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Phytochemical Screening and Antioxidant Activity in Dragon Fruit Plant Extracts as Immunomodulators in Pregnant Women

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ABSTRACT

Introduction: Dragon fruit contains many organic acids, proteins, and minerals such as potassium, magnesium, calcium, iron, and vitamin C. Several natural compounds can increase the activity of the immune system, namely flavonoids, curcumin, limonoids, vitamin C, vitamin E, and catechins. **Objective:** to analyze the levels of phytochemicals and active ingredients from the fruit, skin, stem, and roots of dragon fruit plants. **Methods**: This research was carried out experimentally, carried out at the Pucuk Sirih Jamu Factory. This study used fresh and ripe Dragon Fruit Plants directly taken from the Tanah Laut District garden (South Kalimantan), carried out extract preparation, phytochemical screening, and determination of phytochemical levels of red dragon fruit plants. **Results:** The study found the highest secondary metabolite levels in red dragon fruit for Flavonoids in the stem (0.74%), Alkaloids in the stem (4.21%), Saponins in fruit flesh (0.45%), and Steroids in roots (2.54%). Antioxidant activity in red dragon fruit flesh (78.23%), stem (79.13%), root (8.64%), and skin (11.24%). The highest antioxidant activity in red dragon fruit stems. **Conclusions:** There are groups of secondary metabolites and antioxidants contained in dragon fruit plants (fruit, skin, stems, and roots) from Kalimantan Selatan (Indonesia).

Key words: Phytochemical Screening, Antioxidant, Dragon fruit plant, Immunomodulator, Pregnant Women.

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INTRODUCTION

Dragon fruit is a plant that contains substances that can increase endurance and launch metabolism.[1] Dragon fruit is an important source of phytochemicals such as polyphenols, flavonoids, and vitamin C, which are related to its antioxidant activity, the red and white dragon fruits especially have recently drawn growing attention worldwide not only because of their economic value but also for their health benefits.[2]

Dragon fruit (*Hylocereus* spp.) is a widely consumed tropical fruit that is considered healthy partly due to its high content of phenolic compounds.[3] The phenolic compounds in pulp possess antioxidant activity and have a range of potential health benefits.[4] The global market value of dragon fruit reached 4.9 billion US dollars worldwide in 2016.[5]

Ingredients of natural origin to be used as supplements must be in the dosage form. Natural materials generally have less stable properties in the open air. This allows natural ingredients to be formulated in suitable preparations. Natural ingredients that are formulated in dosage forms will increase the stability of the active ingredients. The purpose of this study was to determine the levels of phytochemicals and secondary metabolites of the Fruit, Stem, and Root of the Dragon Fruit Plant (*Hylocereus polyrhizus*). Dragon fruit flesh has a higher total phenolic content and antioxidant capacity than the skin, while dragon fruit skin contains higher flavonoids and tannins than the flesh. The results showed that dragon flesh planted in Australia has a total phenolic content and antioxidant capacity that is stronger than the skin, while the skin of the dragon fruit contains a higher flamed and tannin content compared to the flesh of the fruit.[6]

Hylocereus polyrhizus is rich in nutrients, and minerals, rich in fito-albumin and betalain, which is highly valued for its antioxidant nature. Betalains also function as a natural food coloring that makes them useful in the food industry.[7] Several studies have shown that fresh fruits and vegetables have excellent antioxidant content and can provide protection against chronic diseases caused by oxidative stress, such as cardiovascular disorders and several types of cancer, phenolic compounds, and flavonoids have been reported to show antioxidant properties.[8,9] The study showed that the extract of the H. *Polyrhizus* rod had excellent Radical Radical and ABTS antidote activities. Stem extract, skin, and flower in watery ethanol 95% show the effect of protection of excellent DNA damage as well. Stem and flower extract 1000 g/ml in watery ethanol 95% increases the nature of cell migration. These results indicate that the stem, leather, and flower H. Polyrhizus is a source of antioxidant polyphenols and has the potential for application in the pharmaceutical, cosmetics, and food industries.[10]

The bioactive phytochemicals betalains, flavonoids, polyphenols, terpenoids, steroids, saponins, alkaloids, and tannins are abundant in dragon fruit. Numerous researches have looked into the antioxidant capacity of dragon fruit and discovered that it has high antioxidant capacity.[11] The presence of phenols and tannins is what gives foods their antioxidant activity.[12] The dragon fruit's phytochemical composition and antioxidant activity fluctuate depending on which section of the fruit you look at. For instance, the red dragon fruit peel has more antioxidants than the white dragon fruit pulp.[13] There are several phytochemicals, including flavonoids, polyphenols, and betalains, which have antioxidative and anti-inflammatory activities,

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present in red dragon fruit, also known as *Hylocereus polyrhizus*.[13-15] The following are some salient results from the search: The red dragon fruit's peel contains the anthocyanin chemicals malvidin, cyanidin, and delphinidin. Using a modified colorimetric test, the total flavonoid contents (TFC) of red dragon fruit extracts were examined. This revealed that red dragon fruit had a high TFC.[13]

Phenol and flavonoid chemical components have been discovered to be present in red dragon fruit peel extract.[14] Red dragon fruit peel phytochemical analysis has revealed the presence of a number of bioactive substances, including flavonoids, polyphenols, and betalains.[15] Flavonoids, a class of antioxidants that can help shield cells from harm brought on by free radicals, are abundant in red dragon fruit. The following are some salient results from the search: Several studies have tested the red dragon fruit extract's overall flavonoid content. According to one study, red dragon fruit extract has a total flavonoid concentration of 0.75 mg EQ/100 g fw.[16] According to a another study, the red dragon fruit powder's total flavonoid content was 210.02 mg/100 g.[17] Red dragon fruit's antioxidant activity is significantly associated with its flavonoid level.[18] Red dragon fruit contains various types of flavonoids, including quercetin, kaempferol, and myricetin.[6] Dragon fruit pulp, rind, seeds, flower buds, and dried flowers all contain high levels of antioxidants, fiber, vitamin C, and minerals. Dragon fruit has many health benefits as anti-inflammatory, antioxidant, anti-diabetic, anti-cancer, and cardioprotective. The significant commercial value of dragon fruit is a result of its nutritional and therapeutic properties.[19] Dragon fruit stems have been found to contain flavonoids, saponins, and steroids.[20] Dragon fruit contains several phytochemicals, including alkaloids, which are present in the seeds and peel of the fruit. The seed of dragon fruit contains catechin, epicatechin, rutin, quercetin, and myricetin. The peel of the red dragon fruit (*Hylocereus polyrhizus*) contains anthocyanins such as malvidin, cyanidin, and delphinidin. Dragon fruit also contains other bioactive compounds such as saponins, terpenoids, flavonoids, tianine, niacin, pyridoxine, kobalamin, phenolic, carotene, and betalains. Alkaloids are known to have various biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties. However, the specific alkaloids present in dragon fruit and their effects on human health are not well understood and require further research.[21]

Research examining the phytochemical and antioxidant content of dragon fruit is essential to understand its health benefits, nutritional value, commercial value, and potential uses by utilizing the locally available and affordable fruit for prevention and alternative medicine as a candidate for Immunomodulator in Pregnant Women.

MATERIAL AND METHODS

Design The study used in this study was experimental (experimental), The research site was at the Pucuk Sirih Herbal Plant. The sample in this study was Dragon Fruit Plants (fruit, bark, stems, and roots) with the criteria of local Dragon Fruit Plants originating from Tanah Laut Regency. (South Kalimantan). Indonesia, Ripe dragon fruit and fresh dragon fruit plant is directly taken from the garden

The tools and materials used are Glassware, Maseri Vessel, Rotary Evaporator, Waterbath, Grinder, UV-Vis Spectrophotometer, and Atomic Absorption Spectrocopic (AAS). Dragon Fruit Plant from Pelaihari, Technical Ethanol, Whatman filter paper no.10, Ethanol pro analysis, Citric acid grade pro analysis, Vitamin c grade pro analysis standard, Aqua sterile, DPPH, Tris Buffer Saline (TBS) solution, Bovine Serum Albumin pro analysis, Pure diclofenac standard

Dragon fruit extract-making procedure

The parts of each plant are separated and then washed thoroughly using running water, then drained. The samples were then cut into smaller sizes, then dried in an oven at 60°C for 3 days. The dried samples were then mashed using a grinder until a powder was obtained.

The dry sample powder was then weighed as much as 1 kg, then each was immersed in 10 liters of ethanol for 3 days. The solvent was changed every 24 hours. The extract solution was filtered using filter paper and a hydraulic press to separate the active ingredients from the pulp. The extract solution was then evaporated using a rotary evaporator to become 1/10 part. Then it was evaporated again using a water bath until a thick extract was obtained.

Process for phytochemical screening

Drying was carried out at a temperature of 60°C which is the maximum temperature for drying Simplicia, for three days to reduce the water content so that the fungus could not grow Then dry sorting is carried out to separate dirt, foreign organic matter and Simplicia which were damaged by the previous process. The last process is the pollination of dry Simplicia to obtain powder for extraction.[22] The extraction results will obtain an extract solution which is then evaporated to remove organic solvents. The solvent evaporation process uses a rotary evaporator and an oven. The extract obtained was then collected to obtain a dry extract consisting of active compounds.

Phytochemical assay procedure

Determination of Total Flavonoid Content with Samples weighed and put into a beaker, extracted repeatedly with 100 ml of methanol at room temperature. Then the entire solution was filtered using Whattman filter paper No. 41. After that, the filtrate was dried by evaporation over a water bath and weighed to obtain a constant weight. The percentage of flavonoids was then calculated.[23]

Determination of Total Alkaloid Concentration A total of 5.0 g of sample was weighed into a beaker and added 200 ml of 10% acetic acid solution was. The mixed solution was covered and allowed to stand for 4 hours. Then filtered to separate the leaf powder and the solution, then evaporated with a water bath up to a quarter of the initial volume. The solution is dripped with concentrated ammonium hydroxide (NaOH) to form a precipitate and allowed to stand until more precipitate is formed. Then the precipitate was filtered and washed with NaOH, then dried and weighed with the filter paper already weighed. The percentage of alkaloids was calculated by the percentage of weight per volume. The assay used in this study was the gravimetric method.[22,24]

Determination of Total Saponin Content with the sample weighed about 1.25 g then refluxed with 50 mL petroleum ether at a temperature of 60-80˚C for 30 minutes. After cooling the petroleum ether solution was removed and the residue left was dissolved in 50 mL of ethyl acetate. The solution was transferred to a separatory funnel to separate the ethyl acetate solution. The residue left was dissolved with n-butanol 3 times. Then the butanolic solution is mixed and evaporated using a rotary evaporator. The remaining evaporation was dissolved with methanol then the solution was dropped into diethyl ether while stirring. The precipitate formed in the mixture is poured on filter paper whose weight is known. The precipitate was weighed at a fixed weight. The difference in the weight of the filter paper before and after filtering was determined as the weight of the saponins. The assay used in this study was the gravimetric method.[25]

Determination of Total Terpenoid Content with About 2g of powder was weighed and added 50 ml of 95% ethanol in a beaker, and allowed to stand for 24 hours. Then filtered and the filtrate was mixed with petroleum ether, then dried at a temperature of 60-80 C. The dry extract of the sample is a total terpenoid.[23]

Determination of total steroid levels in this study using a spectroscopic method.[26,27]

RESULTS AND DISCUSSION

Phytochemical Test results dragon fruit plant extract

Results of Phytochemical Screening on Fruit Extract, Skin and Stem of Local Dragon Fruit (Hylocereus polyrhizus) from Tanah Laut Regency. Phytochemical screening is a preliminary stage carried out to provide an initial description of the class of compounds contained in the plant under study (Table 1). The phytochemical screening method is carried out by a color change reaction or the formation of a precipitate with certain reagents.[28]

Based on table 1, it is known that the phytochemical test results of dragon fruit plant extracts on fruit, skin, stems, and roots of local dragon fruit plants (*Hylocereus polyrhizus*) from Tanah Laut Regency were proven to contain Flavonoids, Alkaloids, Saponins, and Steroids marked by changes in the color of the fruit plant extracts. dragon. Secondary metabolites can overcome various health problems. Secondary metabolites are active ingredients that are responsible for pharmacological activities.

The results of Table 2 show secondary metabolite levels in red dragon fruit plants for the highest flavonoid content in the stem (0.74%), for the highest Alkaloids content in the stem (4.21%), the highest Saponin content in dragon fruit flesh (0.45%, and the highest levels of steroids in fruit flesh (0.81%), the average phytochemical content was higher in the stems of red dragon fruit and the highest levels were dominated by alkaloids which generally have various pharmacological properties as antioxidants.

Table 3 shows that the antioxidant activity was measured using the DPPH method (2,2-diphenyl-1-picrylhydrazyl) by UV-Vis spectrophotometry. The results of the percentage of free radical inhibition from dragon fruit plants at a concentration of 100 ppm or 0.01% it is known that the flesh, stems, roots, and skin of red dragon fruit have inhibitory powers of 78.23%, and 79.13%, respectively. 8.64%, and 11.24%. This shows that the largest antioxidant activity in red dragon fruit stems is 79.13%.

In another study, screening on dragon fruit stems showed that the powder, extract, n-hexane fraction, and ethyl acetate fraction contained flavonoids, steroids, and saponins. While the water fraction contains flavonoid and saponin compounds.[29] The results of the phytochemical analysis showed that the red dragon fruit stem extract contained alkaloids, flavonoids, and steroids. Red dragon fruit stems or *Hylocereus polyrhizus* contain secondary metabolites that play an active role as antioxidants.[30] Betalains, flavonoids, polyphenols, terpenoids, steroids, saponins, alkaloids, tannins, and carotenoids are bioactive compounds that can be extracted from all parts of the fruit plant. dragon includes roots.[31] The results showed that dragon fruit pulp had a higher total phenolic content and stronger antioxidant capacity than peel, while the peel had a higher content of flavonoids and tannins than the pulp. The obtained results indicated that Australian dragon fruit peel by-products and pulp waste are potential sources of phenolic compounds, with potential as antioxidants for the food, cosmetic, pharmaceutical, and nutraceutical industries.[6]

The results of the identification of the content of red dragon fruit peel extract are known to contain flavonoids, tannins, alkaloids, and steroids which were analyzed by the FTIR method.[32] FTIR is used to determine the bonding group of a compound based on the wavelength and wave number of plants. In this study, the method used was slightly different from the research that had been carried out, but the results obtained were the same regarding the class of compounds contained in the skin of the red dragon fruit. Phytochemical screening was carried out as a preliminary test to identify the content of secondary metabolites contained in the red dragon fruit peel extract, followed by TLC to confirm the positive reaction results. The results of screening and TLC obtained positive red dragon fruit peels contain alkaloids, tannins, flavonoids, steroids, and the potential to be used as traditional medicine.[33]

The study showed that the qualitative identification results of phytochemical screening showed that the ethanolic extract of red dragon fruit peel contained tannins, flavonoids, alkaloids, and saponins. The results of the identification of flavonoid compounds using shear reagent and UV-Vis spectrophotometer showed that the ethanol extract of red dragon fruit peel was suspected to contain isoflavone compounds. The results of the identification of the ethanol extract of red dragon fruit peel using FTIR showed the presence of specific functional groups of flavonoid compounds such as OH, C-O alcohol, C=C aromatic, C-H aromatic, C-H aliphatic, C=O, and C-O ether. The phytochemical test of the methanol extract of red dragon fruit peel includes an examination of essential oils, alkaloids, steroids/triterpenoids, saponins, tannins, and flavonoids.[34] In another study, it was found that red dragon fruit flesh contains flavonoids, tannins, alkaloids, and steroids.[32] The phytochemical test results of red dragon fruit (*Hylocereus polyrhizu*) obtained alkaloids, steroids, flavonoids, terpenoids, and phenolic compounds. In thin layer chromatography, no spots were found on the plate. While in GC-MS, there are several structures similar to 5-eicosene, 1-nonadecene, 1,2-benzene dicarboxylic acid, n-henecosane, and gamma-sisterol. Chemical compounds similar to the structure above, have several benefits in the health sector, such as antibacterial, antioxidant, antifungal, and anti-inflammatory.[35]

Red dragon fruit is a rich source of nutrients and minerals such as vitamin B1, vitamin B2, vitamin B3, and vitamin C, protein, fat, carbohydrates, crude fiber, flavonoids, thiamin, niacin, pyridoxine, cobalamin, glucose, phenolic, betacyanin, polyphenols, carotene, phosphorus, iron, and phytoalbumin. It is also rich in phytoalbumin which is highly valued for its antioxidant properties. *Hylocereus polyrhizus* is rich in fiber, vitamin C, minerals, and phytoalbumin which are highly valued for their antioxidant properties. The advantages of red dragon fruit peel are that it is rich in polyphenols and anthocyanins, which are also compounds as a source of antioxidants. The antioxidant activity of the red dragon fruit peel is greater than the flesh.[36]

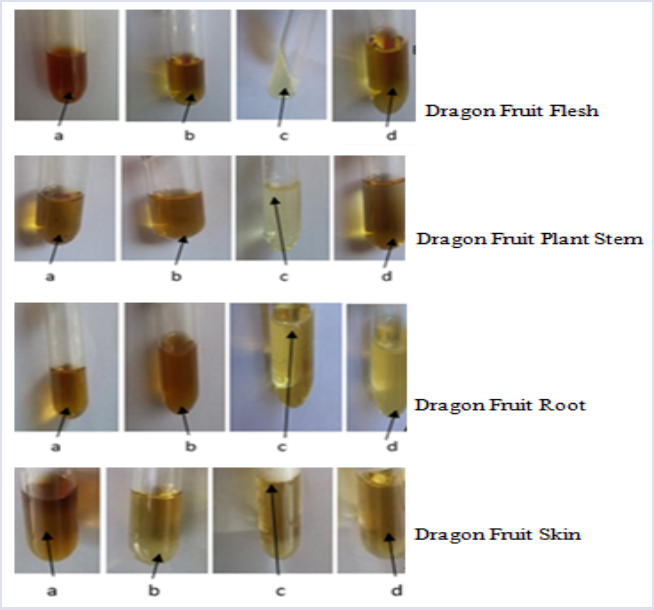

Figure 1: Phytochemical test results of red dragon fruit extract

Image Caption a. Contains Flavonoids, b. Contains Alkaloids, c. Contains Saponins, and d. Contains Steroids

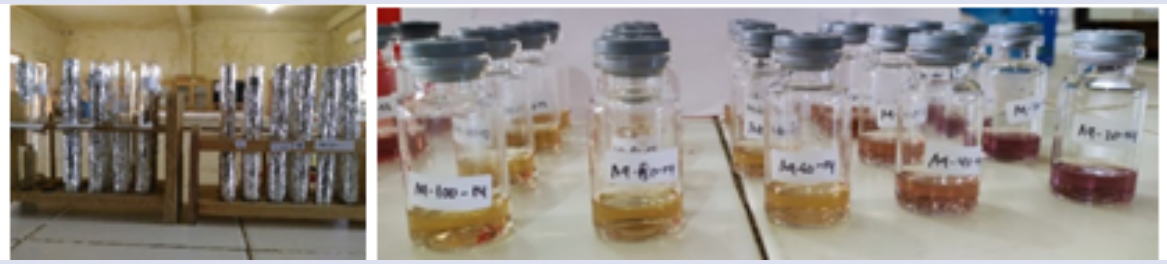

Figure 2: Shows the antioxidant test using the DPPH reagent method spectroscopically, the smaller the IC50 value, the greater the antioxidant ability of a sample.

Table 1: Phytochemical test results of red dragon fruit extract.

No.	Sample	Flavonoid		Alkaloid		Saponin		Steroid	
		Color	Result	Color	Result	Color	Result	Color	Result
1.	Flesh of fruit	Intense yellow	+	Reddish brown precipitate	+	Foam formed	+	Chocolate ring formed	+
2.	Stem	Intense yellow	+	Reddish brown precipitate	+	Foam formed	+	Chocolate ring formed	+
3.	Root	Intense yellow	+	Reddish brown precipitate	+	Foam formed	+	Chocolate ring formed	+
4.	Rind	Intense yellow	+	Reddish brown precipitate	+	Foam formed	+	Chocolate ring formed	+

Table 2: Results of phytochemical levels from dragon fruit plant extract.

No.	Sample	Flavonoid (%)	Alkaloid (%)	Saponin (%)	Steroid (%)
Red Dragon Fruit					
1.	Flesh of fruit	0,62	3,56	0,45	0,81
2.	stem	0,74	4,21	0,24	1,42
3.	Root	0,04	0,81	0,05	2,54
4.	Rind	0,73	2,32	0,13	0,31

Table 3: Results of antioxidant activity of dragon fruit extract.

No.	Sample	Percentage of Free Radical Inhibitory (100 ppm concentration)
1.	Flesh of fruit	78,23%
2.	stem	79,13%
3.	Root	8,64%
4.	Rind	11,24%

The results of the antioxidant test on all parts of the red and white dragon fruit samples showed that at a concentration of 100 ppm or 0.01%, they had varying antioxidant abilities. The strongest antioxidant activity in red dragon fruit stems from free radical inhibition of 79.13%. This shows the ability to inhibit 79.13 parts of 100 parts of free radicals in the tests carried out. This data is not much different from the dragon fruit flesh which has an inhibitory power of 78.23%. The flesh of the fruit has a lower antioxidant capacity than the stem because it is influenced by the low content of vitamin C and secondary metabolites in the flesh. In stems the highest content of flavonoid which is a substance that increases antioxidants, some research results say that the level of vitamin C in the stem is higher, the level of vitamin C is directly proportional to the level of antioxidants so that the higher the content of vitamin C, the higher the antioxidant activity.

In other dragon fruit plants parts such as fruit peels and roots, the free radical inhibition ability is not too high. This is due to the accumulation of vitamin C and the total flavonoids in this section are not too high. Vitamin C can inhibit free radicals due to the presence of a chromophore group in the compound. The chromo from the group can scavenge free radicals. This also applies to the flavonoid group which influences the antioxidant activity of a compound. In the flavonoid structure, it consists of an OH group that can capture free radicals, then can resonate in its structure, so that it can maintain a balance in the flavonoid structure even though it binds free radicals.[37]

The results of this study are also in line with Tsai *et al*'s research in 2019 where the extract of dragon fruit plant stems made with 95%watery ethanol, has the highest phenolic and flavonoid composition and the best DPPH radical antidote activities. The stem extract has excellent radical abts also. Stems, skin, and fruit made using 95%watery ethanol, showed excellent results in protecting themselves from DNA damage, similar to the effects of ferronic acid 0.3 mg/ml. None of the extracts can promote cell proliferation at a concentration of 250 g/ml to 2,000 g/ml in a period of 24 hours. 1000 g of cell migration rod extract is large enough after 24 hours.

The highest flavonoid composition (0.87%) was obtained from H. *Polyrhizus* stem extract in 95%and 50%ethanol solution. Batang extract in watery ethanol 95% has the highest DPPH radical capture activity (IC50 = 224 g/ml). Skin extract does not have good radical antidote activities. In the case of ABTS radical capture activities, stem extract in 50% watery ethanol has the highest ABTS radical capture activity (IC50 = 46 g/ml). Stem extract shows good potential in terms of free radical capture activities. Phenolic compounds and flavonoids are found in plants. This compound has antioxidant properties and the ability to ward off free radicals. Stem and fruit extracts have the highest protein composition and fruit has the highest sugar composition.[10]

The stem extract, which was prepared with 95% aqueous ethanol, had the highest composition of phenolics and flavonoids as well as the best DPPH radical scavenging activity. The stem extract had excellent ABTS radical scavenging activity as well. The stem, peel, and flower extracts, which were prepared using 95% aqueous ethanol, showed excellent results in protecting themselves from DNA damage, similar to the effect of 0.3 mg/mL ferulic acid. None of the extracts were able to promote cell proliferation at concentrations of 250 µg/mL to 2,000 µg/mL in a 24 h period. The 1000 µg/mL stem and flower extracts in 95% aqueous ethanol promoted considerable cell migration after a 24 h period.[10]

The 2017 Manihuruk study showed that the extract of red dragon fruit skin (*Hylocereus polyrhizus*) which contained phytochemical

compounds containing flavonoids, phenol hydroquinone, steroids, triterpenoids, saponins, and tannins as effective as antibacterial and natural antioxidants. The addition of red dragon fruit skin extract to cow sausages is effective in increasing antioxidant activity and reducing the value of tBARS. Sausage Microbiology Quality Meets Indonesian National Standards for Sausages /SNI 01-3820: 1995. Red dragon fruit skin extract at a concentration of up to 40% is not effective in increasing the reddish intensity of the cow sausage, but can improve the yellow color in the cow sausage.[38]

Sulistyarini & Wicaksono's research in 2020 was conducted by dragon fruit research in 2 stages. Stage 1 is the process of pollination of Simplicia, followed by the extraction process with 96%ethanol solvent, and continued with fractionation using N-hexane solvents, ethyl acetate, and water. The second stage is phytochemical screening in the form of color reaction tests, foam formation tests, and depositional reaction tests, both in powder, extracts, and in the dragon fruit stem fraction (*Hylocereus polyrhizus*) to several groups of compounds, including flavonoids, alkaloids, saponins, tannins, and terpenoids. Screening shows that powder, extract, N-hexane fraction, and ethyl acetate fraction contain flavonoids, steroids, and saponins. While the water fraction contains flavonoid compounds and saponins.[29]

HPLC analysis demonstrated that dragon fruit is enriched with bioactive phytochemicals, with significant variations between each part of the fruit. Anthocyanins namely, cyanidin 3-glucoside, delphinidin 3-glucoside, and pelargonidin 3-glucoside were detected in the dragon fruit peel and fresh red pulp. Epicatechin gallate, epigallocatechin, caffeine, and gallic acid were found in the dragon fruit seed. Additionally, 25–100 mg × L 1 of dragon fruit pulp and peel extracts containing enrichment of cyanidin 3-glucoside were found to inhibit the production of reactive oxygen species (ROS), reactive nitrogen species (RNS), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) in cell-based studies without exerted cytotoxicity.[13]

CONCLUSION

Local dragon fruit plants from Tanah Laut Regency contain secondary metabolic compounds (Flavonoids, Alkaloids, Saponins, and Terpenoids) and the highest levels of phytochemicals in dragon fruit stems. It is advisable to do an effective and efficient formulation of dragon fruit extract preparations on experimental animals.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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