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Antioxidant properties of spice extracts

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ABSTRACT

Objective : This study was conducted to determine the antioxidant activities of spice extracts including clove *(Syzygum aromaticum* L.), Indonesian cassia (*Cinnamomum burmanni* (C. Nees& T. Ness)), coriander (*Coriandrum sativum* L), nutmeg (*Myristica fragrans* Houtt), java cardamom (*Amomum compactum* Soland. Ex maton).

Methods : This research was to evaluate antioxidant activities including 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity, the value of superoxide dismutase (SOD), and total phenolic content.

Result : The highest DPPH activity is clove and Indonesian cassia extracts with IC_{50} value 4.16 µg/ml and 5.46 µg/ml respectively. The highest SOD value are Indonesian cassia extract (9.1432 U/ml) at 500 µg/ml, 7.0045 U/ml at 125 µg/ml and 4.6751 U/ml at 31,25 µg/ml. Clove extract was the highest of phenolic content (188.35 µg/mg eugenol equivalent).

Conclusion : Indonesian cassia extract have high antioxidant activities both DPPH scavenging and SOD activities. Clove extract contain the highest eugenol compared with Indonesian cassia, coriander, nutmeg and java cardamom.

1. Introduction

Non communicable diseases (NCD) mainly cardiovascular diseases (CVD), cancers, diabetes mellitus (DM) and chronic respiratory diseases are leading threat to human health. These four diseases are the world's biggest killers, causing an estimated 35 million deaths each year - 60% of all death globally. WHO projects that, globally, NCD deaths will increase by 17% over the next ten years [1]. The free radicals formation is co-related with the pathological conditions including CVD, cancer, neurological, age-related disorders, degenerative disorder [2, 3]. Free radical will attack the DNA, lipid and proteins thus leading to chronic disease [4, 3]. Oxidative stress is an imbalance condition between free radicals and antioxidants, to reduce oxidative stress or high level of free radicals including Reactive Oxygen Species (ROS) and Reactive Nitrogen Species(RNS) can be combated with the involvement of antioxidants both exogenous and endogenous [5].

Spices commonly known as food adjuncts, but they can be used as traditional medicines too [6, 7]. Species such as clove, cinnamon, rosemary, paprika, nutmeg, ginger, garlic, mustard, etc., have antioxidant activities [8]. Antioxidant activity is associated with their phenolic compounds activities to scavenge free radical, transition-metal-chelating, have capacity to quenching singlet-oxygen, and their redox properties [9, 10, 11, 12]. Activities of spices to scavenge ROS and RNS are considered as effective remedies for diabetes [13]. Spices have capability to detoxify the liver, to be anti-inflammatory agent, and infectious protector agent [14].

The aims of this study were to determine antioxidant activities of spices extract including clove, Indonesian cassia, coriander, nutmeg, java

cardamom, and to evaluate the total phenolic content.

2. Materials and Methods

2.1 Plant material

Plant material were collected from several plantation in Indonesia : clove (*Syzygum aromaticum* L.) from Cimahi-Bandung-west Java, Indonesian cassia (*Cinnamomum burmanni* (C. Nees& T. Ness) from Lembang-Bandung- West Java, Indonesia, coriander (*Coriandrum sativum* L) from Cipanas-Cianjur- West Java, nutmeg (*Myristica fragrans* Houtt) from Cicurug-Sukabumi- West Java, java cardamom (*Amomum compactum* Soland. Ex maton) from Cicurug-Sukabumi- West Java. The plants were identified by staff at the herbarium, Department of Biology, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. Seed of coriander, nutmeg, java cardamom and skin of bark of Indonesian cassia and flower of clove were chopped and dried in a dry tunnel (40-45°C) to achieve a stable water level (11% water content), then chopped finely in a blender, producing a 60 mesh size flour [15, 16].

2.2 Extract preparation

The dried flour of samples (400 g) were immersed in 70% ethanol. After 24 h, the filtrates were collected and the residues were immersed again in 70% ethanol for 24 h. The filtrates were evaporated with a rotary evaporator at 40°C, yielded clove extract 13.29%; java cardamom extract 9.71%, Indonesian cassia extract 17.92%, coriander extract 8.80% and nutmeg extract 11.64% [15, 16]. The extracts were stored at 4°C. The ethanol extracts of clove, Indonesian cassia, coriander, nutmeg, java cardamom were dissolved in HPLC-grade methanol (Merck) for DPPH scavenger and phenolic content assay and were dissolved in 10% dimethylsulfoxide (DMSO; Merck) for SOD assay [15, 16]. Pure compounds were used as comparison of antioxidant activities including limonin (Biophurify Phytochemical Ltd.), coumarin (Biophurify Phytochemicals Ltd.), trans-cinnamic acid (Sigma-Aldrich), thymoquinone (Sigma-Aldrich) and cardamom oil (Sigma-Aldrich),

2.3 DPPH scavenging activity assay

Amount of 50 µl extracts and limonin, coumarin, trans-cinnamic acid, thymoquinone and cardamom oil were introduced into a microplate followed by 200 µl DPPH (Sigma-Aldrich) solution (0.077 mmol/l in methanol). The mixtures was shaken vigorously and kept in the dark room for 30 min at room temperature; DPPH scavenging activity was determined with a microplate reader at 517 nm [15, 16, 17]. The radical scavenging activity of each sample was measured according to the following formula:

$$\text{DPPH scavenging \%} = 1 - \frac{\text{Sample absorbance}}{\text{Negative control absorbance}} \times 100$$

2.4 Superoxide Dismutase (SOD) Assay

The SOD assay was performed with SOD assay kit (Cayman) including assay buffer, sample buffer, radical detector, SOD standard, and xanthine oxidase. SOD standards were prepared by introducing 200 µl diluted radical detector and 10 µl SOD standard (7-level standard) per well. Sample wells contained 200 µl of the diluted radical detector and 10 µl of the sample. The reaction was initiated by adding 20 µl of diluted radical detector to all wells. The samples consist of five extracts and five compounds were measured at 3 concentrations in triplicate. The mixtures were shaken carefully for few seconds, incubated for 20 minutes at room temperature, SOD activity was measured on a microplate reader (Multi Go Skan) at 440-460 nm. The linearized SOD standard rate and SOD activity were calculated using the equation which obtained from linear regression of the standard. One unit is defined as the amount of enzyme to yield 50% dismutation of the superoxide radical [16]. Furthermore, standard curves were constructed based on the value of the LR and SOD value be calculated:

The calculation is done by calculating the value of SOD linearized rate/LR (LR Std A= Abs Std A/Abs Std A: LR Std B= Abs Std A/Abs Std B)

$$\text{SOD (U/ml)} = \left[\left[\frac{\text{Sample LR-y intercept}}{\text{Slope}}\right] \text{X } 0{,}23\text{ml}/0{,}01\text{ml}\right] \text{X Sample dilution}$$

2.5 Total phenolic content assay

Total phenolic content was measured according to the Folin–Ciocalteu method. Samples (15 µl) were introduced into microplate; 75 µl of Folin-Ciocalteu's reagent (2.0 M) and 60 µl of sodium carbonate (7.5%) were added. The samples were mixed and incubated at 45°C for 15 min [18,19]. Subsequently, absorbance value was measured at

760 nm using microplate reader (Multi Skan Go). Total phenolic content expressed as Eugenol equivalent was calculated by the following formula:

$$C = \frac{c \times V}{m}$$

C: total content of phenolic compounds (µg/mg) of clove, Indonesian cassia, coriander, nutmeg, java cardamom extract in eugenol equivalent;
c: the concentration of eugenol established from the calibration curve, µg/ml;
V: the volume of extract (ml); m: the weight of spices extract (mg).

Total phenol value was obtained from the regression equation for eugenol :

$Y=0.0024\ X + 0.1228$, with $R^2 = 0.94$

3. Result

3.1 DPPH scavenging activity

The antioxidant activities were measured including DPPH scavenging activity, SOD activity and phenolic content using eugenol as

Table 1. DPPH scavenging activity (IC_{50}) of spice extracts. The DPPH scavenging activity assay was measured triplicate for each sample. Linear equations, coefficient of regression (R^2), and IC_{50} were calculated.

Samples	Linear equation	R^2	IC50 (µg/ml)
Java cardamom extract	Y=0.2071X+13.157	0.8797	177.90
Indonesian cassia extract	Y=6.878X+12.448	0.918	5.46
Coriander extract	Y=0.4873X+2.011	0.9850	98.48
Clove extract	Y=10.342X+7.006	0.9825	4.16
Nutmeg extract	Y=0.861x+16.058	0.878	39.422
Limonin	Y=0.1181X+7.4917	0.8631	359.93
Coumarin	Y=0.0404X+2.7142	0.8387	1170.44
Trans-cinammic acid	Y=0.066X+15.578	0.8849	521.55
Thymoquinone	Y=5.7709X+22.53	0.9471	4.76
Cardamom oil	Y=0.2183X-20.577	0.8472	323.30

standard. DPPH accept a proton from an antioxidant becomes 1 1-diphenyl-2-picrylhydrazyne which is a stable radical [17]. The DPPH free radical scavenging activity of clove, Indonesian cassia, coriander, nutmeg, java cardamom extracts compared to compounds of spice including limonin, coumarin, trans-cinnamic acid, thymoquinone and cardamom oil The IC_{50} is the concentration of antioxidant needed to scavenge 50% of the DPPH free radicals [15].

Based on the IC_{50} values of DPPH scavenging activity (Table 1) showed that clove extract was the most active antioxidant based on DPPH scavenging activity and the lowest was coumarin.

3.2 The SOD activity

Superoxide dismutase (SOD) as an endogenous antioxidant activity would be increased by changing the superoxide anion (O_2^{*-}) into hydrogen peroxide (H_2O_2) and oxygen (O_2) [20]. The SOD activity of extracts and compounds of spices can be seen in Table 2.

Based on the results (Table 2.) showed that the highest SOD value at a concentration of 500 µg/ml, 31.25 µg/ml and 31.25 µg/ml was the Indonesian casia extract 9.1432 U/ml, 7.0045 U/ml and 4.6751 U/ml respectively.

3.3 Total phenol antioxidant activity assay

Phenols have the ideal chemical structure that can scavenge free radicals, so this class of phenolic compounds exhibit higher antioxidant activity than tocopherols and ascorbate [21]. The

total phenolic compound of spice extracts in Eugenol equivalent can be seen in Table 3.

Based on the test results of phenolic compound total (Table. 3) showed that the highest phenolic compound was clove extract contained eugenol 188.35 µg/g.

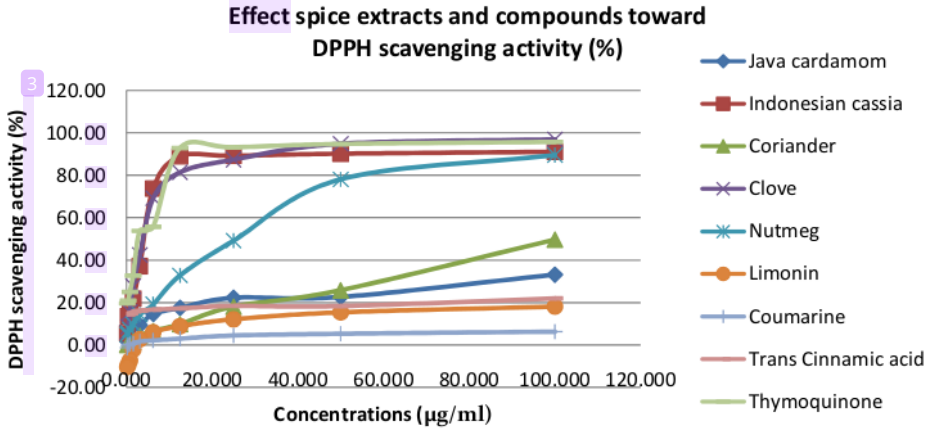

Figure 1. DPPH scavenging activity of spice extracts diluted in methanol to achieve the final concentrations 100; 50; 25; 12.5; 6.25; 3.125; 1.563; 0.781; 0.391; 0.190 µg/ml.

4. Discussion

Based on Table 1,2, and 3 showed that spice extracts have high antioxidant activities and Indonesian cassia extract was the highest SOD value. The most active DPPH scavenging activity was clove extract. The highest phenolic content was clove extract. In previous study, among 30 spices from subtropical country Ireland, clove also had highest antioxidant capacity [8]

Many spices contained high levels of phenolics and demonstrated high antioxidant capacity [21]. The spices and related families with the highest antioxidant capacity were screened, e.g., clove, cinnamon or Indonesian cassia representing potential sources of potent natural antioxidants for commercial exploitation [21]. Clove extract contained very high levels of phenolics (188.35 µg/mg eugenol equivalent). Other spices with high levels of phenolics were cardamom extract

Table 2. Mean, standard deviation and Tukey HSD post hoc test of extracts and compounds of spices SOD activity (U/ml) were measured in triplicate for sample. (Linear equation, coefficient of regression (R^2) of SOD standard and SOD activity of extracts and compunds were calculated.)

Samples	Concentrations (µg/ml)		
	500	125	31,25
Java cardamom extract	2.6903±0.1890 ^{e}	0.9022±0.2242 ^{b}	0.3178±0.1158 ^{a}
Indonesian cassia extract	9.1432±0.7559 ^{g}	7.0045±0.2821 ^{f}	4.6751±0.1732 ^{f}
Coriander extract	1.8448±0.0393 ^{cd}	1.4919±0.0729 ^{cd}	0.9747±0.0587 ^{b}
Clove extract	4.5607±0.2502 f	3.6723±0.0644 ^{e}	2.7509±0.0653 ^{e}
Nutmeg extract	4.3044±0.1368 ^{f}	3.5462±0.0287 ^{e}	2.3532±0.0765 ^{d}
Limonin	0.5615±0.0619 ^{a}	0.3930±0.0415 ^{a}	0.2554±0.0350 ^{a}
Coumarine	0.6960±0.0677 ^{ab}	0.4251±0.0359 ^{a}	0.3423±0.0069 ^{a}
Trans-cinnamic acid	2.3707±0.0253 ^{de}	1.7197±0.0859 ^{d}	1.3663±0.1180 ^{c}
Thymoquinone	1.4034±0.0452 ^{bc}	1.2249±0.0143 ^{bc}	0.9961±0.0181 ^{b}

Cardamom oil	1.1368±0.0777 [abc]	0.9999±0.0479 [b]	0.8883±0.0373 [b]

Table 3. Mean total phenolic compound in spice extracts (Eugenol equivalent/mg)

Sample	Eugenol/ml			Eugenol µg/mg			Average
Clove extract	94.92	92.04	96.29	188.4	184.08	192.58	188.35
Cardamom extract	67.63	67.54	66.21	135.26	135.08	132.42	134.25
Indonesian cassia extract	55.04	69.54	56.88	110.08	139.08	113.76	120.97
Coriander extract	-	-	-	-	-	-	-
Nutmeg extract	47.08	51.04	48.33	94.16	102.08	96.66	97.63

(134.25 µg/mg eugenol equivalent) and nutmeg contained relatively low phenolics (97.63 µg/mg eugenol equivalent). Previous studies also showed that clove had a very strong antioxidant activity and a high level of phenolics [22, 23]. The various antioxidant mechanisms of clove extracts were attributed to a strong hydrogen-donating ability, a metal chelating ability, and their effectiveness as good scavengers of hydrogen peroxide, superoxide, and free radicals [8]. Our results showed that the clove extract was the most powerful phenolic antioxidant and exhibited the strongest radical scavenging activity among five spice extracts.

Spices, like vegetables, fruits, and medicinal herbs, are known to possess a variety of antioxidant effects and properties [24]. The antioxidant effect of phenolic compounds is mainly due to their redox properties and is the result of various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity [11, 21]. These multiple potential mechanisms of antioxidant action make the diverse group of phenolic compounds an interesting target in the search for health-beneficial phytochemicals and also offer a possibility to use phenolic compounds or extracts rich in them in lipid-rich foods to extend shelf life [25]. The presence of antioxidative and antimicrobial of phenolic compounds in many spices gives food-preserving properties [6, 26]. Several studies also showed that black pepper, clove, cinnamon, and coriander exhibited antioxidant properties [22]. In recent decades, a number of phenolic substances were isolated from a variety of spice sources, including phenolic acids (e.g., gallic acid, caffeic acid, etc.), flavonoids (e.g., quercetin, rutin, myricetin, luteolin, naringenin, and silybin), phenolic diterpenes, and volatile oils [27].

5. Conclusion

The most active antioxidant is Indonesian cassia in SOD activity. Clove extract is the highest phenolic content and highest DPPH scavenging activity.

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Conflict of Interest

The authors report no conflicts of interest

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