gentisic acid	Phenolic	5,80	$C_7H_6O_4$	153.01900	154.0266
15.	2-(E)-O-feruloyl-D-	Phenolic	9,37	$C_{16}H_{18}O_{11}$	385.07867	386.0849

	galactaric acid					
16.	Kresoxim-methyl	Alkaloid	10,89	$C_{18}H_{19}NO_4$	312.12485	313.1314
17.	Furagin	Alkaloid	1,17	$C_{10}H_8N_4O_5$	263.04120	264.0495
18.	5'-Oxoinosine	Alkaloid	1,14	$C_{10}H_{10}N_4O_6$	281.05170	282.0600

Based on Table 1, LCMS test found that rosella flower extract contained flavonoids, tannins, phenolic quinic acids, phenolics, and alkaloids. The chromatograms obtained are then processed using the compound discoverer program so that the molecules and mass of each compound can be known and predicted. One chromatogram peak indicates one compound. Prediction of the molecular formula of the spectra can be seen based on the target mass, calculated mass and measured mass values. The measured mass is the mass found from the identified compound, while the calculated mass is the exact mass of a formula. The value of the calculated mass minus the mass of 1 H atom is 1.0078 because, during separation using a column, there is a reduction in H atoms derived from the firing of ESI (-) ions.

DISCUSSION

Rosella (Hibiscus sabdariffa L.) is a plant that has more than 100 varieties spread across different regions of the world. The plant is a natural source rich in flavonoids and belongs to the Malvaceae family. The origin of rosella comes from Asia, especially India to Malaysia as well as the Tropical Africa region. Since the 17th century, the plant began to be recognized in Asia, and 1920, rosella began to be widely cultivated in Indonesia. Nowadays, rosella is widely grown in tropical and subtropical regions, including Indonesia, India, China, the Philippines, Saudi Arabia, Vietnam, Mexico, and Malaysia (Nurnasari & Khuluq, 2018).

Rosella can grow up to a height of about 2.4 meters. The stem is red in color, cylindrical in shape, and has a smooth surface. Roselle leaves vary in size, ranging from 7.5 cm to 12.5 cm in length, and are green in color. Roselle flowers appear in the leaf axils, about 12.5 cm in diameter, yellow or yellowish which then turn pink as they wilt. After the flowers wilt, red-colored petals with five large strands grow and cover the seed-containing capsules. Rosella grows well in areas with tropical and subtropical climates and takes between 4 to 8 months to develop optimally. The plant requires about 13 hours of sunlight exposure day and nighttime temperatures of at least 20°C to support its growth. The quality of rosella is greatly influenced by the type of seed, environmental conditions during growth, harvesting time as well as post-harvest management and drying process (Da-Costa-Rocha et al., 2014).

Rosella is known to have various biological benefits that are important for human health. Many of rosella's pharmacological effects are believed to be related to its anti-inflammatory properties. In addition, roselle flowers also have antibacterial activity that can kill various types of bacteria that cause oral infections. Roselle extracts contain various bioactive compounds with diverse pharmacological potential, including antibacterial, antioxidant, nephroprotective, hepatoprotective, as well as diuretic, anticholesterol, antidiabetic, and antihypertensive effects. One of the main compounds contained in roselle is flavonoids (Da-Costa-Rocha et al., 2014; Ghani et al., 2018). Rosella is believed to have the ability to help cure various diseases, such as hypertension and diabetes, and has a diuretic effect. Roselle petals contain active compounds such as hibiscus glucose, gosipetin, and anthocyanins. Anthocyanins, which give the petals their red color, are part of a group of flavonoids believed to have therapeutic potential in treating degenerative diseases. Rosella also contains other important compounds, such as catechins, vitamins C, B1, B2, carotenoids, organic acids, saponins, and alkaloids. These compounds play a role in damaging bacterial cell proteins, which then cause bacterial cell death. Therefore, rosella can serve as an effective antibacterial agent. Based on its antibacterial content, rosella can kill various types of pathogenic bacteria, such as Pseudomonas aeruginosa, Klebsiella

pneumoniae, Staphylococcus aureus, and Escherichia coli (Aritonang et al., 2021).

Rosella extraction was carried out by maceration method using 96% ethanol solvent (Kusparmanto et al., 2024). In accordance with the research of Badaring et al., (2020) the maceration process is a simple extraction technique without heating (cold extraction) in this process the sample and solvent do not go through a heating process so that they can be used on compounds that are not heat resistant.(Badaring et al., 2020) Research by Ismaningdyah et al. (2016) showed that the best type of solvent detected to have the highest extract concentration was 96% ethanol (Kurniawati et al., 2016).

Liquid Chromatograph Mass Spectrometry (LC-MS) is an analytical technique that combines the physical separation capabilities of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography separates the components of the sample and then charged ions are detected by a mass spectrometer. The results of analysis using LC-MS showed that rosella extract contained flavonoid compounds consisting of rutin, quercetin, and quercetin-3β-D- Glucoside. Tannin compounds consist of 1,5-Anhydro-2-O-(3,4,5-trihydroxybenzoyl)- D-glucitol and caffeic acid. Phenolic-quinic acid compounds consist of 3-O-feruloyl-D-quinic acid, Chlorogenic acid, and Neochlorogenic acid. Phenolic compounds consisting of 2,2-Methylenebis(4-methyl-6-tert butylphenol), Salicylic acid, 2,5- Dihydroxybenzaldehyde, Gentisic acid, and 2-(E)-O-feruloyl-D-galactaric acid. Alkaloid compounds consisting of Kresoxim-methyl, Furagin, and 5'-Oxoinosine.

Flavonoid compounds in rosella extracts, such as rutin and quercetin exhibit various beneficial biological activities such as antioxidant, anti-inflammatory, analgesic, and antimicrobial (Fitriaturosidah et al., 2022). Research by Majeed et al., (2018) on the application of flavonoid extracts from rosella for defects in rat feet showed that flavonoids have potential activity in the process of bone defects by suppressing osteoclast activity and increasing osteoblast formation (Majeed & Ghani, 2018) Raut et al. also mentioned that flavonoids have anti-inflammatory properties to inhibit bone loss (Nicolin et al., 2019).

Rutin, one of the flavonoid compounds identified in rosella extract has PubChem CID 5280805, molecular formula C27H30O16, and molecular weight 610.521 g/mol, also has excellent anti-inflammatory and antioxidant properties. eremia et al., (2020) showed that rutin compounds have anti-inflammatory properties tested on rats in vitro and also have antioxidant abilities that can protect bone injury in rats (Arowoogun et al., 2021).

In addition to rutin, the compound Quersetin was also identified in rosella extract and is known as the largest flavonoid in the flavonol group consisting of quercetin and its glycosides which account for about 60-75% of the flavonoids present (Ikrawan & Muntaha, 2020). Quercetin has PubChem CID 5280343, molecular formula C15H10O7, and molecular weight 302.23 g/mol. Research by Sok Wong et al., (2020) showed that quercetin compounds significantly increased new bone formation and reported that quercetin compounds are osteogenic which has enormous potential to be utilized in any application that requires increased bone formation (Arowoogun et al., 2021). Another study by Zhang et al., (2017) in Sok Wong et al., (2020) determined the protective effect of quercetin against titanium-particle-induced calvarial osteolysis using male rats. Quercetin at 50 or 100 mg/kg per day can inhibit titanium particle-induced osteolysis by increasing bone area (osteoblasts) and decreasing osteoclast formation (Arowoogun et al., 2021).

Fraxetin is a coumarin-derived chemical compound also present in rosella extract, purified from the traditional medicinal plant Fraxinus rhynchophylla, serving as an important ingredient in many herbal supplements. Many studies have confirmed that this compound has various pharmacological benefits, including its neuroprotective properties as an anti-inflammatory. Several studies have proven the anti-inflammatory properties of fraxetin in many inflammatory diseases, such as chronic pancreatitis, hepatocellular carcinoma, and osteoarthritis (Deng et al.,

2022). Fraxetin has antitumor, antibacterial, antioxidant, and anti-inflammatory effects. Xiao et al. (2018) determined the possible protective effect of fraxetin against ethanol-induced liver fibrosis in rats. The data obtained showed that fraxetin attenuated liver fibrosis through its antioxidant and anti-inflammatory properties (Chen et al., 2018).

Ferulic acid, which was also detected in rosella extract, belongs to the group of phenolic acids commonly found in plant tissues. Ferulic acid is a secondary metabolite of various chemical structures and biological properties. One of the most important roles of phenolic acids, especially cinnamic acid derivatives, is their antioxidant activity, which mainly depends on the number of hydroxyl and methoxy groups attached to the phenyl ring (Oresajo, 2020).

Ferulic acid is more easily absorbed into the body and remains in the blood longer than other phenolic acids. Ferulic acid is considered a superior antioxidant. Ferulic acid has low toxicity and has many physiological functions, including anti-inflammatory, antimicrobial, and anticancer (Oresajo, 2020).

Isoquercetin (quercetin-3β-D-Glucoside) was also detected in rosella extract. Having PubChem CID 5280804, its molecular formula C21H20O12, and its molecular weight of 464.4 g/mol, it has antioxidant and anti-inflammatory properties. According to Choi et al., and De Araujo in Seyun Lee et al., (2019) quercetin and isoquercetin (quercetin-3β-D-Glucoside) have antioxidant, antiproliferative, and anti-inflammatory properties. Seyun Lee et al., (2019) showed that isoquercetin alleviated ethanol-induced hepatotoxicity, oxidative stress, and inflammatory responses via the Nrf2/ARE antioxidant signaling pathway (Lee et al., 2019).

Tannins, which are also found in rosella extract, are a group of compounds that can affect the bone remodeling process. Sukmana et al., (2017) stated that the extract of mango kasturi bark (Mangifera casturi) contains tannin compounds that can reduce IL-1β expression during bone remodeling and increase BMP-2 expression during bone remodeling where both of these are very influential in the process of bone density. IL-1β plays a role in cells that experience inflammation at the stage of bone resorption and BMP-2 induces the formation of osteoblasts (Sukmana et al., 2017).

Caffeic acid (CA) is one of the phenolic compounds in rosella extract that also shows a variety of potential biological activities. Caffeic acid is a hydroxycinnamic and phenylpropanoid metabolite that is commonly synthesized by all plant species.(Ekeuku et al., 2021) Caffeic Acid has PubChem CID 689043 and its chemical compound name is (E)-3-(3,4-dihydroxyphenyl) prop-2-enoic acid. Its molecular formula is $C_9H_8O_4$ and its molecular weight is 180.159 g/mol. CA and its main derivatives including caffeic acid phenethyl ester (CAPE) and caffeic acid 3,4-dihydroxy-phenethyl ester (CADPE) were reported by Espindola et al., Magnani et al., and Armutcu et al., in the study of Sophia et al., (2020) to have potential antibacterial, antidiabetic, antioxidant, anti-inflammatory, and cardioprotective activities.(Ekeuku et al., 2021) Sudina *et al*., Jung *et al*., Arasoglu *et al*., and Erdemli *et al*., in Henrique Silva (2020) also stated that caffeic acid compounds have antioxidant, anti-inflammatory, antibacterial, and antiviral properties (Silva & Lopes, 2020).

Phenolic compounds, including caffeic acid, are a group of compounds known to have very strong antioxidant activity. These compounds, found in rosella, can help boost the immune system and fight degenerative diseases. Several studies have shown that regular consumption of phenolic compounds can also help minimize the appearance of various deadly diseases and excessive oxygen reactivity which can lead to anticancer, anti-inflammatory, antidiabetic, antiallergic, and antiviral properties (L. Lestari et al., 2023).

Alkaloids found in rosella extracts, such as Kresoxim-methyl, Furagin, and 5'-Oxoinosine are secondary metabolite compounds having nitrogen atoms, which are found in plant tissues. Alkaloids have a variety of pharmacological benefits, including antibacterial and antifungal activities. Alkaloids play a role in metabolism and control development in

plant life systems. Most alkaloid compounds are sourced from plants. Alkaloids are compounds that have antifungal activity by inhibiting DNA esterase and RNA polymerase (Fatma et al., 2021; Maisarah et al., 2023). Dewi & Wuryandari (2019) in Mesy Maisarah (2023) explained that alkaloids as antifungals work by damaging cell membranes where alkaloids will bind strongly to ergosterol forming holes that cause leakage of cell membranes this will cause permanent damage to cells and cause death to fungal cells (Fatma et al., 2021; Maisarah et al., 2023).

Alkaloids have the ability as antibacterial by inhibiting enzymes that play a role in the replication process of bacterial DNA, so that it will cause bacteria to be unable to divide. Alkaloids contained in the extract of a plant will inhibit the wall layer of bacterial cells from forming intact and cause cell death by disrupting the cell components that make up the peptidoglycan of bacteria. Alkaloids also can help heal wounds because the active compounds of alkaloids have antioxidant activity (Hakim et al., 2023).

Neochlorogenic acid (NCA), which is also found in rosella extract, has anti-inflammatory and antitumor potential. Research conducted by Jianhuan Che et al., (2020) on the effects of NCA and Pingyangmisin (PYM) on oral squamous cell carcinoma (OSCC) cells. The results of this study showed that NCA treatment significantly enhanced the suppressive effect of PYM on OSCC cell proliferation and apoptosis and also showed that NCA could enhance the antitumor effect of PYM by regulating TOP2A. In addition, NCA can reduce the toxicity of PYM on normal oral epithelial cells. Thus, the use of NCA may enhance the therapeutic effect of PYM chemotherapy in patients with OSCC. This study also mentioned that NCA showed significant anti-inflammatory effects, antitumor, anticancer, and immune-enhancing effects (Che et al., 2021).

Salicylic acid (SA), also detected in rosella, is a phenolic compound that belongs to a group of phytochemicals that have beneficial effects on human health. Salicylic acid is a phenolic compound and can be found in plants that have an important role in defense against pathogenic agents. Salicylic acid is more popular as a major metabolite and anti-inflammatory drug that has been used in clinical practice for more than 100 year (Gąsowska-Bajger et al., 2023). Cronstein in Nuzul Wahyuning et al., (2020) stated that salicylic acid has anti-inflammatory potential. In line with what Mitchell in Nuzul Wahyuning et al. (2020) salicylic acid as the main metabolite of aspirin is known to provide analgesic and anti-inflammatory effects through mechanisms that are still unidentified (Diyah et al., 2020).

Gentisic acid (GA), another phenolic compound found in rosella extract has various health benefits including antioxidant, anticancer, and hepatoprotective properties. Research conducted by Abedi et al (2019) stated that this compound was found to have antioxidant, anticancer, hepatoprotective, antimicrobial, and anti-inflammatory properties. GA may act as an adjuvant to enhance the efficacy and potency of anticancer drugs and reduce the side effects of chemotherapy and radiotherapy, but more comprehensive research and evidence are needed to confirm the role of GA in cancer prevention or treatment (Abedi et al., 2020).

CONCLUSION

Rosella extract contains chemical compounds that have antioxidant, anti-inflammatory, antibacterial and antiviral activities. LCMS test on rosella flower extract contains flavonoid compounds consisting of: routine, quercetin, quercetin-3β-D-Glucoside, Fraxetin, Isoferulic Acid, tannin compounds consisting of 1,5-Anhydro-2-O-(3,4,5- trihydroxybenzoyl)- D-glucitol and caffeic acid, phenolic compounds- quinic acid consisting of 3-O-feruloyl-D-quinic acid, chlorogenic acid, neochlorogenic acid, phenolic compounds consisting of 2,2-Methylenebis (4-methyl-6-tert butylphenol), salicylic acid, 2,5Dihydroxybenzaldehyde, gentisic acid, 2-(E)-O-feruloyl-D-galactaric acid and alkaloids consisting of kresoxim-methyl, furagin, and 5'-Oxoinosine. Rosella has great potential as a natural ingredient with various pharmacological applications.

The discussion shows that the

chemical compounds of rosella extract have many pharmacological benefits, but further research is still needed to understand the deeper mechanisms and the potential clinical use of rosella in the treatment of various diseases, especially in dentistry.

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