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Anthocyanin Compounds from Ethanol Extract of Red Fruit (*Pandanus conideus* L.): A Functional Group Analysis

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Abstract: Red Fruit (*Pandanus conoideus* L.) is a typical Papuan plant that is rich in bioactive compounds such as anthocyanins, which have the potential to act as antioxidants. This research aims to analyze the chemical structure of anthocyanin compounds from red fruit ethanol extract using Fourier Transformed Infrared (FTIR) spectroscopy. FTIR is a spectroscopic tool widely used to characterize or detect functional groups and qualitatively identify compounds in samples. Anthocyanins act as antioxidants and have various biological activities such as preventing cancer, cardiovascular disease, and diabetes. Extraction was carried out using the maceration method using 70% ethanol solvent, and characterization was performed using FTIR in the wave range 4000-450 cm^{-1}. The results of the research that has been carried out show that the ethanol extract of red fruit contains secondary metabolites such as flavonoids, alkaloids, and saponins. The FTIR results of red fruit extract show the presence of functional groups characterized by the presence of the O-H, C-O-C, C-O, and C=C functional groups which are important indicators of the antioxidant properties of anthocyanins. This research confirms the potential of Red Fruit as a natural source of anthocyanins which are useful as antioxidants.

Keywords: Anthocyanins, Red fruit, *Pandanus conoideus* L., FTIR

Introduction

Indonesia is a tropical country that is rich in various kinds of typical plants that are easy to find in certain areas. The distribution of endemic plants in a region has an attractive value for the plants themselves. One plant that has attractiveness and high selling value is red fruit (*Pandanus conoideus* L.). Red Fruit (*Pandanus conoideus* L.) is a typical Papuan plant which is known to be rich in bioactive compounds. Red fruit is traditionally consumed by local people in the form of fruit juice or oil (Tethool, Ollong, and Koibur 2021).

Previous research stated that red fruit (*Pandanus conoideus* L.) contains 500 ppm total α tocopherol, 700 ppm beta-carotene, 11,000 ppm tocopherol, 12,000 ppm carotenoids, and contains fatty acids such as 8.8% linoleic acid, 7.8% linolenic acid, oleic acid 58%, and decanoic acid 2.0% (Wabula, Dali, and Widiastuti 2019). Anthocyanin is a type of flavonoid found in plants, both in flowers and fruit, and can provide various colors such as purple, blue, red, and orange. The main part of this anthocyanin compound is characterized by two aromatic benzene rings (C_6H_6) and is connected via three carbon atoms that form a ring. The main component of anthocyanins consists of aglycones (anthocyanidins) which are esterified with one or more sugar groups (glycones). The core structure of anthocyanins consists of 2-phenyl-benzopyrylium or flavilium with several methoxy and hydroxy groups (Ifadah, Wiratara, and Afgani 2022). The stability and color of the anthocyanin compound depends on its overall molecular structure. The color of anthocyanins depends on the pH of the solution. In acidic conditions the anthocyanin is red, in neutral conditions the anthocyanin is purple, and in

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alkaline conditions the anthocyanin is blue. (Perdani 2019). The stability of anthocyanin compounds depends on temperature, light, pH, and chemical structure (Herfayati, Pandia, and Nasution 2020). In general, the addition of hydroxy groups reduces the stability of anthocyanins, while the addition of methyl groups tends to increase the stability of these compounds. The pH of the solution can also affect the color and stability of anthocyanins which tend to be more stable in acidic conditions than alkaline. Temperature also affects the stability of anthocyanins. High temperatures can damage the structure of this compound. Therefore, the anthocyanin heating process should be carried out at a temperature ranging between 50-60°C, the optimal temperature for heating. Besides that, light can also affect the stability of anthocyanins in the process of formation and rate of degradation, so that these compounds are more stable when stored in a place protected from light (Surianti, Husain, and Sulfikar 2019).

Anthocyanins, which act as natural colorants in drinks and food, can increase the added value of a product and attract more consumers. The extraction results from various methods that have been researched previously starting from maceration extraction, with the help of heat, and ultrasonic extraction with accelerated solvents produce an attractive color from this compound. This pigment has low toxicity, and some studies even report that this pigment has no toxic properties. This compound is also safe to consume because it has various health benefits which act as antioxidants, antidiabetics, antimicrobials, and preventing heart disease (Perdani 2019).

In society, red fruit is often used as a natural coloring agent in traditional foods and drinks, because it is valued more safe compared to synthetic dyes which can contain dangerous chemicals. So it can reduce the use of synthetic dyes among the public. However, the biochemical content in red fruit cannot yet be ascertained, therefore it is necessary to carry out testing using FT-IR to confirm the chemical compound content in red fruit.

Methods

Tools and materials

The tools used in this research were a water bath, analytical balance, glass funnel, oven, hot plate, and functional group analysis using Fourier Transform Infra Red spectroscopy. The materials used in this research were red fruit, 70% ethanol, concentrated HCl, lead, NaOH, potassium iodide, mercury 2 chloride, iron, and distilled water.

Sample Preparation

The red fruit samples (*Pandanus conoideus* L.) used came from the Osok area of Sorong City, Southwest Papua Province. The part used is the red fruit seeds. The samples that have been obtained are then subjected to wet sorting to separate the dirt that sticks to them when taking the sample. The samples were washed using running water to remove adhering dirt. Sample drying was carried out at a temperature of 45°C to maintain the stability of the compound content contained in the sample. Dry sorting is carried out to separate unwanted parts during drying. Sample refinement is carried out to obtain a size that makes the extraction and sieving process easier.

Preparing Extract from Red Fruit

The sifted simplicia powder was then extracted using the maceration method with 70% ethanol solvent. This method was chosen because it is a simple extraction method carried out without any heating process, thus helping to maintain the stability of compounds that are not resistant to heating. The extraction process lasted for 3 days with daily stirring. After 3 days, the maceration results were filtered to obtain a liquid extract, then continued with the re-maceration process for 3 days and stirred once a day. After getting the liquid extract from the maceration and re-maceration process, the next step is the evaporation process to get a thick extract.

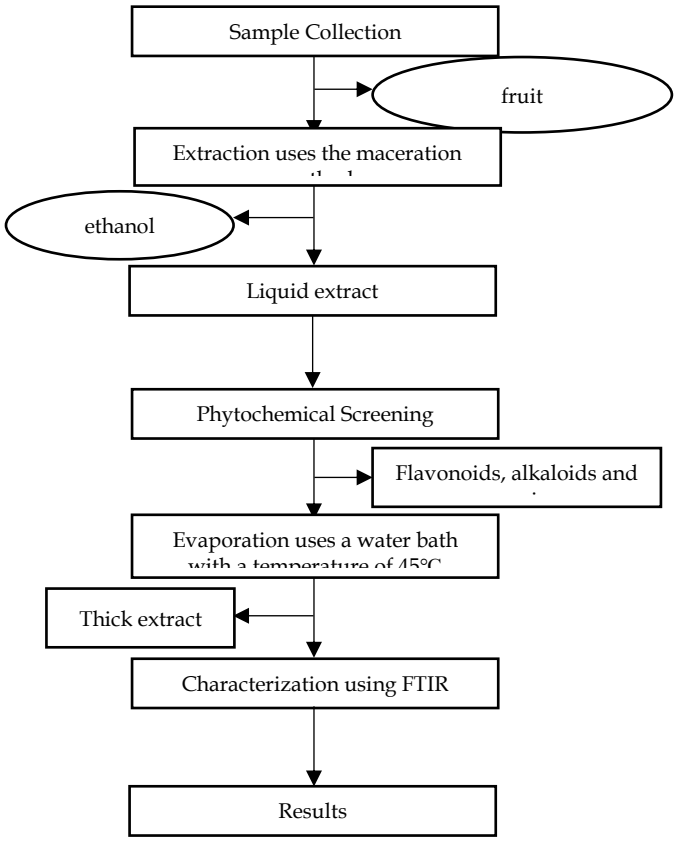

Figure 1. Research Flow Chart

Phytochemical Screening of Red Fruit Extract

Phytochemical tests were carried out to determine the compound content in red fruit extract:

Identification of saponin compounds

A sample of 4 mL was added with distilled water and then shaken vigorously, and it was seen that a stable foam was formed with a height of approximately 1 cm with a stable time of 30 seconds. The formation of stable foam for 30 seconds indicates that the sample contains saponin compounds (Ismawati, Ismawati, and Destryana 2021, Lanipi, R.P., 2022, (Sulistyarini et al. 2016, and Erawati, R., et al. 2024).

Identify alkaloid compounds

A total of 3 mL of sample was put into a test tube and given Mayer's reagent. If a white or yellowish-white precipitate appears after adding Mayer's reagent, then alkaloid compounds are detected in the sample (Sulistyarini et al. 2016, and Erawati, R., et al. 2024).

Identification of flavonoid compounds

A total of 3 mL of sample was put into a test tube, then 0.5 mL of concentrated HCl was added and heated over a water bath for 15 minutes. If the color changes from dark red to purple, this indicates that the ethanol extract of red fruit contains flavonoid compounds in the anthocyanidin group. (Susiloningrum and Indrawati 2020, (Sulistyarini et al. 2016, and Erawati, R., et al. 2024).

3 mL of sample was put into a test tube and added with PB-2-acetate reagent. The formation of a brownish-yellow color after adding the reagent indicates that the sample contains flavonoid compounds (Musiam et al. 2022, and Fabanyo, S., et. al., 2023).

A total of 3 mL of sample was put in a test tube, then added with 2n NaOH reagent. If the color changes to yellow, the ethanol extract of red fruit contains flavonoid compounds (Murni and Santoso 2018, and Fabanyo, S., et. al., 2023).

Sample Characterization using FTIR

After carrying out the phytochemical screening test, the sample was then tested using a Fourier Transformed Infrared (FTIR) spectroscopy instrument by taking a small sample and placing it in a mold. Then the sample was characterized using Fourier Transformed Infrared (FTIR) spectroscopy at wave numbers (4000-450 cm^{-1}). The results of FTIR characterization of red fruit ethanol extract are displayed in graphic format. The spectrum displayed on the graph was then analyzed by comparing it with the literature to determine the functional groups of the red fruit ethanol extract.

Result and Discussion

Phytochemical Analysis

The results of the phytochemical test showed that phytochemical screening of the ethanol extract of red fruit (*Pandanus conoideus* L.) showed the presence of saponin, alkaloid, and flavonoid compounds. This test is carried out to identify the presence of compounds in the sample which is generally known as a qualitative test.

Table 1. Results of phytochemical screening of red fruit extract (*Pandanus conoideus* L.)

Variable	Reagent	Observation	Results
Saponins	Aquades	Formation of foam	+
Alkaloids	Mayer	There is a white precipitate.	+
Flavonoids	Pb2 acetate	Changes to a yellow-brown color	+
	Concentrated HCl	Changes to red color	+
	NaOH	Changes to yellow color	+

The ethanol extract of red fruit (*Pandanus conoideus* L.) was used to test the saponin compound. The results (table 1) showed positive results, which were indicated by the formation of stable foam for 30 seconds. This is because the saponin compound has the physical property of being simple to dissolve in water and will form foams when shaken (Geeta 2016), The alkaloid test using Mayer's reagent yielded positive results as well, as evidenced by the appearance of a white precipitate. This is because mercury ions are heavy metal ions that can precipitate alkaloid compounds which are alkaline in nature (Sulistyarini et al. 2016, Fabanyo, S., et. al., 2023).

pb-2-acetate, concentrated HCl and NaOH 2n were the three chemicals used in the flavonoid compound testing. Owing to the bond breakage at the C_3 atom, the reaction products utilizing the PB-2-acetate reagent will take on a brownish-yellow hue (Musiam et al. 2022).

A strong HCl solution is added and cooked over a water bath for 15 minutes in the Bate-Smith procedure. To hydrolyze flavonoids and separate them into aglycones by severing the O-glycosyl, strong HCl is added. The H^+ from the acid will replace the electrophilic O-glycosyl (Susiloningrum and Indrawati 2020). The phytochemical screening results demonstrated the presence of flavonoid components in the red fruit ethanol extract, as evidenced by a red color shift. Anthocyanidin group flavonoid chemicals are present when red color forms, as demonstrated by research done in 2015 (Rahayu, Kurniasih, and Amalia 2015), Positive results were achieved in the test for

flavonoid compound identification using NaOH reagent, as evidenced by the sample solution taking on a yellow color (Murni and Santoso 2018).

FTIR Analysis

By analyzing the absorption values and functional groups of the red fruit (*Pandanus conoideus* L.) ethanol extract, FTIR spectroscopy was used to estimate the concentration of anthocyanin chemicals in the extract.

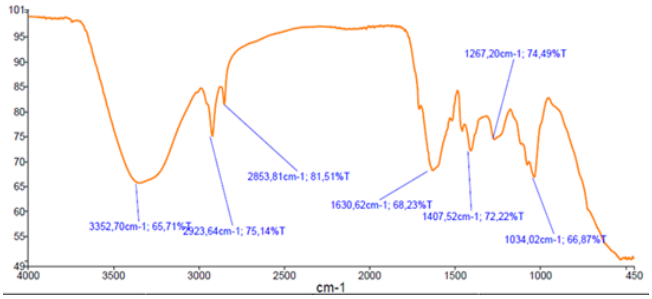

Figure 2. Results of FTIR analysis of red fruit ethanol extract

Based on the test results using an FTIR spectrophotometer, the results showed absorption at wave numbers (4000 – 450 cm^{-1}). The ethanol extract of red fruit shows absorption in the wave number range (3650 – 3200 cm^{-1}) for the stretching vibrations of the O-H group. Another absorption band is observed at the absorption length (2960 - 2850 cm^{-1}) for the stretching vibration of the C-H group, respectively for C-H3 and C-H2. The absorption that appears at the wave number (1680 - 1620 cm^{-1}) indicates the presence of the group aliphatic C=C . The presence of the C=C aromatic group can be seen in the absorption with wave numbers (1600, 1500 – 1400 cm^{-1}). The C-O-C group was detected at wave numbers (1230 – 1270 cm^{-1}). And the wave number (1000 – 1300 cm-1) indicates the presence of the C-O group. However, the FTIR spectrum obtained from the ethanol extract of red fruit also showed the absorption of other compounds that were observed. This was influenced by the fact that the sample used in this research was ethanol extract of red fruit which was not in the form of pure anthocyanin compounds.

The results of the phytochemical screening of red fruit (*Pandanus conoideus* L.) showed positive results using 3 different reagents, namely PB-2 acetate, NaOH, and using the bate smith method using concentrated HCl which showed that the ethanol extract of red fruit contains flavonoid compounds in the phenol group, flavonols, flavonones, and anthocyanidins which are aglycone forms of anthocyanin compounds.

Anthocyanin itself is a compound that has various functions for health, one of which is as an antioxidant. Antioxidant compounds are needed by the body to ward off free radicals and protect normal cells, fats and proteins from cell damage caused by free radicals. The properties of anthocyanin compounds as antioxidants are due to their ability to donate H atoms to free radicals due to the presence of an OH group attached to the aromatic ring. Apart from its function in the health sector, anthocyanin also functions in the food sector, namely as a natural coloring that is safe to use in food or drinks. The use of anthocyanin compounds as a natural coloring is due to its nature which is easily soluble in water (Kunnaryo and Wikandari 2021).

Table 2. Results of anthocyanin absorption characterization in FTIR spectroscopy

Functional group	Wavenumber value (cm^{-1})	Frequency area	Intensity
O-H (hydroxyl)	3352.7	3200 - 3650	strong
C=C (aromatic)	1407.52	1450, 1500 - 1600	strong
C-O-C (ether)	1267.2	1230 - 1270	strong
C-O (alcohol)	1034.02	1000 - 1350	strong

The results of the characterization of anthocyanin compounds using the FTIR instrument show that there is a typical absorption which illustrates that the ethanol extract of red fruit has hydroxyl groups and aromatic groups, where the absorption of these functional groups is the typical absorption of anthocyanin compounds (Wahyuningsih et al. 2017), So the ethanol extract of red fruit has the characteristics of an anthocyanin compound due to the presence of O-H, C-O-C, C-O, and C=C groups in the FTIR analysis spectrophotometer.

Conclusion

Pandanus conideus L. ethanol extract contains secondary metabolites such as flavonoids, alkaloids, and saponins. The ethanol extract of *Pandanus conideus* L. also contains anthocyanin compounds which were characterized using FTIR spectroscopy characterized by the presence of the functional groups O-H, C-O-C, C-O, and C=C which are typical absorptions of anthocyanin compounds.

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Author Contributions

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Conflict of Interest
The author declares no conflict of interest.

References

Erawati, R., Muslihin, A., & Hardia, L. (2024). Uji Aktivitas Antioksidan Fraksi Ekstrak Etanol Tali Kuning (Anamirta cocculus) Dengan Metode DPPH. *urnal romotif reventif*, *7*(2), 381-391. https://doi.org/10.47650/jpp.v7i2.1264

Fabanyo, S., Hardia, L., Muslihin, A., Budiyanto, A., & Irwandi, I. (2023). Analisis Fitokimia dan Gugus Fungsi Kulit Kayu Akway (Drymis sp.). *urnal romotif reventif*, *6*(6), 976-982. https://doi.org/10.47650/jpp.v6i6.1165

Geeta, Verma. 2016. "FTIR Spectroscopy - A Technique for the Evaluation of Edible Oil Oxidation." *International Journal of Science and Research (IJSR)* 5(1):294–96.

Herfayati, Putri, Setiaty Pandia, and Halimatuddahliana Nasution. 2020. "Karakteristik Antosianin Dari Kulit Buah Nipah (Nypa Frutican) Sebagai Pewarna Alami Dengan Metode Soxhletasi." *Jurnal Teknik Kimia USU* 9(1):26–33. doi: 10.32734/jtk.v9i1.2831.

Ifadah, Raida Amelia, Pinasthika Rizkia Warapsari Wiratara, and Chairul Anam Afgani. 2022. "Ulasan Ilmiah : Antosianin Dan Manfaatnya Untuk Kesehatan." *Jurnal Teknologi Pengolahan Pertanian* 3(2):11–21. doi: 10.35308/jtpp.v3i2.4450.

Ismawati, Lisa, Ismawati, and R. Amilia Destryana. 2021. "Identifikasi Senyawa Saponin Pada Ekstrak Rumput Mutiara (Hedyotis Corimbosa L. (Lamk)) Dengan Pelarut Yang Berbeda." *Prosiding SNAPP* 1(1):150–54.

Kunnaryo, Herman Joseph Bimo, and Prima Retno Wikandari. 2021. "Antosianin Dalam Produksi Fermentasi Dan Perannya Sebagai Antioksidan." *Unesa Journal of Chemistry* 10(1):24–36. doi: 10.26740/ujc.v10n1.p24-36.

Lanipi, R. P., Hardia, L., Astuti, R. A., & Budianto, A. B. (2022). Uji Fitokimia Ekstrak Daun Katuk (Sauropus adrogynus (L) Merr). *Jurnal Etnofarmasi*, *1*(1), 1-6.

Murni, Maria Angelina, and Bilal Subchan Agus Santoso. 2018. "Skrining Fitokimia Jus Apel Manalagi Dari Daerah Punten Kota Batu." *Akademi Farmasi Putra Indonesia Malang*.

Musiam, Siska, Erna Prihandiwati, Eka Kumalasari, and Aisyah. 2022. "Penetapan Kadar Flavonoid Total Ekstrak Dan Fraksi Kulit Buah Citrus Reticulata." *Jurnal Farmasi Indonesia* 19(2):257.

Perdani, Arum Widyastuti. 2019. "Mini Review : Ekstraksi Antosianin Sebagai Pewarna Makanan Dengan Bantuan Ultrasonik Dan." *Food and Nutrition Research*.

Rahayu, Siti, Nunung Kurniasih, and Vina Amalia. 2015. "Ekstraksi Dan Identifikasi Senyawa Flavonoid Dari Limbah Kulit Bawang Merah Sebagai Antioksidan Alami." *Al-Kimiya* 2(1):1–8. doi: 10.15575/ak.v2i1.345.

Sulistyarini, Indah, Arum Sari, Dan Tony, Ardian Wicaksono, Sekolah Tinggi, Ilmu Farmasi, " Yayasan, Pharmasi Semarang, Jl Letjend, Sarwo Edie Wibowo, and Plamongansari Semarang. 2016. "Skrining Fitokimia Senyawa Metabolit Sekunder Batang Buah Naga Skrining Fitokimia Senyawa Metabolit Sekunder Batang Buah Naga (Hylocereus Polyrhizus)." *Jurnal Ilmiah Cendekia Eksakta* 56–62.

Surianti, Surianti, Halimah Husain, and Sulfikar Sulfikar. 2019. "Uji Stabilitas Pigmen Merah Antosianin Dari Daun Jati Muda (Tectona Grandis Linn f) Terhadap PH Sebagai Pewarna Alami." *Chemica: Jurnal Ilmiah Kimia Dan Pendidikan Kimia* 20(1):94. doi: 10.35580/chemica.v20i1.13623.

Susiloningrum, Dwi, and Dania Indrawati. 2020. "Penapisan Fitokimia Dan Analisis Kadar Flavonoid Total Rimpang Temu Mangga (Curcuma Mangga Valeton & Zijp.) Dengan Perbedaan Polaritas Pelarut." *Jurnal Keperawatan Dan Kesehatan Masyarakat Cendekia Utama* 9(2):126. doi: 10.31596/jcu.v9i2.593.

Tethool, Angelina Novita, Abdul Rahman Ollong, and Johan Fredrik Koibur. 2021. "Pengaruh Sari Buah Merah (Pandanus Conoideus Lam) Terhadap Abnormalitas Spermatozoa Ayam Kampung." *Jurnal Ilmu Peternakan Dan Veteriner Tropis (Journal of Tropical Animal and Veterinary Science)* 11(2):92. doi: 10.46549/jipvet.v11i2.107.

Wabula, Rheytno Asdin, Seniwati Dali, and Harti Widiastuti. 2019. "Aktivitas Antioksidan Ekstrak Etanol Buah Merah (Pandanus Conoideus Lam.) Dengan Metode FRAP." *Window of Health : Jurnal Kesehatan* 2(4):329–37. doi: 10.33368/woh.v0i0.203.

Wahyuningsih, S., L. Wulandari, M. W. Wartono, H. Munawaroh, and A. H. Ramelan. 2017. "The Effect of PH and Color Stability of Anthocyanin on Food Colorant." *IOP Conference Series: Materials Science and Engineering* 193(1). doi: 10.1088/1757-899X/193/1/012047.