Determination of chemical, bioactive compounds and volatile composition in different extracts and the parts of pomegranate (punica granatum l.) as a natural feed additive

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Research Article

Determination of Chemical, Bioactive Compounds and Volatile Composition in Different Extracts and the Parts of Pomegranate (*Punica Granatum* L.) as a Natural Feed Additive

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Abstract | Antibiotics and microbial resistance have become important issues in the poultry industry. Therefore, alternative natural feed additives that replaced antibiotics and have positive effects as growth promoters continue to be studied. This study aimed to provide the biochemical composition and volatile composition of pomegranate (*Punica granatum* L.) with the peels, arils (including seed), and whole fruit (peels and arils) using six replicates for each part of the pomegranate. Four types of maceration were used, such as aqueous, ethanol, n-hexane, and chloroform for each part of the pomegranate. The experimental design was completely randomized design, if differences were found, it was continued with Duncan's test. The parameters of the study were water, carbohydrates, protein, fat, fiber, ash, polyphenols, flavonoids, tannins, antioxidant activity, and oil volatile composition. The findings showed that the water content (1.73%), fat (0.39%), and protein (3.51%) of peel ethanol extract were substantially lower than those of arils and whole fruit, in contrast, the ash (36.67%), fiber (37.67%), carbohydrate (72.51%), polyphenols (12.34 mg/g), flavonoid (5.57 mg/g), tannins (243 mg/g), and antioxidant (42.03%) were significantly higher ($p \leq 0.05$) than other parts of pomegranate. Pomegranate peel ethanol extract has a higher percentage of volatile compounds 2-furan carboxaldehyde5 (34,34%) and 2-Furancarboxaldehyde (4,82%) as antimicrobial agents and anti-inflammatory, and 4H-pyran-4-one (3.19%) as an antioxidant compound. Pomegranate peel ethanol extract has a chemical composition, bioactive compound, and volatile compound which is suitable as a natural feed additive to improve poultry health and production.

Keywords | Feed additive, Pomegranate, Chemical, Bioactive, Growth promoter

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INTRODUCTION

Unpredictable extreme weather increases mortality and productivity due to decreased immunity, impaired energy regulation, and cell synthesis, causing stunted growth, and reduced production performance in broiler industries (Kpomasse *et al.*, 2021). As a solution to extreme weather problems, antibiotic growth promoters (AGPs) maintained production performance to reduce the population of pathogenic bacteria, leading to pathogenic microbial resistance and antibiotic residues (Fancher *et al*., 2020). The solution to maintaining the performance of poultry production without AGPs is to use natural ingredients.

Various studies have been conducted using multiple plants as natural ingredients and have shown positive effects (Pohl and Kong, 2018). The advantage of using natural ingredients is that they are safe for long-term human consumption and do not contain chemical residues (Jiang and Xiong, 2016). Phytobiotics are active substances that form secondary metabolites in plants that have pharmacological effects obtained from essential oils, and extracts from plants (Bagno *et al*., 2018). Due to their natural, residue-free, and less toxic nature, the use of phytobiotics as poultry feed additives has increased (Ayalew *et al*., 2022). Phytobiotics in broiler diets improve gut function (Urban *et al*., 2024), increase nitrogen retention, fiber digestibility, improve growth performance (Alghirani *et al*., 2021), reduce inflammation (Kikusato, 2021), enhance anti-oxidative (Duskaev *et al*., 2023), and antimicrobial activity (Hashem *et al*., 2022). Phytobiotics prevent intestinal diseases, increase growth rate, and immunological parameters (Kikusato *et al*., 2021).

Pomegranate (*Punica granatum* L.) is a plant of the Punicaceae family. Supplementation of pomegranate peel in low doses had antioxidant activity (Al-Gubory *et al*., 2016). Pomegranate peel in diet reduced triglyceride and total cholesterol in broilers, and low-density lipoprotein concentrations in serum increased high-density (Abu Hafsa *et al*., 2024). Pomegranate peels have anti-parasitic properties in broilers (Khorrami *et al*., 2022). The products include seeds and peels (Ko *et al*., 2021). Pomegranates contain polyphenols including phenolic acids (gallic, ellagic, caffeic, citric, tartaric, malic, and ascorbic) (Kalaycıoğlu and Erim, 2017), flavonoids, flavonols as cathecin, epicatechin, and gallocatechin as well as anthocyanins (Abd El-Ghany, 2023), hydrolyzed tannins (ellagitannin, punicalin, punicalagin, pedunculagin, gallotannin, and condensed tannins (Türkyılmaz *et al*., 2024). Polyunsaturated fatty acids, vitamins, and minerals are other components of pomegranate (Bozkurt and Ergun, 2021). The components of pomegranate seed oil are cis-9, trans-11, cis-13 octadecatrienoic (punicic) acid, and one conjugated linolenic acid (Białek *et al*., 2017). The nutritional value and health-promoting effects of pomegranate can be used as an alternative poultry feed (Mnisi *et al*., 2022). In addition, pomegranate and its by-products exhibit growth-promoting effects (Abdel Baset *et al*., 2022).

Numerous studies have examined the impact of pomegranate in feed, but the studies have limitations on the use of pomegranate peels and have not examined the other parts of the pomegranate arils and the whole fruit so that the effects of other parts of the pomegranate cannot be verified. Existing research has not conducted extraction methods on pomegranate so it has not quantitatively validated the bioactive compounds present in pomegranate that are important as natural feed additives. In this study, extraction was carried out using four solvents, such as aquadest, ethanol, hexane, and chloroform.

The study aims to provide the determination of chemical, bioactive compounds, and volatile composition in different extracts of pomegranate (*Punica granatum* L.). The study validated the parts of the pomegranate and the types of solvents that will provide results from the pomegranate to be used as natural feed additives to replace antibiotics.

MATERIALS AND METHODS

Preparation of Samples

The study began by manually separating the pomegranate into peels, arils, and whole fruit (peels and arils). The separated materials were cleaned, dried at 60^0C, and ground into flour by the method of Gumolung *et al*. (2019). Samples were ground using a blender at medium speed and grinding time for 2 min, then sieved using 40 mesh and 100 mesh (Amalina *et al*., 2023). The peels, arils, and whole fruit (peels + arils) were extracted with four solvents: aqueous, chloroform, ethanol, and n-hexane using six replicates for each part of the pomegranate.

Proximate analysis according to reference (Aller *et al*.,2017). Analysis of carbohydrate content using the reference methods of Kole *et al*. (2020). The polyphenols analysis by methods of Sun *et al*. (2022). The flavonoid analysis and tannin by the method of Bae *et al*. (2012). The antioxidant activity analysis is based on references from Shahidi and Zhong (2015).

These different extracts were subsequently analyzed by GC-MS to characterize the volatile and majority compounds in these extracts. Analysis of volatile components of macerated samples consisted of identification and determination of the content of volatile components obtained by GC-MS. The GC-MS using a modified analysis and identification process produces bioactive compounds seen from chromatogram peaks as identification of chromatography and mass spectrometry (MS) data on each molecular weight of bioactive compounds (Rani and Kapoor, 2019).

Statistical Analysis

SPSS software version 24 was used to analyze the data in a completely randomized design and expressed mean ± standard deviation. Treatment means were separated by Duncan's multiple range test. Significant values were selected at the ($p \leq 0.05$) level.

RESULTS AND DISCUSSION

Chemical Composition

The research on the chemical composition of pomegranates

Table 1: Chemical composition of peels, arils and whole fruit with different extracts.

Chemicals	Peels				Arils				Whole Fruit			
	Ethanol	n-hexane	Chloro-form	Aqueous	Ethanol	n-hexane	Chloro-form	Aqueous	Ethanol	n-hexane	Chlo-roform	Aqueous
Water, %	1.73^{a}±0.01	1.42^{a} ±0.01	3.95 ^{b}± 0.02	3,03 ^{b}± 0.02	5.44 ^{c}± 0.04	4.74 ^{c}± 0.03	5.58 ^{c}± 0.04	5.12 ^{c}± 0.04	1.16 ^{a}± 0.01	4.04 ^{b}± 0.02	4.44^{c}± 0.03	3.45 ^{b}± 0.02
Ash, %	36.67 ^{c}± 4.35	8.52 ^{a}± 1.15	10.33 ^{a}± 1.12	13.25 ^{b}± 2.21	17.92 ^{b}± 3.25	7.67 ^{a}± 1.18	9.77 ^{a}± 2.20	11.94 ^{a}± 2.16	17.49 ^{b}± 3.19	14.35 ^{b} ± 2.22	6.11 ^{a}± 1.14	9.97 ^{a}± 1.16
Fat, %	0.39 ^{a}± 0.01	0.38 ^{a}± 0.02	0.05 ^{a}± 0.01	4.24 ^{a}± 0.12	12.77 ^{c} ± 0.23	12.75 ^{c}± 0.24	6.29 ^{b}± 0.34	7.63 ^{b}± 0.42	1.99 ^{a}± 0.24	1.98 ^{a}± 0.31	0.29 ^{a}± 0.04	0.62 ^{a}± 0.05
Protein, %	3.51 ^{a}± 0.12	2.66 ^{a}± 0.09	2.63 ^{a}± 0.08	4.86 ^{b}± 0.25	14.77 ^{d} ± 0.80	8.31 ^{c}± 0.02	8.29 ^{c}± 0.35	7.63 ^{c}± 0.18	8.60 ^{c}± 0.20	5.75 ^{b}± 0.32	5.39 ^{b}± 0.38	2.68 ^{a}± 0.28
Fiber, %	37.67 ^{d}± 5.80	8.52 ^{a}± 1.16	10.33 ^{b}± 2.02	13.25 ^{b}± 1.42	17.92 ^{c} ± 2.65	7.67 ^{a}± 2.31	9.77 ^{b}± 2.42	11.94 ^{b}± 2.59	17.49 ^{c}± 2.83	14.35 ^{b} ± 2.87	6.11 ^{a}± 1.66	9.97 ^{b}± 2.74
Carbohy-drate, %	72,51 ^{b}± 2.65	72.04 ^{b}± 3.15	78.45 ^{c}± 2.67	70.76 ^{b}± 2.93	65.52 ^{a} ± 3.44	75.02 ^{c}± 2.15	73.55 ^{b}± 1.35	64.29 ^{a}± 4.42	66.58 ^{a}± 3.28	71.18 ^{b} ± 3.65	79.47 ^{c} ± 2.89	79.47 ^{c}± 3.68

Different superscripts in the same row and column indicate significant differences (p≤0.05).

is focused on validating the chemical composition of various parts of the pomegranate using different extractions. The results of chemical composition analysis in the form of water ash, protein, fat, carbohydrates, and fiber on various parts of the pomegranate are presented in Table 1.

Water Content

The water content in arils (5.12 to 5.44%) was higher (p≤0.05) than all parts of the pomegranate. According to Wanderley *et al.* (2023), the water content of the arils tends to be higher than the peels.

Extraction is the process of selective transfer of compounds between an organic solvent and an aqueous solvent (Patel *et al.*, 2019). The extraction method depends on the texture and water content of the sample material being extracted and the compounds being isolated (Jha and Sit, 2022). The water content of the peel ethanol extract was the lowest (1.73%), and the highest water was in the arils chloroform extract (5.58%). The results of this study are close to the research of Al-Musodi (2023) who reported that the lowest moisture content in the peel was 4.22% and the highest in whole fruit was 5.55%.

Among different parts of the pomegranate, the water content of the peel was significantly different in all parts of the pomegranate (p≤0.05). The water content significantly affects quality. The high water content will be used by bacteria, fungi, and other microbes to multiply resulting in chemical changes. Fruit peel has a low water content that causes microbes to not develop, so the feed is safe to store for a long period (Saroj *et al.*, 2020).

Ash Content

Ash is the total mineral determined by burning the sample at 550-600°C, all organic material is burnt and leaves a residue in the form of ash or inorganic material (Kuswa *et al.*, 2024). Among the pomegranate parts, the ash of the peel was the highest (p <0.05) compared to other pomegranate parts, while the arils and whole fruit did not differ significantly (p >0.05), this is close to the report of Wanderley *et al.* (2023). The ethanol extract of the peels has the highest ash content (36.67%), while the lowest ash content was in the whole fruit chloroform extract (6.11%) (p≤0.05). The ash content of the peels above the research of Ranjitha *et al.* (2018) was 4.32%. Ethanol is relatively non-toxic compared to acetone and methanol be used in various extraction methods (Plaskova and Mlcek, 2023). The carbon chain of ethanol dissolves a wide variety of molecules - both slightly polar and slightly nonpolar, ethanol is an effective solvent for bioactive compounds (Onuki *et al.*, 2016). Percent extraction yield indicates the effectiveness of the extraction process. The effectiveness of extraction is influenced by the amount of surface area interacting with the solvent, the contact time of the sample and extractor, the volume of solvent used, and the distribution coefficient (Spietelun *et al.*, 2013). Ash content indicates the total minerals in a material. The ash content of the peel indicates that the total minerals present in the peel are the highest of all pomegranate parts.

Fat Content

Arils had the highest fat content (7.63 to 12.77%) of all fruit parts (p≤0.05). The fat content of peels (0.05 to 4.24%) and whole fruit (0.29 to 1.99%) did not differ significantly (p >0.05). The highest fat content was in arils ethanol extract (12.77%) and n-hexane extract of arils (12.75%). The fat content of the peel tends to be low because the peel usually serves as a protective barrier for the inside of the fruit, which is more susceptible to damage or infection (Knoche and Lang, 2017). The fat content of the peels is close to the results of the study by Ranjitha *et al.* (2018) was 0.85%.

Table 2: Bioactive composition of peels, arils and whole fruit with different extracts.

Bioactive	Peels				Arils				Whole Fruit			
	Ethanol	n-hexane	Chloroform	Aqueous	Ethanol	n-hexane	Chloroform	Aqueous	Ethanol	n-hexane	Chloroform	Aqueous
Polyphenols, mg/g	12.34 ^{c} ± 1.12	8.99 ^{c} ± 2.23	8.72 ^{c} ± 3.35	11.68 ^{c} ± 1.01	3.24 ^{a} ± 1.03	3.07 ^{b} ± 1.15	2.98 ^{a} ± 0.96	2.28 ^{a} ± 1.01	4.42 ^{b} ± 1.43	3.77 ^{b} ± 0.93	3.54 ^{a} ± 0.86	3.72 ^{b} ± 0.85
Flavonoid, mg/g	5.57 ^{d} ± 1.15	4.26 ^{c} ± 1.01	4.32 ^{c} ± 1.16	4.40 ^{c} ± 1.24	2.37 ^{a} ± 1.06	2.14 ^{a} ± 1.01	3.28 ^{b} ± 1.07	3.32 ^{b} ± 1.05	3.38 ^{b} ± 0.99	2.16 ^{a} ± 0.81	3.30 ^{b} ± 0.90	3.34 ^{b} ± 0.94
Tannins, mg/g	243 ^{a} ± 34.02	143 ^{b} ± 27.87	149 ^{b} ± 39.77	169 ^{b} ± 33.79	132 ^{b} ± 28.55	78 ^{a} ± 12.24	68 ^{a} ± 9.45	88 ^{a} ± 7.42	115 ^{b} ± 7.47	122 ^{b} ± 8.43	123 ^{b} ± 6.03	165 ^{b} ± 5.86
Antioxidant, %	42.03 ^{c} ± 4.34	37.21 ^{c} ± 3.38	36.83 ^{b} ± 4.86	40.21 ^{c} ± 4.08	29.32 ^{a} ± 1.68	26.82 ^{a} ± 3.05	26.73 ^{a} ± 3.21	27.89 ^{a} ±23.44	30.23 ^{b} ± 1.81	26.97 ^{a} ± 2.73	26.78 ^{a} ± 2.01	28.02 ^{a} ± 1.07

Different superscripts in the same row and column indicate significant differences ($p \leq 0.05$).

The main function of the peel is to protect the fruit from water loss, microorganism attack, and physical damage. The peel usually contains more fiber, antioxidants, and other compounds, but less fat (Navarro-González *et al*., 2011). The low crude fat content of pomegranate peels was confirmed by the findings of Omer *et al*. (2019). However, the fat content is higher than similar findings of Egbuonu and Osuji (2016).

Protein Content

Arils have protein content (7.63 to 14.77%) followed by whole fruit (2.68 to 8.60%), and the lowest protein content in the peels (2.63 to 4.86%). Arils contain high protein-causing organoleptic flavors (Elfalleh *et al*., 2012). The protein content of the peels in this study was higher than the results of De Graaf (2000) was 3.74%. Arils ethanol extract (14.77%) had the highest protein content compared to all extracts ($p < 0.05$). According to the report Ko *et al*. (2021) arils of pomegranate have high protein and higher amino acids.

Fiber Content

The fiber content of the peel (8.52 to 37.67%) was higher than other pomegranate parts. The fiber content in this study was higher than Rajintha *et al*. (2018) at 17.31%. Pomegranate peel is a major waste that has no commercial value as food due to its high fiber content (Hasnaoui *et al*., 2014). Crude fiber increases the rate of digestion, slows nutrient absorption, and affects broiler performance (Tejeda and Kim, 2021). Fiber plays an important role in the morphological and histological changes of the gastrointestinal tract, which is characterized by an increase in size (Chater *et al*., 2015). Crude fiber consists of cellulose, hemicellulose, and lignin is closely related to the development of digestive organs. Based on the analysis of pomegranate fruit parts, the peel contained the highest fiber ($p < 0.05$) compared to aril and whole fruit, while the ethanol extract had the highest fiber compared to all extracts ($p < 0.05$). Pomegranate peel is a major waste due to its high fiber content (Brownlee, 2011).

Carbohydrate Content

According to the solvent extract, the highest carbohydrate was in chloroform ($p < 0.05$). The effectiveness of the extraction process is influenced by the type of solvent used as a distillation liquid, the method, and the duration of extraction. In this study, carbohydrates were highest ($p < 0.05$) in whole fruit (79.47%) followed by peel (78.45%) and arils (73.55%). Carbohydrate content in this study was higher than Rajintha *et al*. (2018) was 66.31%. Carbohydrates are the most widely available biomolecules in the world composed of carbon, hydrogen, and oxygen. Carbohydrates are a source of energy for vital activities in the cell (Ochoa *et al*., 2014). Carbohydrates in pomegranate have nutritional and functional potential (Yang *et al*., 2023). The peel of pomegranate is a source of carbohydrates.

Bioactive Composition

Natural antioxidants in fruits have health potential, but the health risks of synthetic antioxidants. Several studies have explored the potential of fruits as a source of natural bioactive compounds with antioxidant potential. Bioactive ingredients provide physiological benefits. Therefore, a diet that presents bioactive ingredients is extremely important for improving health (Fernandes *et al*, 2019). The bioactive compounds of pomegranate including flavonoids, total phenols, total tannins, and antioxidants are presented in Table 2.

Polyphenols Compound

Widely studied bioactive compounds are polyphenols, flavonoids, and tannins (Fernandes *et al*, 2019). Phenolic compounds are one of the most important categories of natural antioxidants and much evidence is derived from antioxidant potency. In recent studies, the antioxidant and the content of phenolic compounds were found in the peel and arils of some fruits. Phenolic compounds are the main secondary metabolites and are composed of one aromatic ring with more hydroxyl substituents. The main phenolic substances are tannins, flavonoids, and phenolic acids. Some plant parts are major sources of bioactive compounds. Pomegranate peel has a high content of phenolic compounds (Gullon *et al.* 2016).

Phenolic compounds in pomegranate have high biological activities such as antioxidant, antimicrobial, antimutagenic, and anticarcinogenic (Asif, 2015). Phenolic compounds are associated with antioxidant activity (Mousavinejad *et al.*, 2009). The presence of phenolic compounds such as anthocyanins, ellagic acid, flavonoids, and tannins in different parts of pomegranate has been confirmed with high antioxidant activity. Polyphenol content in pomegranate is between 7.94 mg/g (Elfalleh *et al.*, 2012). In this study, polyphenol was highest in the peel ethanol extract (12.34 mg/g). The extraction method of polyphenols is one of the important factors in the availability of these compounds. In most studies, conventional solvent extraction methods were used to obtain pomegranate peel extracts. The order of polyphenol content was ethanol extract, followed by aqueous extract, chloroform extract, and n-hexane extract (Wang *et al.*, 2011).

Flavonoid Compound

Flavonoids are secondary metabolites of polyphenols, found widely in plants having various bioactive effects including anti-viral, anti-inflammatory, and antioxidant (Marzouk, 2016). Flavonoids were highest ($p < 0.05$) in the peel ethanol extract (5.57 mg/g), above the research of Elfalleh *et al.* (2012) was 3.30 mg/g. It has been demonstrated that adding flavonoids to chicken diets improves microbiological qualities, lowering the cholesterol and triglyceride, and changing the fatty acid profile of meat and eggs. These results also suggest that flavonoids, an antioxidant, are an essential tool for altering the functional architecture of the small intestine (Shen *et al.*, 2022).

Tannins Compound

Tannins are phenolic compounds in plants that can dissolve in water and organic solvents. The most useful tannin components in pomegranate are ellagitanin, punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavones (Khadivi *et al.*, 2024). In this study, tannins were highest ($p < 0.05$) in the peel ethanol extract (243 mg/g), the results of this study are higher than the report of Elfalleh *et al.* (2012) tannin content was 62.71 mg/g. The type of extraction that produces the highest tannins uses ethanol. Bioactive compounds from the peel have a synergistic effect with different compounds to produce physiological activity. Pomegranate peel was rich in several phenolate-like tannin structures that are water-soluble and mostly hydrolyzable. The tannin levels in this study in the range of 143 to 243 is close to the results of Mo *et al.* (2022) is between 84 to 193 mg/g.

Antioxidant Compound

Pomegranate fruits, peels, and roots have been widely used, in addition to health benefits, pomegranates contain antioxidant activity. The antioxidant activity of pomegranate depends on several factors, such as cultivar, plant part (fruit, flower, and leaf), and fruit part (peel, aril, and rind) (Magangana *et al.*, 2020). Antioxidant level was highest ($p<0.05$) in the peel ethanol extract (42.03%).

Volatile Composition

Secondary metabolites with volatile characteristics are compounds that help plants survive in the natural environment. Volatile compounds have low molecular weight, evaporate easily, and diffuse in the gas phase and biological systems. The volatile compounds in the ingredients will disappear due to oxidation and hydrolysis reactions. Volatile compounds can still be retained through the treatment given to the extraction of volatile compounds after the drying process at 60^0C followed by a cooling process for 2 hours before maceration for 24 hours (Phong *et al.*, 2022).

Despite the encouraging findings of these studies about pomegranate's potential as a feed additive, further study is required to completely study the benefits of pomegranate added in poultry feed, advance investigates utilizing Gas Chromatography-Mass Spectrometry (GC-MS), separation, and characterization of particular compounds inside pomegranate that will give benefits when included in poultry diet. Gas chromatography is an instrument that can be used for the analysis of feed composition and is suitable for volatile and semi-volatile compounds (Ranjan *et al.*, 2023). Quantitative determination is based on the area on the chromatogram obtained (Majchrzak *et al.*, 2018). The validation process uses quantification of volatile compounds using GC-MS to improve the sensitivity, accuracy, and precision of the results (Portari *et al.*, 2008). The GC-MS is used to validate the main compounds in pomegranate as a feed additive. The volatile compounds present in various parts of the pomegranate with different extraction methods are shown in Table 3.

The high content of volatile compounds in pomegranate of this study such as 2- Furancarboxaldehyde, 1,2-Benzenedicarboxylic, 9-Octadecenamide (CAS), Propanoic acid, 1-Octadecanol (CAS), 1-Hexadecanol, 4H-pyran-4-one,2-hydroxy, gamma.-Tocopherol, 2-Amino-9-(3,4-Dihydroxi), 1,2,3-propanetriol (CAS), 2,3-dihydro-3,5-dihroxy-6, Glucitol, 5-(hydroxymethyl)- (CAS), d-manitol, 3-pentene-1-ol,2,2,4-tri-, Tetracontane, Oxyrane, 2-hidroxymethyl-3-methyl, Gl-glyceraldehyde, 5,5-D2-Trans-3,4-, Cytidine (CAS), Dl-glyceraldehyde, Hydroxylamine,o.o'-1,2, Hexadecanoid acid (CAS), Pentadecanoid acid (CAS), N-methyltaurine, Methane, trinitro- (CAS), 1,2,3-Benzenetriol (CAS), Stigmasta-5,22-dien, Phenol,2,4-BIS(1,1), Dotriacontane (CAS).

The extraction of volatile compounds in plants produces different amounts of compounds (Jha and Sit, 2022). The result

Table 3: Volatile compound of peels, arils, and whole fruit with different extracts.

Volatile	Peels				Arils				Whole Fruit			
	Peak Report (%) Area											
	Ethanol	n-hexane	Chloroform	Aqueous	Ethanol	n-hexane	Chloroform	Aqueous	Ethanol	n-hexane	Chloroform	Aqueous
2- Furancarboxaldehyde5	34.34	-	-	40.24	15.60	-	19.70	15.30	40.83	-	9.70	35.71
1,2-Benzenedicarboxylic	-	-	8.01	-	-	7.78	-	-	-	10.46	8.58	-
9-Octadecenamide (CAS)	4.98				6.21	6.22	-	-	-	5.56	10.43	-
Propanoic acid	-	-	9.61	-	-	8.07	7.72	-	-	11.59	7.92	-
1-Octadecanol (CAS)	-	-	5.87	-	-	31.64	5.25	-	-	8.90	-	-
1-Hexadecanol			20.15				16.14			22.45	23.64	
4H-pyran-4-one,2-hydroxy	3.19	-	-	-	7.08	-	-	5.47	4.23	-	-	7.35
gamma.-Tocopherol	-	16.45	23.17	-	-	-	12.68	-	-	-	-	
2-Amino-9-(3,4-Dihydroxi	7.42			4.97				12.50	13.33			
1,2,3 -propanetriol (CAS)	-	-	-	-	5.41	-	-	-	6.16	-	-	4.44
2- Furancarboxaldehyde (CAS)	4.82	-	-	4.09	-	-	-	-	--	-	-	-
2,3-dihydro-3,5-dihroxy-6				2.99								7.37
Glucitol				4.31								4.72
5-(hydroxymethyl)- (CAS)												13.04
d-manitol									5.12			
3-pentene-1-ol,2,2,4-tri									5.00			
Tetracontane										6.24		
Oxyrane,heptadecyl											8.20	
2-hidroxymethyl-3-methyl								24.65				
Gl-glyceraldehyde								6.088				
5,5-D2-TRANS-3,4-								4.69				
Cytidine (CAS)					14.11							
Dl-glyceraldehyde					9.53							
Hydroxylamine,o.o'-1,2					7.87							
Hexadecanoid acid (CAS)						5.10						
Pentadecanoid acid (CAS)							5.09					
N-methyltaurine				17.71								
Methane,trinitro-(CAS)	12.06											

1,2,3-Benzenetri-ol (CAS)	4.35	
Stigmas-ta-5,22-dien		33.71
Phe-nol,2,4-BIS(1,1)		9.64
Dotriacontane (CAS)		6.26

of the aqueous extract (Table 3) contained 2-furan carboxaldehyde compounds, the highest level in whole fruit was (35.71%), followed by peels (4.69%), while in arils in the form of 5-hydroxymethylfurfural (15.30%). The results of chloroform extract (Table 3) on peels were the highest compound 1-Hexadecanol (20.15%), while in arils (16.14%) and 1-octadecanol (5.25%), in whole fruit only contained in the form of 1-octadecanol (23.64%). Peels and arils contained .gamma.-Tocopherol at 23.17% and 12.68%, respectively, and were not found in whole fruit. The compound 1,2-Benzenedicarboxylic acid was found in peels (8.01%) and whole fruit (8.58%), but was not the main volatile compound in arils. The highest volatile compound from hexane extraction (Table 3) was stigmasterol in peels (33.71%), while in arils it was 1-octadecanol (21.67%), and whole fruit had the main volatile compound 1-hexadecanol (22.45%). The results of the ethanol extract of peel contained 2-furan carboxaldehyde compounds (34,34%), and 2-Furancarboxaldehyde (4,82%). These compounds form antimicrobial agents, also used in the preparation of anti-inflammatories, also used in the preparation of anti-inflammatory diarylpentanoid analogues. Furthermore, it contains 4H-pyran-4-one (3.19%) belongs to the flavonoids, which are classified as high anti-oxidants (Hameed *et al*., 2015) and improve body health (Čechovská *et al*., 2011).

Pomegranates have bioactive substances that can enhance the nutritional profile of poultry diets, especially their by-products such as peel extracts. These substances have been found to have a good impact on broiler performance (Lioliopoulou *et al*., 2023).

CONCLUSIONS AND RECOMMENDATIONS

Pomegranate peel ethanol extract contains bioactive substances such as polyphenols (12.34 mg/g), flavonoids (5.57 mg/g), and tannins (243 mg/g) showed potential antioxidant activity (42.03%). Pomegranate peel ethanol extract is a valuable resource of chemical, bioactive compounds, and functional properties to be applied to animal feed. These findings suggest the use of pomegranate peel ethanol extract in poultry feed provides health benefits. The pomegranate peel ethanol extract should be used as a natural feed additive instead of antibiotics.

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NOVELTY STATEMENT

Nutritionists will be able to develop more efficient diets that increase poultry performance while maintaining animal welfare if they have a better understanding of the precise metabolites responsible for the reported benefits. This study verified the part of pomegranate fruit and the most optimal extraction method and findings ethanol extract of pomegranate peel as a feed additive in poultry presents a promising avenue for enhancing poultry health and production.

AUTHOR'S CONTRIBUTIONS

Merry Muspita Dyah Utami: conceptualization, methodology, writing review, and editing, supervised this research.
Aryanti Candra Dewi: data curation, writing original draft preparation
Rosa Tri Hertamawati: project administration, collected and processed research data.
Dillenia Jasmine, Nala Wafia, Jini Saputri, Kornelius hangga Septiyanto: collected and processed research data and compiled the article.
All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Determination of chemical, bioactive compounds and volatile composition in different extracts and the parts of pomegranate (punica granatum l.) as a natural feed additive

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