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Phytochemical Screening and in Vivo Test of Dewandaru (Eugenia uniflora L) Fruit Extract on Mice Exposed to Cigarette Smoke

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Abstract---*Cigarette smoke contains various toxic chemical compounds which can trigger oxidative stress. Biomarkers to show the degree of oxidative stress that occurs are levels of malondialdehyde (MDA) The n-butanol extract of Eugenia uniflora fruit contains compounds that have antioxidant activity of phenolic and non-phenolic compounds. The purpose of this study was phytochemical screening and the effect of giving n-butanol extract of fruit Eugenia uniflora L on oxidative stress mice exposed to cigarette smoke. The research design was an experimental randomized post-test control group design, The control group of mice was only given exposure to cigarette smoke 1 stick per day (K), the second group was given exposure to cigarette smoke 1 stick per day P1 dose of 100 mg /KgBB, the third group was given exposure to cigarette smoke 1 stick per day P2 dose 200 mg /KgBB, n-butanol Dewandaru fruit extract. The treatment of experimental animals was given for 30 days, malondialdehyde was analyzed using the Elisa method. The results of the study using the One Way ANOVA and Post Hoc Tukey test showed that there was a significant difference ($p < 0.05$) in the provision of n-butanol extract of Eugenia uniflora fruit to levels of MDA exposed to cigarette smoke with the highest mean levels of MDA found in the group control and the lowest mean levels of MDA were in the P2 group. Based on the results of the study it can be concluded, phytochemicals consist of compound alkaloid, flavonoid, saponin, terpenoid, tannin, and in vivo test of the n-butanol extract can inhibit the increase in MDA levels in mice exposed to cigarette smoke. Eugenia uniflora fruit butanol extract has the potential as a natural antioxidant.*
Keywords---*cigarette smoke, Eugenia uniflora, fruit extract, MDA*

Introduction

Cigarette smoke (AR) induces inflammation and oxidative stress, which causes chronic inflammatory lung disease. AR contains about 1 × 1016 oxidants / free radicals per suction and thousands of different chemical compounds, including reactive aldehydes (Yao et al., 2014). AR also induces endogenous production of reactive oxygen species (ROS) from inflammatory cells that infiltrate the lungs. This results in the formation of more reactive and unstable free radicals, such as superoxide anions, nitric oxide, and hydroxyl radicals, causing tissue damage and an uncontrolled inflammatory response to the body such as nicotine, tar, nitrosamines. In addition, cigarettes also contain various carcinogens and mutagens in the form of polonium, benzopyrene, dimethylbenz anthracene, dimethylnitrosamine, and naphthalene (Wibowo et al., 2017). The substances contained are free radicals that cause oxidative stress.

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Cigarette smoke is a source of free radicals that are very harmful to the body which can cause an inflammatory response in the peripheral airways and lung parenchyma. Free radicals in cigarette smoke that enter the airway directly can interfere with the body's antioxidant defense mechanism, due to free radicals that enter the body more antioxidants, which can result in oxidative stress, damage or oxidation of lipids, proteins, and DNA in cells. characterized by increasing levels of malondialdehyde in blood serum, as a marker of lipid peroxidation Abdul-(Rasheed & Al-Rubayee, 2013; Yoshida et al., 2013). can be measured from the breath condensate that is exhaled (Janicka et al., 2010). In stating that in obese people and smokers there is an increase in levels, in his research, he also states that levels can be measured through biological fluids such as plasma and human urine, levels also increased in obese cats (Jeusette et al., 2009). Increased levels of occurring in Several flavonoid compounds of Dewandaru fruit such as myricetin 3-O-pentoside, myricetin 3-O-rhamnoside, quercetin 3 from the fruit have high antioxidant activity, (Celli et al., 2011), the red color of the fruit Dewandaru evidenced by, test the metabolic syndrome, lipid-lowering fruit extracts dewandaru peroxidation and prevents degradation of endogenous antioxidant activity of superoxide dismutase (SOD) and catalase (Oliveira et al., 2017).

Materials and Methods

Materials

A sample that is used in this research is Eugenia uniflora fruits which were taken from Gianyar Regency Bali which has been determined at LIPI. Chemical materials which used in this research such as, n-butanol (Merck), MDA kit, kit, micropipette, tools glas, centrifuge, Elis reader, experimental animals in the form of mice with an average weight of 20 grams in healthy condition as many as 30, certificate of ethical clearance number 46 / UN14.2.9

Phytochemical screening

Eugenia uniflora fruits are picked from Bali areas (Gianyar Regency), the pulp of dark red Dewandaru is then dried in the oven at a temperature of 50ºC. After that, they are blended into powder. 500-gram simplistic of Dewandaru fruits powder is weighed. Then it is macerated by using solvent n-butanol, as much as 2000 ml in a glass jar, and stirred constantly for one hour. And then it is covered by aluminum foil and plastic wrap and hushed for three days (it must be stirred every day for 30 minutes). The filtrate was then evaporated by using a rotary evaporator at a temperature of 60 ° C. The thick extract obtained was then evaporated in an oven at a temperature of 50 ° C until the dry extract was obtained (Sopori & Kozak, 1998; Hasday et al., 1999).

Alkaloid examination

2 ml of the test solution was evaporated on a porcelain cup to obtain a residue. The residue was then dissolved with 5 ml of 2N HCl. The solution obtained was then divided into 2 test tubes. The first tube serves as a blank. The second tube was added 3 drops of Dragendroff's reagent, a positive reaction formed an orange precipitate in the tube (Santoso et al., 2018).

Flavonoid examination

A total of 40 mg of extract was added with 100 ml of hot water, then boiled for 5 minutes, and then filtered. 5 ml filtrate then add 0,05 mg of Mg powder and 1 ml of concentrated HCl, then shake vigorously. A positive result is indicated by a change in the solution to red, yellow, or orange (Santoso et al., 2018).

Saponin examination

A total of 40 mg of extract was added to 10 ml of water then shaken for 10 minutes, then 2 drops of 1 N HCL were added, if the foam formed remained stable for ± 7 minutes, it means that the extract was positive for saponins (Santoso et al., 2018).

Terpenoid examination

As much as a total of 100 mg of extract was weighed and then dissolved using 10 ml of water. Then 2 ml of the soluble extract was taken and then added with 3 drops of concentrated HCl and 1 drop of concentrated H2SO4. A positive result is indicated by the formation of a red or purple color (Ergina et al., 2014).

Tanin examination

A total of 40 mg of extract was dissolved in 4 ml of water, then 2 ml of the dissolved extract was taken and then 1 ml of 10% FeCl3 was added. A positive reaction is indicated by the formation of a dark blue or greenish-black color (Simaremare, 2014).

Experimental

The study pure experimental planning of post-test only control group design. Thirty-male mice were divided into three groups consist of 10 mice each. The mice went through an adaptation process for seven days. The first group was set as control (C); the mice have given smoke one cigarette per day and. The second group (P1) was given smoke one cigarette per day, and 100 mg /KgBB Eugenia uniflora extract. The third group (P2) was given smoke one cigarette per day, and 200 mg/KgBB Eugenia uniflora extract The treatment was given for 30 days. For sampling collection, the mice were anesthetized (with ketamine + xylazine, 0.1 ml, im), and then 1 ml of their blood was collected to be observed. MDA was analyzed using the Elisa method. data were analyzed by using SPSS software version 25. one way ANOVA test was used too (Lykkesfeldt, 2007; Wyatt et al., 2012; Syakur et al., 2018).

Result and Discussion

The result and discussion should be combined in the manuscript. It should be described concisely. Text, tables, and figures must be internally consistent. Discussion should involve the significant findings presented with relevant and extensive discussion (Band et al., 2002; Oliveira et al., 2006). The Dewandaru fruit (Eugenia uniflora L.) used in this study was taken from the Gunung Kawi area, Malang, East Java and has been determined at UPT LIPI Plant Conservation Center "Eka Karya" Bedugul Bali. The purpose of the determination is to ensure that the true plant used is Dewandaru fruit (Eugenia uniflora L.). The extraction process was carried out by the maceration method using n-Butanol as solvent. After evaporation of the filtrate from the maceration, a thick extract with a weight of 23,4 grams was obtained. The maceration extraction method was chosen because this method is easy and does not require heating so that natural ingredients are less likely to be damaged or decomposed (Susanty & Bachmid, 2016), and relatively cheap, while the drawback is that it takes a long time and a lot of solvents so it is not efficient (Kiswandono, 2017).

Skrining fitokimia

Table 1
Phytochemical screening of Dewandaru fruit n-butanol extract

Compound	Reactor	Observation result	Ket.
Alkaloid	Number of samples added 5 ml of 2N HCl and 3 drops of Dragendroff's reagent	An orange precipitate appears	(+)
Flavonoid	A total of 5 ml The sample was added with 0,05 mg of Mg powder and 1 mL of concentrated HCl, then shaken vigorously.	A red solution is formed	(+)
Saponin	A total of 10 ml of the sample was shaken vigorously for 1 minute, then 2 drops of 1 N HCl were added.	Stable foam is formed when left for 7 minutes	(+)
Terpenoid	A total of 2 ml The sample was then added with 3 drops of concentrated HCl and 1 drop of concentrated H_2SO_4.	A red solution is formed	(+)
Tanin	A total of 2 ml of the sample was then added	Formation of a dark blue	(+)

with 1 ml of 10% FeCl3.	or greenish-black solution.

Information:
(-) : Does not contain these secondary metabolites.
(+) : Contains these secondary metabolites.

The results of phytochemical screening of the n-butanol extract of the Dewandaru fruit for each extract contained in the table indicate that the n-butanol extract of the Dewandaru fruit (Eugenia uniflora L.) is positive for alkaloids, flavonoids, tannins, steroids, and triterpenoids. These results are consistent with the journal (Onwudiwe et al., 2010), which stated that the n-butanol extract of Dewandaru fruit (Eugenia uniflora L.) was positive for alkaloids, glycosides, tannins, saponins, terpenoids and had high levels of antioxidants.

Table 2
Mean level of MDA, of each group

	Group			
Marker	K	P1	P2	p value
MDA (µmol / L)	13.69± 0.46	8.67 ± 0.53	5.58 ± 0.43	0.0001

Note:
K : control is only given exposure to cigarette smoke without extract
P1 : given exposure to cigarette smoke and 100 mg/kg
P2 : of Dewandaru fruit extract, given exposure to cigarette smoke and Eugenia uniflora fruit extract 200 mg/kg

Table 3
Post Hoc test

Group		p value	Description
Kontrol	P1	< 0.05	There is a significant difference
	P2	< 0.05	There is a significant difference
P1	Kontrol	< 0.05	There is a significant difference
	P2	< 0.05	There is a significant difference
P2	Kontrol	< 0.05	There is a significant difference
	P1	< 0.05	There is a significant difference

The results showed that exposure to cigarette smoke per day for 30 days caused increased levels of MDA and seen in the control group (K), the highest average MDA level was 13.69± 0.46, the mean level was 6.82. ± 0.61, in contrast to the P1 and P2 treatment groups, the overall results are presented in Table 2 . In normal circumstances free radicals that are formed in the body are very slow and slow when free radicals increase beyond the endogenous antioxidant defenses oxidative stress occurs. Oxidative stress causes excessive lipid peroxidation. The results of lipid peroxidation are MDA so that increased lipid peroxidation can cause levels of MDA and in the body to increase (Winarsi, 2007). Damage to the lungs as the main target directly exposed to cigarette smoke can be explained by exposure to chemical agents in cigarette smoke. However, the effect that causes chronic disease in other organ systems is probably the result of indirect exposure (Yanbaeva et al., 2007). The gas-phase of cigarette smoke can contain up to 1014 free radicals and reactive substances per cigarette smoke. Free radicals and oxidants present in the gas phase of cigarette smoke have a short half-life, but these compounds can enter the bloodstream and cause macromolecular oxidative damage (Swan & Lessov-Schlaggar, 2007).

The gas-phase of cigarette smoke also contains saturated and unsaturated aldehydes which are more stable than free radicals and hydrogen peroxide. As a result, the tissue that is far from the lungs can also experience increased oxidative stress (Tohomi, 2014). Increased oxidative stress can cause normal metabolic disorders and lead to various diseases such as by attacking and killing pathogens (Ozougwu, 2016). MDA levels and in cancer, Parkinson's, Alzheimer's, atherosclerosis, heart failure, and myocardial infarction. In not excess amounts, free radicals are useful as the body's defense system Eugenia uniflora fruit extract treatment group decreased. Eugenia uniflora fruit which is

purplish-red has strong antioxidant activity because it contains high total phenol and anthocyanin content (Bagetti et al., 2011). The content of flavonoids, phenols which are thought to be active antioxidants has an antioxidant effect by preventing the formation of ROS, directly capturing ROS, protecting lipophilic antioxidants, and stimulating an increase in enzymatic antioxidants (Birben et al., 2012). The effect of n-butanol extract which contains several compounds as antioxidants can reduce levels in mice. This effect is caused by flavonoids, polyphenols, and carotene compounds, which are antioxidants that break down lipophilic chains, which play a role in protecting cell membranes by preventing lipid peroxidation (Mustafa et al., 2010). Flavonoids inhibit enzymes involved in the formation of ROS, namely microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, NADH oxidase.

Flavonoids, beta-carotene, and vitamin C have antioxidant properties, which protect LDL cholesterol from oxidation, due to non-cyclooxygenase. A plant that contains flavonoid compounds has been shown to have antioxidant activity (Murray & Morrison, 1993; Moldoveanu & Kiser, 2007). Flavonoids are non-enzymatic anti-oxidant compounds, act as antioxidants by chelating metals, are in the form of glucosides (have glucose side chains) or in a free form called aglycones. Flavonoid compounds are non-enzymatic antioxidants or chain-breaking antioxidants. In vitro flavonoids have been shown to have a very strong biological effect (Schapoval et al., 1994; Kwak & Lim, 2014). The mechanism of flavonoids can reduce the bad effects of free radicals, by inhibiting lipid peroxidation through peroxidase activation. Flavonoids suppress reactive oxygen formation, chelated trace elements involved in free-radical production, scavenge reactive species, and up-regulate and protect antioxidant defense (Agati et al., 2012).

Conclusion

Based on this study it was found that n-butanol extract of dewandaru fruit has compound alkaloid, flavonoid, saponin, tannin, terpenoid and inhibit the increase in MDA levels and blood of mice exposed to cigarette smoke for 30 days

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