IN VITRO, OVERVIEW OF THE ANTIOXIDANT ACTIVITIES FROM THE PINEAPPLE, (*Ananas comosus* (L) Merr): A REVIEW ARTICLE

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ABSTRACT: *Free radicals are reactive oxygen compounds that have unpaired electrons and look for their partners by binding to electron molecules around them. Many diseases are caused by an excess of free radicals. (Ananas comosus (L) Merr) is a plant that has been recognized worldwide as a potential anti-antioxidant. This has been proven from several studies where testing the antioxidant activities using different solvents and with several methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazole-6-sulfonic acid (ABTS), ferrous reducing antioxidant power (FRAP), and using Indian mackerel steak was found almost equal to its inhibitory potential as compared to its standards used, such as gallic acid and BHA.*

Keywords*: Free radicals, antioxidants, Ananas comosus*

INTRODUCTION

Free radicals can be defined as molecules or molecular fragments that contain one or more unpaired electrons in their outer atomic or molecular orbitals and are capable of existing independently [1]. The unpaired electrons produce certain general properties shared by most radicals. An odd number of electrons makes radicals unstable, short-lived, and highly reactive [2, 3] Free radicals are divided into two types, both reactive oxygen species (ROS) and reactive nitrogen species (RNS), originating from endogenous sources (mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells, etc.) and exogenous sources (pollution, alcohol, smoke). tobacco, heavy metals, transition metals, industrial solvents, pesticides, certain drugs such as halothane, paracetamol, adriamycin, bleomycin, mitomycin C, nitrofurantoin, and chlorpromazine. and radiation [3,4].

Around 10,000-20000 free radicals attack every cell every day some of which are good for health which allows the human body to fight inflammation, kill bacteria, control smooth muscles that regulate the functioning of internal organs and blood vessels [4]. In other conditions, free radicals cause oxidative stress which is reported to be involved in several conditions such as diabetes mellitus, neurodegenerative disorders (Parkinson's disease, Alzheimer's disease, and Multiple sclerosis), cardiovascular disease (atherosclerosis and hypertension), respiratory disease (asthma), cataracts, rheumatoid arthritis and various cancers (colorectal, prostate, breast, lung, bladder cancer) [3].

Antioxidants are substances that, when present at a low concentration compared to an oxidizable substrate, significantly delay or prevent the oxidation of that substrate. The term oxidized substrate includes nearly everything found in living cells including proteins, lipids, carbohydrates, and DNA [5]. Other literature states that antioxidants are molecules that have the ability to prevent or slow down the oxidation of macromolecules. The role of antioxidants is to reduce or stop this chain reaction by eliminating free radicals or inhibiting other oxidation reactions by oxidizing themselves. So, antioxidants are often reducing agents [6].

Pineapple (*Ananas comosus* (L) Merr) is a plant that is known to the world [7]. Pineapple is a member of the Bromeliaceae family which is divided into sixsubfamilies, namely, pitcarnioideae, Tillandsioideae, and Bromelioideae [8, 9]. Pineapple has a fresh taste, fragrant, and high in nutrients so that pineapple is called the queen of fruit Pineapple is consumed or served fresh, cooked, juiced, and can be preserved. Various foods such as pumpkin, syrup, jelly, candy are produced from pineapple as well as vinegar, alcohol, citric acid, calcium citrate, etc. Also produced from pineapple. Thus pineapple fruit can be used as additional nutritious fruit for good personal health [10].

Figure 1. Pineapple (*Ananas comosus* (L) Merr). [11]

Pineapple is known to be very effective in curing constipation and irregular bowel movements because pineapple is rich in fiber, which makes bowel movements regular and easy [12]. Pineapple is also reported to reduce cholesterol, increase male fertility, treat joint and muscle pain, strengthen bones, treat coughs and colds, treat wrinkles, fever, cardiovascular disease, and many other uses [13, 14].

The composition contained in pineapples is very much such as water, energy, fat, protein, carbohydrates, fiber, potassium, phosphorus, iron, sodium, magnesium, calcium, zinc, selenium, vitamin C, vitamin A, vitamin B1, vitamin B2, vitamins E, niacin, folic acid [13]. Secondary metabolites are also found in pineapples such as alkaloids, tannins, flavonoids, steroids, saponins, and terpenoids, phenols, and quinines [15, 16, 17]. Pineapple plants also produce several proteolytic enzymes, including bromelain (from stems and fruit), ananin, comosaine, and several non-protein enzymes such as amylase, peroxidase, and phosphatase [18].

METHODS

Data Collection

In compiling the reviews for this article, the technique used is to use literature studies by searching for sources or literature in the form of primary data in the form of official books and international journals in the last ten years. The main reference search used in this review article is through authentic web sites, such as NCBI, ResearchGate, GoogleScholar, and other published and trustworthy articles.

RESULTS AND DISCUSSION

Pineapple plants have an antioxidant effect from the flesh to the skin. In table 1, the antioxidant test of pineapple using different solvents (hexane, dichloromethane, and methanol) with the maceration process and the 2,2-diphenyl-1- picrylhydrazyl (DPPH) method shows that the methanol extract has a higher inhibitory power of 96.91% compared to the two solvents. others where its activity was almost comparable to that of gallic acid as control of 97.93%. This means that the methanol extract of pineapple fruit has a very high antioxidant activity compared to other solvents [19].

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Table 1. Summary of antioxidant activity of pineapples

Plant Section	Solvent	Dose	Result	Ref
Fruit	Hexane Dichloromethane methanol	3 x 700 mL 3 x 700 mL 3 x 700 mL	The effectiveness of pineapple as an antioxidant	[19]
Fruit	Methanol Ethyl acetate Water	25, 50, and 100 μL	Reduces the purple DPPH effect to yellow	[20]
	Methanol Ethyl acetate Water	50 and 100 μg / mL	inhibits β-carotene bleaching levels by neutralizing linoleic free radicals and other free radicals that form in the system	
Skin	Ethanol Water Ethanol - water (50-50) Ethanol - water (50-50) Ethanol Water	-	Has a low inhibitory value of 13.65 mg/ml	[21]
Skin	n-hexane Dichloromethane Ethyl Acetate Methanol Water	-	Has good antioxidant activity	[23]
			Has high inhibitory power so that its antioxidant power is high	
Skin	Methanol Ethyl acetate Water	-	Protects fish from lipid oxidation	[26]
Waste (pulp, skin, core, and crown) fermented and non-fermented	Ethanol Toluene Petroleum ether Acetone Methanol Water	2-10 μg / mL	Pineapple extract waste is an electron donor, which can react with free radicals to convert it into a more stable product and stop radical chain reactions.	[27]
Fruit skin Leaf	Methanol	60% 80% 100%	The highest antioxidant activity was found in the skin of the fruit with absolute methanol	[28]
Weevil	Ethanol 70%	200 μg / ml 100 μg / ml 50 μg / ml 25 μg / ml 12.5 μg / ml 6.25 μg / ml	The higher the concentration, the higher the free radical scavenging activity in DPPH	[29]
Fruit	50% Methanol 70% Methanol 50% Ethanol 70% Ethanol 50% Acetone 70% Acetone Water	-	- There are phenolic compounds and vitamin C which contribute to the process of scavenging free radicals. - The ability of the extract to donate electrons to the iron ion - There was an increase in the iron-chelating ability of the extract with increasing concentration.	[30]

The role of antioxidants is their interaction with oxidative free radicals. The essence of the DPPH method is that antioxidants react with stable free radicals namely α, α-diphenyl-β-picrylhydrazyl (dark purple color) and convert them to α, α-diphenyl-βpicrylhydrazine with color change. The free electrons present in DPPH pair with antioxidants and their absorption is reduced due to the loss of purple DPPH to yellow[20, 21] The degree of a color change indicates the potential for free radical scavenging of the antioxidant sample. Research by Hossain & Rahman, 2011 shows that pineapple extract can remove DPPH color and the potential for free radical scavenging is shown in methanol extract, ethyl acetate extract, and pineapple water extract. Thus, pineapple extract is believed to be able to donate hydrogen to act as an antioxidant [20].

In the method with the β-carotene-linoleic system, the addition of pineapple extract and butylated hydroxyanisole at a concentration of 50 μg / mL prevents β-carotene bleaching to different degrees. The β-carotene in this model system undergoes rapid color changes in the absence of antioxidants. This is due to the combined oxidation of β-carotene and linoleic acid, which results in free radicals. Linoleic acid-free radicals formed in the abstraction of a hydrogen atom from one of the diallik methylene groups attack the highly unsaturated β-carotene molecule. As a result, β-carotene is partially oxidized and broken down, then the system loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically. In our current research, The extract from pineapple was found to inhibit β-carotene bleaching levels by neutralizing linoleic free radicals and other free radicals that build up in the system. Methanol extract, ethyl acetate extract and water extract showed antioxidant activity of 84.3%, 55.4% and 51.8% respectively at a concentration of 100 μg/mL [20]

Free radical scavenging methods using the DPPH and ABTS methods have become the most commonly used to evaluate the antioxidant activity of compounds because of their simple, fast, sensitive, and reproducible procedures [22]. Testing the antioxidant activity of pineapple peels using the DPPH method with different solvents (water, methanol, ethyl acetate, dichloromethane, and n-hexane) also has an antioxidant effect. The highest activity was shown in water extract and methanol extract of pineapple rind with 61.48 and 59.05% inhibition power, respectively. Based on research with percentage inhibition, water extract had a higher IC50 (266.02 μg/mL) than methanol extract (281.33 μg/mL). It is known that the lower the IC50 and EC50 values, the higher the antioxidant activity [21. 23]. In pineapple fruit skin, it is reported that there are polyphenolic compounds such as catechins, epicatechins, gallic acid, and ferulic acid which are likely to be potential antioxidants [24, 25].

Alias & Abbas's research, 2017 uses waste from pineapples (skin). In this study, compounds believed to be antioxidants are phenolic compounds. This study shows a good correlation between phenolic compounds and antioxidants with a low EC50 value [21].

The 2,2'-azinobis-3- ethylbenzothiazole-6-sulfonic acid (ABTS) method is also a method often used in antioxidant testing. Testing the antioxidant activity of pineapple peels using the ABTS method showed that methanol extract had the highest activity among other fruit peel extracts. The methanol extract had a lower IC50 value compared to the water extract of pineapple peel [23]. This shows that the methanol extract has a higher activity than the water extract of pineapple skin.

Natural antioxidants extracted from pineapple peel are also applied as a preservative against lipid oxidation in Indian mackerel steaks that are kept cold for 15 days. IC50 of pineapple peel extract on free radical scavenging using the DPPH method is 0.55 mg/ml-1. While the reducing power (OD = A700nm) of pineapple peel ethanol extract was 0.272 at a concentration of 500µgml-1 [26]

DISCUSSION

Lipid changes during refrigerator storage after being given pineapple peel extract were monitored by measuring the value of peroxide (PV), free fatty acid (FFA), and thiobarbituric acid reactive substance (TBARS). These parameters indicate that Butylated hydroxyanisole (BHA) which is used as a comparison standard is more effective than pineapple peel extract. However, when the extract-treated samples were compared with changes in control samples, the pineapple peel extract was able to protect the fish against lipid oxidation. Thus, it can be concluded that the antioxidant compounds found in pineapple peels can be extracted and used as natural antioxidants even though the effect is not comparable to the BHA used as a comparison [26].

Many of the antioxidant components in pineapple waste are fermented rather than unfermented, which can react quickly with DPPH radicals, and reduce almost all DPPH radical molecules according to the available hydroxyl groups. These results indicate that the two extracts were effective at cleaning up DPPH radicals. The ability of the extract to scavenge these radicals indicates that it contains compounds that are electron donors, which can react with free radicals to turn them into more stable products and stop radical chain reactions. So, DPPH scavenging may be related to the inhibition of lipid peroxidation. Likewise using the systemβ-carotene, and the reduction capacity also has the highest antioxidant effect found in fermented pineapple waste. However, this is different in the method using ion rating, pineapple waste whether fermented or not both show good antioxidant effects [27].

Pineapple plant parts are not only the skin, leaves, and fruit which are studied as antioxidants. Tests on pineapple weevils using the DPPH method also showed a significant effect on the extract concentration. The higher the concentration, the greater the DPPH free radical scavenging activity. Although this study shows a low antioxidant effect when compared to luteolin compounds, the ethanol extract of pineapple weevils shows its activity as an antioxidant. The content contained in pineapple hump extract is tannins and triterpenoids [29]. The content of tannins and triterpenoids has antioxidant activity [31, 32].

Various variations of pineapple plants have also been conducted on antioxidants, including Josephine, Morris, and Sarawak, which were tested for their activity using the DPPH method, Ferric Reducing Power (FRP), Ferrous Ion Chelating (FIC). The highest antioxidant activity was obtained in the Morris variety, followed by Josephine and Sarawak. This is because pineapple has phenolic compounds that are responsible for antioxidant activity. In addition, vitamin C is thought to also contribute to antioxidant activity. Even though it is classified as a weak antioxidant, the pineapple plant in this study still shows its effect as an antioxidant and is a natural antioxidant. The best solvent system to extract phenolic compounds from the pineapples is 50% methanol to extract phenolic compounds [30].

CONCLUSION

Based on the description above, pineapple has the potential as an antioxidant. The greatest potential is obtained from methanol extract. However, testing on food is not recommended to use methanol extract because methanol is not allowed to be applied in the food sector. polyphenolic compounds such as catechins, epicatechins, gallic acid, and ferulic acid are most likely potential antioxidants. Likewise with tannin compounds and triterpenoids. This shows that pineapple is a good antioxidant remedy to serve as a potential source of natural antioxidants for food and nutraceutical applications.

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