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The Effect of N-Hexane, Ethyl Acetate, and Water Fractions of Peanut Seeds (Arachis Hypogaea L.) on Antioxidant Activity with the DPPH Method

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KEYWORDS

peanut seeds, DPPH, EC50 (ppm), quercetin

ABSTRACT

Free radicals are among the main causes of various degenerative diseases, such as cardiovascular disorders, hypertension, stroke, liver cirrhosis, cataracts, diabetes mellitus, and cancer. This study aimed to determine the antioxidant activity, maximum concentration, *EC50* (ppm), and the differences in antioxidant activity of the *n-hexane* fraction, ethyl acetate fraction, and water fraction of peanut seeds *in vitro*. The extraction method used was *remaceration* with 96% ethanol solvent for 3 days, followed by fractionation to separate the compounds. Each fraction was tested through phytochemical screening and thin-layer chromatography (*KLT*), and quantitative analysis of antioxidant activity was carried out using the DPPH method at a maximum wavelength of 517.5 nm. The results showed that the average extract yield was 24.15%, with the *n-hexane*, ethyl acetate, and water fractions producing 20.33%, 25.61%, and 33.28%, respectively. Phytochemical screening indicated positive results for flavonoids, which contain antioxidants *in vitro*. The average *EC50* values for standard quercetin, the *n-hexane* fraction, ethyl acetate fraction, and water fraction were 12.02 ppm, 10.62 ppm, 12.25 ppm, and 11.75 ppm, respectively. Statistical testing using one-way ANOVA (*ANAVA I*) showed a significance value greater than 0.05 (p = 0.105), indicating that the differences were not statistically significant.

INTRODUCTION

The danger of exposure to free radicals causes cell damage that can lead to various diseases such as cancer, cardiovascular disorders, and premature aging. Free radicals can be counteracted by compounds that act as antioxidants. However, concerns about the side effects of synthetic antioxidants, which may be carcinogenic, have made natural antioxidants a preferred option (Sri, 2013). The body therefore requires compounds called antioxidants that can capture and neutralize free radicals, stopping the chain reactions that cause oxidative stress, preventing cell damage, or inhibiting the induction of disease. Antioxidant termination reactions usually occur by capturing hydroxyl (*OH*) radicals during the peroxidation stage of fats, proteins, or other molecules in normal cell membranes, thereby preventing damage (Maharani et al., 2021).

Antioxidants are essential in the human body, but additional antioxidants from external sources are also needed. Examples of natural antioxidant enzymes in the body include catalase, superoxide dismutase (*SOD*), glutathione peroxidase, and glutathione S-transferase (Lobo et al., 2010). Antioxidants from food and beverages can be natural—such as those contained in vegetables, fruits, and drinks—or synthetic, which are deliberately added as additives. Butyl Hydroxy Anisol (*BHA*), Butyl Hydroxy Toluene (*BHT*), Propyl Galate (*PG*), and Tert-Butyl

Hydroxy Quinone (*TBHQ*) are synthetic antioxidants widely used in foods and beverages. However, according to Amarowicz, cited in Parwata (2016), long-term use of synthetic antioxidants may cause side effects such as inflammation, liver damage, and increased risk of carcinogenesis in test animals.

Increased consumption of natural antioxidants from fruits, vegetables, flowers, and other plant sources can help prevent degenerative diseases. Micronutrients such as vitamins A, C, and E, folic acid, anthocyanins, phenolic compounds, and flavonoids are effective substitutes for synthetic antioxidants. This is supported by research showing that peanut seeds contain phenolic compounds, carotenoids, and vitamin C, which function as natural antioxidants (Annadira et al., 2021). Peanuts are an important crop cultivated worldwide, mainly for oil production. However, their by-products contain beneficial substances such as protein, fiber, polyphenols, antioxidants, vitamins, and minerals, which are widely used as additives in processed foods. In addition, peanuts contain bioactive compounds including resveratrol, phenolic acids, flavonoids, and phytosterols, which function to prevent cholesterol absorption from food. Peanuts also contain 20 amino acids, with arginine being the highest, and are a good source of Co-enzyme Q10. These bioactive compounds are known to have disease-preventing properties and may contribute to longevity. Processing methods such as roasting and boiling have been shown to increase the concentration of these compounds (Arya et al., 2016).

External oxidants can still cause cell damage that endogenous antioxidants alone cannot prevent. This imbalance allows free radicals to easily enter the body and cause harm. Peanuts (*Arachis hypogaea L.*), originally from Brazil (South America), have spread to many tropical and subtropical regions. In Indonesia, peanuts are a highly strategic agricultural commodity, especially on the islands of Java, Nusa Tenggara, Sulawesi, South Sumatra, and North Sumatra, where they generate significant income for farmers on dry land (Zulchi & Puad, 2017). The extraction method used in this study is *remaceration*, chosen for its simplicity, practicality, and ability to provide high yields. Following extraction, fractionation is performed to separate compounds based on polarity. In principle, polar compounds are extracted with polar solvents, while non-polar compounds are extracted with non-polar solvents (Harborne, 1987). This study uses *n-hexane*, which is effective for extracting non-polar compounds due to its volatility, stability, and selectivity. Ethyl acetate is used because it is semi-polar, meaning it can attract both polar and non-polar mixtures, is volatile, and has a low hazard level. Water is used as a polar solvent.

The *n-hexane*, ethyl acetate, and water fractions were tested using *KLT* (Thin Layer Chromatography), a phytochemical separation method based on adsorption, partition, or both. This technique employs a thin, uniform layer on a plastic, aluminum, or glass plate coated with an adsorbent (stationary phase). When the mobile phase passes through the adsorbent, chromatographic development occurs. The antioxidant content in peanuts is largely found in the seed shells in the form of phenolic compounds such as resveratrol, catechin, epicatechin, and quercetin. Phenolic compounds are characterized by one or more hydroxyl groups attached to aromatic rings. There are more than 8,000 known types of phenolic compounds, ranging

from simple, low-molecular-weight structures to complex molecules exceeding 30,000 Da (Annadira et al., 2021).

This study uses the DPPH method to evaluate antioxidant activity, as this method is simple, fast, sensitive, and effective for samples with low concentrations. The principle of the DPPH method is that antioxidant compounds donate hydrogen atoms to DPPH radicals, reducing them to a non-radical form. Antioxidants are vital because they donate electrons to oxidant compounds, thereby neutralizing harmful effects. While some antioxidants are produced endogenously, the body requires additional antioxidants from external sources, especially plant-based foods rich in polyphenols and flavonoids (Annadira et al., 2021).

Based on this background, the present study aims to evaluate the differences in antioxidant activity among the *n-hexane*, ethyl acetate, and water fractions of peanut seed extract *in vitro*. Specifically, this study addresses three main questions: (1) do the fractions of *n-hexane*, ethyl acetate, and water from peanut seeds exhibit antioxidant activity *in vitro*? (2) what are the *EC50* values of each fraction, reflecting antioxidant activity? and (3) is there a significant difference in antioxidant activity among the three fractions? The objectives are to determine the antioxidant activity of each fraction, establish their *EC50* values, and assess the differences in activity. The findings are expected to provide valuable information to the public regarding the potential of peanut seeds (*Arachis hypogaea L.*) as a natural antioxidant source, contribute to scientific development, and serve as a reference for future studies.

RESEARCH METHOD

The object of this study was the *EC50* value (ppm) of the *n-hexane*, ethyl acetate, and water fractions of peanut seeds (*Arachis hypogaea L.*), which was measured using the DPPH method. The peanut seeds used were of the BIMA variety, obtained from Pasucen, Trangkil, Pati, Central Java. The sampling technique applied was simple random sampling, which provided each member of the population with the same opportunity to be selected. The study variables consisted of independent variables, namely the fractionation of ethanol extract of peanut seeds (*n-hexane*, ethyl acetate, and water); dependent variables, which included the antioxidant reaction of ethanol extract against the *EC50* ppm parameter; and controlled variables, which included a sample weight of 150 grams, the *remaceration* extraction method, a soaking duration of 3 × 24 hours, 96% ethanol solvent, and antioxidant reaction measurement using the DPPH method.

The tools used in this study included beakers, measuring cylinders, and a UV-Vis spectrophotometer, while the materials required included *n-hexane* fractions and reagents such as DPPH, ethanol, and HCl. The work procedure began with plant determination, material collection, preparation of *simplicia*, and production of ethanol extract, followed by phytochemical screening to identify active compounds. Quercetin flavonoid assays were conducted using *KLT* (Thin Layer Chromatography), followed by determination of the antioxidant activity of the extract and the ethanol fractions of peanut seeds using the DPPH method. This process involved the preparation of DPPH solution, preparation of sample solutions, and absorbance measurement to calculate the inhibition percentage, which served as an indicator of the antioxidant potential of the extract.

RESULT AND DISCUSSION

The purpose of this study is to determine the effect of the administration of n-hexane fraction, ethyl acetate fraction, and water fraction of peanut seed extract on antioxidants using the DPPH method, as well as to determine the concentration of n-hexane fraction, ethyl acetate fraction, and water fraction of peanut seed extract containing antioxidants with the DPPH method. and empirically there are antioxidants.

Free radicals can be obtained from inside and outside the body. Free radicals from inside and outside the body, namely ROS (reactive oxygen species). ROS comes from the respiratory burst of activated macrophages. This causes the use of glycosis to increase for its reduction from NADP to NADPH through cross-pentose phosphate. To oxidize NADPH and produce superoxide it is necessary to increase oxygen consumption and to kill microorganisms that have been phagocytes need radical halogens as cytotoxic agents.

Mitochondria are important in mitochondrial production in various disease pathogenesis. Because the formation of ROS in the mitochondrial daklam is triggered by chronic leakage of the normal respiratory chain and intra-mitochondrial respiratory bursts, cytoplasms, and ROS that come from the outside.

The source of ROS formation from the outside, namely radiation from X-rays and ultraviolet rays, can both liquefy water into OH radicals. Metal ions such as Fe2+, CO2+, and CU+ also react with oxygen or hydrogen peroxide (Fakriah, Kurniasih., Eka, Adriana., Rusydi. et al., 2019).

Free radicals can attack susceptible compounds such as lipids and proteins, resulting in dangerous diseases. Free radicals threaten the health of the body because of their reactive and unstable nature, free radicals react with the closest molecules after entering the body and produce other free radicals, so that they become a chain reaction that can threaten the health of the body, for example the formation of cancer. The important role of free radicals in tissue damage and also pathological processes in living organisms (Khaira, 2010).

The sample used was peanut seeds from peanut plants that had been determined at the Pharmaceutical Biology Laboratory, College of Pharmaceutical Sciences, Yayasan Pharmacy Semarang. Plant determination aims to identify and to find out the correctness of the sample that will be used for research, so that there are no errors and the collection and collection of plant samples. The determination results can be ascertained that the plants used in this study are peanut plants shown by the plant determination letter presented in appendix 1.

The peanut seeds used come from the village Pasuken, Trangkil District, Pati Regency, Central Java, bima variety. Peanut seeds that can be sorted. The next step is to wash with running water to remove dirt and dry with a dryer. The purpose of drying is to reduce moisture content, prevent the growth of mold and mildew, and prevent enzymatic reactions by eliminating enzyme activity that can decompose the content of active substances so that simplicia is not easily damaged and can be stored in the long term (Ministry of Health of the Republic of Indonesia, 1986). The use of a drying machine aims to prevent contamination of simplicia with direct UV rays that can damage the active substances in it, avoiding contamination or the entry of insects and dust that can damage simplicia. The powder is sifted

using sieve number 30 and sieve number 40. This aims to obtain a uniform particle size with a large surface area (passing sieve number 30) but the size of the powder is not too small (it does not pass sieve number 40) because the belt size is too small it is feared that clumping will occur at the time of extraction (Helwig et al., n.d.).

Simplicia powder sifting with a 30/40 mesh sieve aims to equalize the particle size because the size of the powder particles can affect the extraction process of a simplicia. The small particle size of the powder can expand the contact surface between the cell wall and the filter fluid, so that the active substances contained in peanut seeds can be dissolved in the filter fluid. Simplicia powder that is too fine can interfere with the filtering process because it will form a compact period that is dense and difficult for the filtering liquid to pass. Simplicia powder that is too coarse has an effect on inhibiting the penetration process of the filter fluid in penetrating the cell cavity containing the active compound (Ministry of Health of the Republic of Indonesia, 1986: 10).

The extraction process is carried out using the remaceration method. Remaceration is a modification of the maceration method. The principle of this method is the immersion of simplicia with a suitable softening fluid, for which every day the softening fluid must be replaced new. Replacement of the filter fluid aims to obtain an extract with the maximum content of active compounds because the withdrawal of these compounds occurs optimally. Remaceration is carried out by soaking simplicia powder in a container for 3 days and replacing the solvent every 1 x 24 hours. The remaceration process requires periodic stirring so that the active substance can enter the cell cavity. Used in simplicia powder, there are secondary metabolites, most of which are dissolved in a filter solution (Rahmawati, 2020).

The separation of active compounds from within the plant through a method called extraction. Extraction is a way of separating compounds from their mixtures that usually use a specific solvent with the principle of difference in solubility. According to Winarno et al. (1973), the mechanical extraction method aims to separate the mixture into separate components. Each component has a considerable difference in solubility in the solvent. The manufacture of peanut seed extract is chosen by the repeated maceration or remaceration method. The remaceration method was chosen because it can obtain more extracts and attract more active compounds so that the sample can be extracted perfectly. Efforts to prevent saturation during filtration are carried out by changing the same type of solvent every step of remaceration (Edy et al., 2016). 50 grams of peanut seed powder x 5 soaked with 96% ethanol solvent of 1500 ml x 5 left for 1 x 24 hours while stirring occasionally. 1x24 hours are separated between the filtrate and its residue using filter paper. The result obtained between the filtrate and the residue, the residue is again macerated with a new 96% ethanol solvent for 1 x 24 hours. Once the time has reached the maximum time limit, the filtrate and residue are separated with filter paper. The total filtrate is evaporated using a rotary evaporator so that a thicker filtrate is obtained.

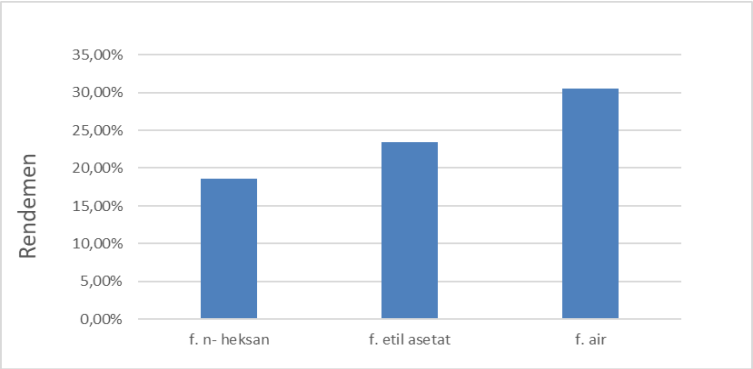

Figure 1. Yield of n-Hexane Faction, Ethyl Acetate Faction, and Water Faction

Ethanol is 96% the solvent used in this study. According to Masyhuri (2012) research, 96% ethanol is very suitable for extracting peanut seeds because ethanol has a safe tendency to have a low boiling point and has a high polarity level, and produces greater yield and antioxidant levels in peanut seeds compared to non-polar solvents such as n-hexane. The remaceration process is carried out for 3 days, the results of the remaceration are collected into one then the maserrate is concentrated using a rotary evaporator to obtain a concentrated extract. The choice of using a rotary evaporator is because it has a small effect on the damage of compounds in the extract, as well as the small possibility of contact with air so that the condensation method occurs faster, and the solvent used can be recovered. The temperature used in rotary evaporators is 70o C because the boiling point of 96% ethanol is less than 78.4°C (Ministry of Health of the Republic of Indonesia, 1993:83). The results are thickened in a waterbath until a thick extract is obtained, then weighed to obtain a yield.

The first step is to conduct an ethanol-free test. This test is carried out to find out that the condensed extract is free of ethanol, meaning there is no solvent left.

Table 1. Ethanol-Free Yield in Peanut Seed Ethanol Extract

Test	Treatment	Positive results	Research results	Information
Smell test	Extract + acetic acid + H2SO4 (p)	Ester smell	No ester odor	(-)
Smell test	Extract + salicylic acid + H2SO4 (p)	Ester smell	No ester odor	(-)
Color test	Extract + sulfanilac acid + HCl+ NaOH	Solution red frambos	Solution of non-red frambos	(-)

Ethanol-free test odor test treatment is based on an esterification reaction, where carboxylic acid compounds are acetic acid and salicylic acid. If added with ethanol, it will produce a volatile ester characterized by the distinctive smell of each ester (Dwipa et al., 2014).

Thick extracts are carried out a fractionation process that aims to separate the compounds in the sample based on their polarity. Non-polar solvents can dissolve non-polar compounds while polar solvents can dissolve polar compounds. A thick extract of peanut seeds is dissolved first with aquades. N-hexane is inserted in a separation funnel that already contains viscous and aquiadeas. Scrubbed until the n-hexane fraction is cloudy. Remove the n-hexane fraction and water. Repeat until the n-hexane fraction is clear. Ethyl acetate is inserted into a separate funnel

containing a water fraction, scrubbed until the ethyl acetate fraction becomes cloudy. Repeat until the ethyl acetate fraction becomes clear. All fractions are in a rotary evaporator and heated in a waterbath until a thick fraction is obtained (Runtuwene et al., 2021).

Color reactions were identified to n-hexane fractions, ethyl acetate fractions and water fractions to find out what compounds each fraction can attract. The results of the identification of the color reaction of the n-hexane fraction, the ethyl acetate fraction, and the water fraction of peanut seeds are shown in table 2.

Table 2. Results of Identification of Reactions of n-Hexane Fraction, Ethyl Acetate Fraction, and Water Fraction of Peanut Seeds

Test	Treatment	Faction *n-Hexane*	Ethyl acetate fraction	Water fraction	Negative control (-)	Result
Flavonoid	Sample+powder +Mg+HCl(p)+ Amil alcohol	(+) Ring-shaped, orange color	(+) Ring-shaped, orange color	(+) Ring-shaped, orange color	No rings formed, clear solution	All fractions contain flavonoids
Phenolic	Sampel+FeCl3	(+) Brownish -green color formed	(+) Brownish -green color formed	(+) Formed brownish -green color	not Brownish -green color formed	All fractions contain phenolics
Saponins	Sample+hot water+HCl 2N	(+) In other words, it is stable	(+) In other words, it is stable	(+) In other words, it is stable	Unstable foam	All fractions contain saponins
Tannins	Sample+gelatin 0.5%	(+) Gelatin deposits are formed	(+) Gelatin deposits are formed	(+) Gelatin deposits are formed	No gelatin deposits	Aemua fraction contains tannins
Alkaloid	Sampel + HCl 2N+ air + mayer	(+) Brown deposits are formed	(+) Brown deposits are formed	(+) Brown deposits are formed	No deposits formed	All factions Contains alkaloids
Alkaloid	Sample + HCl 2N+air+bouchardat	(+) White deposits are formed	(+) White deposits are formed	(+) White deposits are formed	No deposits formed	All fractions contain alkaloids
Polyphenols	Polyphenol KfeCN + FeCl3	(+) Blue solution	(+) Blue liquid	(+) Blue solution	Larutan bening	All fractions contain polyphenols
Terpenoid/ Steroids	Samples+ether+concentrated sulfuric acid+anhydrous acetic acid	(+) Brown	(+) Brown	(+) Brown	Colorless	All fractions contain terpenoids

The flavonoid color reaction test uses the wilstater method, which uses Mg metal. In the Wilstater method, red, yellow and orange colors are formed by flavones or isoflavones . The results of the n-hexane fraction, ethyl acetate fraction, and water fraction testing produced an orange color on the amyl alcohol layer, this allows it to contain flavones or isoflavones (Arreneuz et al., 2015).

The identification of phenolic compounds aims to determine the presence or absence of phenolic groups in the n-hexane fraction, ethyl acetate fraction, and water fraction of peanut seeds. The identification of phenolic compounds is carried out by means of a sample dripping with a solution of FeCl3, FeCl3 reacts with phenolic groups to form green, purple, blue and black colors (Robinson, 1995:87). Compounds containing phenol groups will form Fe3+ bonds resulting in Fe-Phenolate complexes that have 3 ionic bonds and 3 covalent coordination bonds (Mutiara and Wildan, 2013). The reaction between phenolic compounds and FeCl3 can be seen in figure 2.

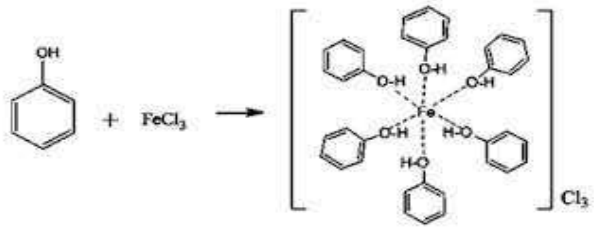

Figure 2. Reactivity between Phenolic Compounds and FeCl3 (Bayani, 2016)

Saponin identification is done by adding hot water to the sample in this case the n-hexane fraction, the ethyl acetate fraction, and the water fraction are then vigorously shaken for 10 seconds. A steady foam is formed 1-10cm high, not less than 10 minutes and does not disappear with the addition of 2N hydrochloric acid indicating the presence of saponins (Sulistyarini et al., 2019).

Active compounds whose surfaces can cause foam if shaken in water are polar because they are in the form of glycosides. These saponins have polar and non-polar groups so that micelles are formed. This micellar is formed so that it causes a non-polar group to face inwards and vice versa a polar group facing outwards, this is what results in the formation of foam (Padmasari et al., 2013).

Tannin identification is carried out by adding a gelatin solution in the sample. The positive result is the formation of white deposits. This is because the formation of copolymers so that they are not soluble in water resulting in the reaction of gelatin, which is a protein caused by tannins (Harbone, 1996).

Alkaloid testing was carried out by adding Bouchardat solution and Mayer solution to samples that are n-hexane fractions, ethyl acetate fractions, and water fractions from peanut seeds. The presence of brown deposits in the bouchardat solution and white deposits in the Mayer solution indicates the presence of alkaloids (Rumape et al., 2023).

Metal ions with the formation of coordinate covalent bonds are useful when alkaloids contain nitrogen atoms. The formation of potassium alkaloid complex deposits is caused by the reaction between the k+ metal ions and nitrogen present in the alkaloid by tetraiodomercurates

(II), this is an estimate in alkaloid experiments using a Mayer reagent. The aim is to find coordinate covalent bonds found in nitrogen atoms that have free electrons present in alkaloids. The formation of red deposits of mercury (II) iodide occurs due to the reaction of a solution of mercury (II) chloride with potassium iodide. So that the Mayer reagent is formed. If the compound contains alkaloids it will form a white precipitate predicted it is a potassium alkaloid complex.

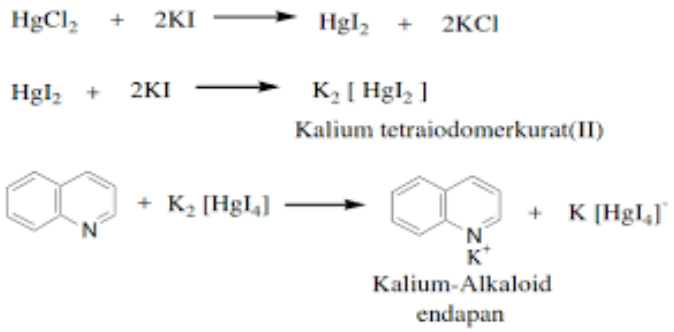

Figure 3. Alkaloid Reaction with Mayer Reagent (Adeanne C. Wullur, Jonathan Schaduw, 2017)

The polyphenol compound test was carried out by means of a sample dripping with a mixture of K3(Fe(CN)6) and FeCl3. Polyphenol compounds will reduce hexasianoferic ion III to hexasianoferic ion II. Hesasianoferic ion II will react with FeCl3 to form Kfe (Fe(CN)6) (Wahyuni and Aini, 2021). The reaction is shown in the picture below:

$$Fe(CN)_{63-} + ArOH \longrightarrow Fe(CN)_{64-} + ArO^{-} + H+$$
$$Fe(CN)_{64}^{-} + Fe^{3+} + K+ \longrightarrow KFe(Fe(CN)_6)\ \text{(Prussian blue)}$$

Figure 4. Reaction of Polyphenol Compounds with Hexasianoferrate Ions II
(Mutiara and Uning, 2019)

Identification of triterpenoids and steroids was carried out by adding samples with concentrated sulfuric acid and anhydrous acetic acid. The positive results shown in the triterpenoid test are the formation of red, purple, and brown colors, while the positive results shown in the steroid test are blue or purple. All samples showed positive results for triterpenoids (Habibi et al., 2018).

Triterpenoids are based on the ability of the compound to form colors with concentrated sulfuric acid in anhydrous acetic acid solvents. The positive effects of triterpenoids in the sample were characterized by the formation of a brownish color. This color change is caused by the oxidation reaction in the triterpenoid and steroid groups through the formation of conjugated double bonds (Dewi et al., 2013: Tomahayu, 2014).

The motion phase used in the flavonoid test was toluene:ethyl acetate:formic acid (4:3:0,2). In the quercetin standard, the n-hexane fraction, the ethyl acetate fraction, and the peanut seed water fraction form a yellow stain color after being given ammonia vapor spots. This shows that the extract, the n-hexane fraction, the ethyl acetate fraction, and the water

fraction contain flavonoid compounds. Based on the color and Rf value obtained by the standard quercetin, the fraction contains flavonoids (Robinson, 1995:211).

The yellow color that forms in flavonoids is phenolic when reacted with a base such as ammonia, will form color. This color occurs due to the conjugation system of phenolic groups (Markham, 1988).

Table 3. KLT Test Results of Flavonoid Compounds

Sample	Phase of motion	UV light 245nm (Rf)	Sinar UV 245nm (color)	Uap ammonia (RF)	Uap ammonia (color)	Information
Baku kuersetin	Toluene:ethyl acetate:formic acid (3:4:0,2)	0,56	Purple	0,56	Yellow	+ contains flavonoids
Faction N-Hexan	Toluene:ethyl acetate:formic acid (3:4:0,2)	0,50	Purple	0,50	yellow	+ Contains flavonoids
Ethyl acetate fraction	Toluene:ethyl acetate:formic acid (3:4:0,2)	0,50	Purple	0,50	Yellow	+ contains flavonoids
Water fraction	Toluene:ethyl acetate:formic acid (3:4:0,2)	0,56	Purple	0,56	Yellow	+ contains flavonoids

Description: Flavonoids are positive if they form a light yellow to orange color after being treated with ammonia vapor (Robinson, 1995:211).

The change in color from purple to yellow due to the donation process of hydrogen or electron donation accompanied by a decrease in the absorbance of 2,2-diphenyl-1-pykrihydrazil proportional to the change in color gradation is the reaction principle of the DPPH method. The greater the decrease in absorbance is due to the greater the activity of antioxidant compounds in DPPH radical capture. DPPH free radicals will be captured by flavonoid compounds and flavonoids will be oxidized by free radicals resulting in a more stable radical form with low reactivity. The presence of antioxidant compounds results in a decrease in absorbance from 2,2-diphenyl-1-pykrihydrazil radicals which are measured using UV-Vis spectrophotometers which is one of the principles of the DPPH method. Absorbance calculation at the standard operating time of the quercetin comparator for 12 minutes with a maximum wavelength of 517.5 nm (Hasan et al., 2022).

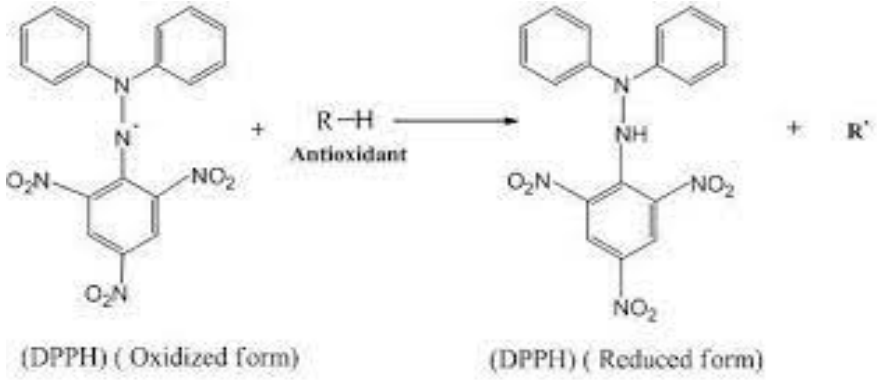

Figure 5. Reaction of DPPH with Antioxidants (Molyneux, 2004)

The DPPH method was chosen because it has several advantages, namely it can be applied directly to samples without the need for the addition of additional compounds or reagents, it is sensitive and requires only a small sample for the evaluation of antioxidant activity from natural ingredient compounds, commonly used to test the antioxidant activity of plants using ethanol, methanol, and water solvents. In addition, the DPPH method has a linear relationship between antioxidant concentration and the resulting decrease in absorbance.

The DPPH method has a weakness, namely that DPPH radicals and other radical compounds can react. The highly reactive molecule of 2,2-diphenyl-1-pykrilhydrazil can only be soluble in organic solvents especially alcohol and methanol so it cannot be reacted with uncompounded ones using solutions of natural origin, highly reactive molecules of 2,2-diphenyl-1-pykrihydrazil are sensitive to compounds containing lewis bases, the processing time is longer because it requires a longer OT than the FRAP method, The shelf life of DPPH is shorter than FRAP, which is 1x24 hours at cold temperatures and the absorbance of DPPH in methanol can be reduced when exposed to light (Kedare and Singh, 2011).

The weakness of the DPPH method experienced in this study, which is related to light, can be overcome by storing DPPH in a measuring flask wrapped in aluminum foil. The instability of DPPH to temperature was overcome by storing DPPH in a basin filled with ice cubes during the research process (Badaring et al., 2020).

The sample is measured using visible light using a visible spectrophotometer because the sample to be measured is a color sample that can be absorbed in the wavelength range of 400-800 nm. The measurement begins with a search for the maximum wavelength, with the aim of finding out the wavelength produced by the maximum absorbance. The measurement results in this study obtained a maximum wavelength of 517.5 nm. This is consistent with the color visible light spectrum which states that the purple color in the DPPH solution can be absorbed at a wavelength of 517.5 nm.

The next step is to determine the operating time which is used to determine a stable measurement time, which is when it reacts perfectly with the reagent, so that the maximum absorbent is obtained. Based on the measurement results, the operating time was obtained at 12 minutes.

The n-hexane fraction, ethyl acetate fraction and peanut seed water fraction were measured in antioxidant activity with the aim of finding out whether the three fractions have antioxidant ality. The experiment was preceded by measuring the initial concentration of the DPPH solution to quantitatively determine the concentration of the DPPH solution before it was added with the n-hexane fraction, the ethyl acetate fraction, and the peanut seed water fraction. The purpose of DPPH solution measurement is to quantitatively determine the concentration of DPPH solution. The n-hexane fraction, ethyl acetate fraction, and water fraction are dissolved in methanol because the quercetin raw used for the experiment is also dissolved with methanol so that the n-hexane fraction, ethyl acetate fraction, and water fraction can mix and react with DPPH (Maravirnadita, 2019).

The concentration of fraction samples used included 4,6,8,10,12 ppm. And each of these concentrations is taken 2 ml of DPPH put in a test tube and then add 1 ml of sample mixed with the vortex.

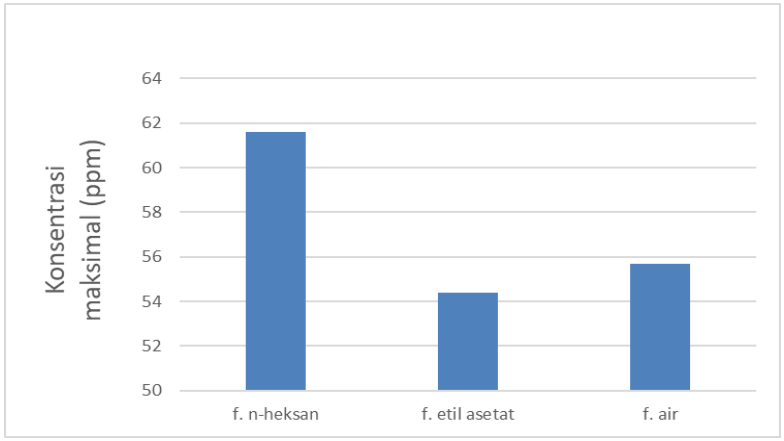

Figure 6. Maximum Concentration of n-Hexane Fraction, Ethyl Acetate Fraction, and Peanut Seed Water Fraction

The test solution is left in a dark place protected from light for 12 minutes according to its operating time. The goal is to stay in a dark place to be protected from the light. Read the absorption with the UV-Vis 1700 spectrophotometer at a wavelength of 517.5 nm. The absorption of the concentration of n-hexane fraction, ethyl acetate fraction, and water fraction of peanut seed extract is then calculated as a percent inhibition up to EC50 can be seen in Figure 22.

The data analysis in this study is statistically using the statistical product and service solution version 23 with a confidence level of 95% which begins with a normality and homogeneity test, the purpose of the normality test is to find out the normal distribution data or not while the homogeneity test is to find out the variety of treatment between homogeneous groups or not. This study obtained the results of n-hexane fraction, ethyl acetate fraction, and water fraction from peanut seed extract with normal and homogeneous distribution with a significance value of more than 0.05 (appendix 17)

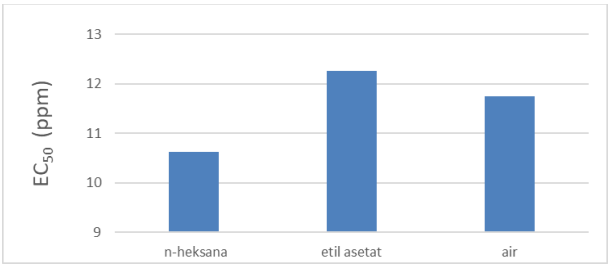

Figure 7. EC50 n-Hexane Faction, Ethyl Acetate Faction, and Peanut Seed Water Faction

The effect of n-hexane fraction, ethyl acetate fraction, and water fraction of peanut seed extract on antioxidants was carried out 1-way anava test. Preceded by the normality test and the homogeneity test. The condition of the data normality test is that if the significance (probability of error) is less than or equal to 0.05, then the sample is abnormal, but if the significance (probability of error) is more than 0.05, then the sample is declared normal. The data normalization test with the shapiro-wilk test, obtained a result greater than 0.05 which indicates that the distribution data is normal. The purpose of the data normality test is to find out whether the data is normal or not. The test results found that the n-hexane fraction, ethyl acetate fraction, and water fraction of peanut seed extract were distributed normally because the significance value was more than 0.05.

The next test was carried out with a 2-way anava test. The results of the 2-way anava test showed the influence of the n-hexane fraction, ethyl acetate fraction, and water fraction of peanut seed extract on antioxidants marked with a significance value of less than 0.05, namely to find out the location of the difference in influence between each of the influence concentrations of n-hexane fraction, ethyl acetate fraction, and water fraction from peanut seed extract, post hock test was carried out. The results of the post hock test between the antioxidant groups after the addition of n-hexane fraction, ethyl acetate fraction, and water fraction are shown in table 4.

Table 4. Post Hock Test Results Between Antioxidant Groups After the Addition of N-Hexane Fraction, Ethyl Acetate Fraction, and Water Fraction

	KUERSETIN					**N-HEKSANA *Faction***					**ETHYL ACETATE FRACTION**					**WATER FRACTION**				
	4	6	8	10	12	4	6	8	10	12	4	6	8	10	12	4	6	8	10	12
4		BS	BS	BS	BS	TBS	TBS	BS	BS	BS	TBS	TBS	BS	BS	BS	TBS	BS	BS	BS	BS
6	BS		BS	BS	BS	BS	TBS	BS	BS	BS	BS	TBS	BS	BS	BS	BS	TBS	BS	BS	BS
8	BS	BS		BS	BS	BS	BS	TBS	BS	BS	BS	BS	TBS	BS	BS	BS	BS	BS	BS	BS
10	BS	BS	BS		BS	BS	BS	BS	BS	BS	BS	BS	BS	TBS	BS	BS	BS	BS	BS	TBS
12	BS	BS	BS	BS		BS	BS	BS	BS	TBS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS
4	TBS	BS	BS	BS	BS		BS	BS	BS	BS	TBS	BS	BS	BS	BS	TBS	BS	BS	BS	BS
6	BS	TBS	BS	BS	BS	BS		BS	BS	BS	BS	TBS	BS	BS	BS	BS	BS	BS	BS	BS
8	BS	BS	TBS	BS	BS	BS	BS		BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS
10	BS	BS	BS	BS	BS	BS	BS	BS		BS	BS	BS	BS	BS	BS	BS	BS	BS	TBS	BS
12	BS	BS	BS	BS	TBS	BS	BS	BS	BS		BS	BS	BS	BS	BS	BS	BS	BS	BS	TBS
4	TBS	BS	BS	BS	BS	TBS	BS	BS	BS	BS		BS	BS	BS	BS	TBS	BS	BS	BS	BS
6	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS		BS	BS	BS	BS	BS	BS	BS	BS
8	BS	BS	TBS	BS	BS	BS	BS	BS	BS	BS	BS	BS		BS	BS	BS	BS	BS	BS	BS
10	BS	BS	BS	TBS	BS	BS	BS	BS	BS	BS	BS	BS	BS		BS	BS	BS	BS	BS	BS
12	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS		BS	BS	BS	BS	BS

	KUERSETIN					N-HEKSANA *Faction*					ETHYL ACETATE FRACTION					WATER FRACTION				
4	TBS	BS	BS	BS	BS	TBS	BS	BS	BS	BS	TBS	BS	BS	BS	BS		BS	BS	BS	BS
6	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS		BS	BS	BS
8	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS	BS		BS	BS
10	BS	BS	BS	BS	BS	BS	BS	BS	TBS	BS	BS	BS	BS	BS	BS	BS	BS	BS		BS
12	BS	BS	BS	BS	TBS	BS	BS	BS	BS	BS	TBS	BS	BS	BS	BS	BS	BS	BS	BS	

Table 4 can imply that most of the groups of n-hexane fractions, ethyl acetate fractions, and water fractions of peanut seed extracts differ significantly. Based on the calculation of the post-anava test between groups, most of the data showed a significant difference, which was significantly less than equal to 0.05, so it can be concluded that each group of n-hexane fraction, ethyl acetate fraction, and water fraction of peanut seed extract had differences in antioxidant tests.

CONCLUSION

From the results of the experiments conducted, it can be concluded that there is antioxidant activity in the *n-hexane* fraction, ethyl acetate fraction, and water fraction of peanut seed extract *in vitro*. The average *EC50* (ppm) values obtained for standard quercetin, the *n-hexane* fraction, ethyl acetate fraction, and water fraction were 12.02 ppm, 10.62 ppm, 12.25 ppm, and 11.75 ppm, respectively. Statistical testing showed a significance value of 0.105, indicating that the differences in antioxidant activity among the *n-hexane*, ethyl acetate, and water fractions were not statistically significant. Based on these findings, the researcher suggests that further experiments should be conducted on isolated compounds from peanut seeds to better evaluate their antioxidant properties. In addition, testing on experimental animals is recommended to determine the effects of peanut seed fractions on antioxidant activity.

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