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Chemical Composition and Antioxidant Activities of the Essential Oil from the Clove Buds (*Syzygium Aromaticum*) Toward Various Oxidative Stresses in Vitro

***Ke Zhang*[1]**

[1]College of Physical Education, Shanxi Normal University, Linfen, Shanxi, China

Abstract

The chemical composition and antioxidant activities of the essential oil from clove buds toward various oxidative stresses in vitro were investigated in this work. The results showed that 22 compositions of the essential oil were identified by gas chromatography (GC) and GC-mass spectrometry (MS), and engenol (76.23%) was found to be the main components of the essential oil, followed by β-caryophyllene (11.54%), caryophyllene oxide (4.29%), and eugenyl acetate (1.76%). The essential oil from clove buds had the better scavenging capacity against 2, 2'-azino-bis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salts (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, high reducing power, and inhibiting lipid peroxidation reaction in lard emulsion, as well as exhibited different protection effects against DNA damage caused by Fe^{2+} and 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH). And the antioxidant activities of essential oil showed a dose-response relationship to some degree. However, the essential oil did not have the protection against protein damage, but had a prooxidant effect on protein oxidative damage.

Keywords: Chemical composition, Antioxidant activity, Essential oil, Clove buds, DNA, Protein

Contents

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by many redox processes [1]. However, the excessive amounts of ROS and RNS can attack important biological molecules such as carbohydrates, proteins, lipids, DNA and RNA, which lead to cell death and tissue damage [2]. One of the principal causes of food quality deterioration is the oxidation of unsaturated lipids initiated by free radicals [3]. In addition, as it is known, the presence of high amounts of polyunsaturated fatty acids in oils and fats make them more susceptible to oxidation in the course of the time depending on external factors [4]. As a result of autoxidation especially which occurs with the effect of oxygen in the air, unpleasant taste and smell that are known as the signs of rancidity in oil occur [5]. In particular, the use of safer antioxidants from various natural materials is recommended because of the side effects of synthetic antioxidants. The flower bud of *Syzygium aromaticum* (L.) Merr. & Perry. (Family Myrtaceae), commonly known as clove, is a well known food flavor and a popular remedy for dental disorders, respiratory disorders, headache and soar throat in traditional medicines of Australia, and Asian countries [6]. Clove buds comprise around 20% of volatile oil rich in eugenol. Clove oil has been listed as a 'Generally Regarded As Safe' substance by the United States Food and Drug Administration when administered at levels not exceeding 1500 ppm in all food categories [7]. Clove bud essential oil still remain a research priority due to their wide range of pharmacological and biological activities such as antioxidant [8], antibacterial, antifungicidal, antiviral, and anesthetic effects [9]. However, these informations are still limited. In this study, the chemical composition and antioxidant activities of essential oil from the clove buds toward various oxidative stresses in vitro are investigated in order to provide a reference for the comprehensive development and utilization of clove buds.

2. Material and Methods

2.1. Plant Materials and Reagents

The dried buds of clove were purchased as commercial products from the local market. TPTZ were purchased from Fluka. 2,2'-azino-bis ABTS, DPPH and AAPH were from Sigma. Other chemicals used were all of analytical grade.

2.2. Extraction of Essential Oil

The dried clove buds were ground and hydrodistilled for 4 h using a Clevenger-type apparatus. The oil was separated from water and dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 °C until use.

2.3. GC-MS Analysis

The analysis of the essential oil were performed using a Hewlett-Packard 5890 II GC, equipped with a HP-5 MS capillary column (30 m×0.25 mm; film thickness, 25 μm) and a HP 5972 mass selective detector for the separation. The mass selective detector was operated in electron-impact ionization (EI) mode with a mass scan range from *m*/*z* 30 to 500 at 70 eV. Helium was the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 250 and 200 °C, respectively. The oven temperature was programmed from 40 °C for 2 min, raised to 180 °C at a rate of 3 °C/min, held isothermal for 2 min, and finally raised to 250 °C for 5 min. A sample of 0.1 μL of the essential oil and oleoresin were injected manually using a 1:50 split ratio.

2.4. DPPH Assay

The DPPH radical scavenging activity was determined according to the method of Xu, et al. [10]. The scavenging rate on DPPH radicals was calculated according to the formula, scavenging rate (%) = [(Ao-A_1)/Ao]×100, where Ao is the absorbance of the control solution, A_1 is the absorbance in the presence of samples in DPPH solution. The scavenging activity of the sample on DPPH radicals was expressed by IC_{50} value. IC_{50} value is the effective concentration at which DPPH radicals are scavenged by 50% and is obtained by interpolation from regression analysis.

2.5. ABTS Assay

The ABTS cation radical scavenging activity was determined according to the method of Xu, et al. [10]. The scavenging rate and IC_{50} value were calculated using the equation described for DPPH assay.

2.6. Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing ability was determined by using FRAP assay according to the method of Xu, et al. [10]. The FRAP of the sample was expressed by IC_{50} value. The IC_{50} value is the effective concentration at which the absorbance is 0.5 and is obtained by the equation described for DPPH assay.

2.7. Antioxidant Activities in Lard Emulsion

The antioxidative effects of the essential oil on lipid peroxidation were evaluated according to the method of Kamkar, et al. [11] with some modifications. Each emulsion sample was transferred to a series of capped glass test tubes. Then, essential oil (0.25, 1 and 4 g) and BHT (0.01 g) were added to the test tubes and put in a dark oven at 70 °C respectively. The stability of emulsion to oxidation was evaluated 5 times over an 11-day period by analyzing the peroxide values (PVs) measured.

2.8. DNA Damage Protective Effect Assay

Protection of DNA from oxidative damage of Fe^{2+} The ability of samples to protect supercoiled pBR322 plasmid DNA against Fe^{2+} and H_2O_2 was estimated with the DNA nicking assay as described by Xu, et al. [10].

Protection of DNA from oxidative damage of AAPH The ability of samples to protect supercoiled pBR322 plasmid DNA against AAPH was measured following the method described by Zhang and Omaye [12] with some modifications. To assay inhibition of DNA damage induced by essential oil, 0.5μg of pBR322 DNA was incubated with various concentrations of compounds and 2 μL of 25 mM AAPH in PBS for 30 min at 37 °C. Then the samples were electrophoresed on 0.8% agarose gel containing 0.5 μg/mL ethidium bromide and the DNA was photographed under ultraviolet light.

2.9. Determination of Protein Oxidation

Protein oxidation was assayed as described by Xiang, et al. [13] with minor modifications. Briefly, 2 μL 3mg/mL of bovine serum albumin (BSA) in PBS was mixed with 2μL of 0.5 mM $CuSO_4$ solution and 2 μL of 10 mM H_2O_2 solution and inhibited by various concentrations of test samples. After incubation for 2 h at 37 °C, the samples were then assayed with normal SDS–PAGE.

2.10. Statistical Analysis

All experiments were conducted three times independently. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant differences ($p < 0.05$) between the means.

3. Results and Discussion

3.1. GC-MS Analysis

The chemical compositions of the essential oil from clove buds are analyzed by GC and GC-MS, and the results are presented in Table 1. In total, 22 components in essential oil were identified, representing 95.80% of the total amount. The engenol (76.23%) was the major component of the essential oil, followed by β-caryophyllene (11.54%), caryophyllene oxide (4.29%), and eugenyl acetate (1.76%). Similar to our finds, some studies reported that eugenol was the main component of clove essential oil, but its content was different. For example, Tang, et al. [14] identified 19 components in the essential oil of clove buds and the content of engenol was 62. 21 %; Guan, et al. [15] compared essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods, and found that there were differences in the components and content of essential oil of clove buds by various methods, but the content of engenol was more than 50%; Jirovetz, et al. [16] identified 23 components of clove leaf essential oil and found that eugenol (76.8%) was the main component, followed by β-caryophyllene (17.4%), α-humulene (2.1%), and eugenyl acetate (1.2%). Although we can find in the literature studies using the essential oil from clove buds, these differences in the content and components of essential oil are difficult to compare because the species, geographic regions, and extract methods used are different among studies.

3.2. DPPH Radicals Scavenging Capacity

The scavenging capacity assayed herein on DPPH radicals is shown in Table 2. The IC_{50} values of essential oil on DPPH radicals was 396.25 μg/mL, and the scavenging rates of essential oil on DPPH radical increased by 3.89-fold from 14.31% to 70.06% with the increase of its concentrations, showing a concentration-dependent scavenging of the DPPH radicals at certain concentrations, which was also consistent with the previous reports [8, 16].

3.3. ABTS Radicals Scavenging Capacity

The profile of scavenging capacity of essential oil on ABTS was similar to the result of the scavenging capacity on DPPH radicals (Table 2). Somewhat differently, the IC_{50} value on scavenging ABTS radicals was 60.34 μg/mL. Similarly, ABTS radicals scavenging capacity of essential oil increased dose-dependently at concentrations ranging from 10 to 100 μg/mL, which may be attributable to its hydrogen-donating ability. These differences in data between DPPH and ABTS assays were likely due to different experimental conditions.

3.4. Ferric Reducing Antioxidant Power (FRAP) Assay

Table 2 showed that the reducing power of essential oil from clove buds was in a concentration-dependent manner and increased with concentrations. The IC_{50} value was 683.4 μg/mL. These results suggested that essential oil from clove buds had a remarkable potency to donate electron to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction.

3.5. Antioxidant Activities in Lard Emulsion

The PVs of lard emulsion increased gradually within 7 days after treating, but no obvious distinction among different treatments was found (Fig. 1). Thereafter, the PVs of control significantly and rapidly increased, and were much higher than the treated samples. These results indicated that the essential oil from clove buds had inhibiting lipid peroxidation reaction, and the antioxidative effects depend on its concentration and action period, even though far lower than that of the BHT.

3.6. Protection of DNA Damage

To assay the potential of the essential oil to prevent DNA damage, oxidative DNA strand breakage induced by AAPH and Fe^{2+} were measured with pBR322 DNA. The damage of plasmid DNA produces a relaxed open circular form and further a linear double-stranded DNA molecule [17]. The plasmid DNA was mainly of the supercoiled form in the absence of Fe^{2+} and H_2O_2 (Fig. 2, Lane 1). During the addition of Fe^{2+} and H_2O_2, the supercoiled form of DNA converted into the open circular and linear forms (Fig. 2, Lane 2). From the gel analysis, the protection effects against DNA damage offered against DNA damage by essential oil (15.62–250 mg/mL) was concentration dependent, but a lower concentration of essential oil did not seem to have the protection effects (Fig. 2, Lane 3, 4). The results indicated that the essential oil might prevent the reaction of Fe ions with H_2O_2, on the other hand, and it

probably quenched hydroxy radicals by donating hydrogen-atom or electron, and therefore protecting the supercoiled plasmid DNA from hydroxy radicals dependent strand breaks [17].

As shown in Fig. 3, similar results were found in protective effect assay of DNA from oxidative damage of AAPH. The protection offered against DNA damage by essential oil (3.9–62.5 mg/mL) was also concentration dependent, and the essential oil exhibited a stronger protective effect against DNA damage caused by AAPH with a lower completely prevented concentration of 3.9 mg/mL (Fig. 3, lane 3). The differences in protective effect of essential oil against DNA damage caused by Fe^{2+} and AAPH may come from different determination method [18]. Nonetheless, these results showed that the essential oil from clove buds owned a higher potential to prevent DNA damage.

3.7. Inhibition of Protein Oxidation

Oxidation of cellular proteins has been described under many pathological conditions. The vulnerability of various amino acid residues of proteins to oxidation varies with reactive oxygen species [19]. The protection effect against protein oxidative damage was determined by the oxidation of BSA initiated by Cu^{2+} and the results are shown in Fig. 4. The results showed not only non-protective effect against oxidation of BSA but also a prooxidant effect, which was augmented by increasing concentrations of the essential oil. Similar to our findings, Xiang, et al. [13] reported that carnosic acid had the potential to prevent BSA oxidant damage in low concentrations but showed a prooxidant effect against BSA when the concentrations of carnosic acid were higher and the effect was concentration related. Some researches [20, 21] reported that polyphenol have prooxidant effect with the existence of mental ions. Nakagawa, et al. [22] suggested that Cu(II) ions could convert (+)- catechin from an antioxidant to a prooxidant in protein oxidation. According to our results of chemical composition analysis, engenol that was the major composition of the essential oil, as a phenolic compound, may be the most possible reason of producing prooxidant effect. However, the prooxidation mechanism of the essential oil from clove buds needs to be further studied.

4. Conclusions

In conclusion, this work showed that eugenol was the main component in essential oil from clove buds, and the essential oil exhibited different antioxidant activities toward various oxidative stresses in vitro. The essential oil from clove buds had the better scavenging capacity against DPPH and ABTS radicals, high reducing power, and inhibiting lipid peroxidation reaction in lard emulsion during storage, as well as it exhibited different protection effects against DNA damage caused by Fe^{2+} and AAPH. And all the antioxidant activities of essential oil from clove buds showed a dose-response relationship to some degree. However, the essential oil did not show the protection against protein damage as we expected, but had a prooxidant effect on protein oxidative damage.

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Table-1. Chemical composition of essential oil from clove buds

Compound	Peak area (%)	Compound	Peak area (%)
2-Pinene	0.02	Engenol	76.23
α-Pinene	0.03	β-Caryophyllene	11.54
Eucalyptol	0.14	α-Caryophyllene	0.64
Methyl salicylate	0.06	(-)-b-Cadinene	0.12
Chavicol	0.09	β-Selinene	0.25
4-Allylanisole	0.13	α-Selinene	0.16
Anethol	0.11	Caryophyllene oxide	4.29
α-Muurolene	0.01	Jasmone	0.07
α-Copaene	0.05	Ledol	0.03
Valencen	0.01	Globulol	0.04
Eugenyl acetate	1.76	Cedrene	0.02

Table-2. Radicals scavenging capacity and FRAP of essential oil

	Scavenging rate (%) and FRAP (Abs) at different concentrations (μg/mL)					Regression equation	IC_{50} (μg/mL)
DPPH	100	250	500	750	1000	y=24.73Ln(x)-98.98 R^2 = 0.9974	396.3
	14.3±1.2d	37.7±0.9c	56.1±2.1b	65.4±3.5a	70.2±4.3a		
ABTS	10	25	50	75	100	y= 0.7959x + 1.9747 R^2 = 0.9903	60.4
	9.8±0.5e	22.9±2.4d	44.5±3.1c	61.6±2.8b	82.8±5.3a		
FRAP	100	250	500	750	1000	y = 0.0005x + 0.1583 R^2 = 0.9982	683.4
	0.22±0.03e	0.28±0.05d	0.41±0.03c	0.54±0.07b	0.68±0.04a		

Values represent means of three independent replicates ± SD. R^2 refer to the regression coefficients. Different letters within a row indicate statistically significant differences between the means ($p < 0.05$) for different concentrations essential oil.

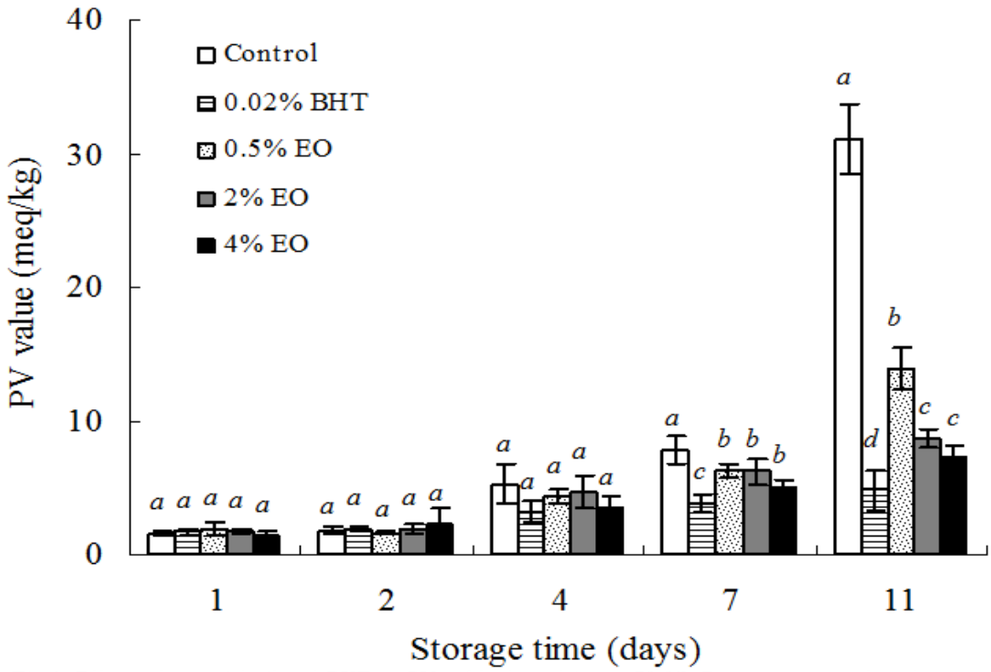

Fig-1. Effects of essential oil and BHT on PVs of lard emulsion at 70 °C. Data are expressed as the mean values of three independent replicates ± SD. Different letters indicate statistically significant differences between the means ($p < 0.05$) for different treatments at the same treatment time.

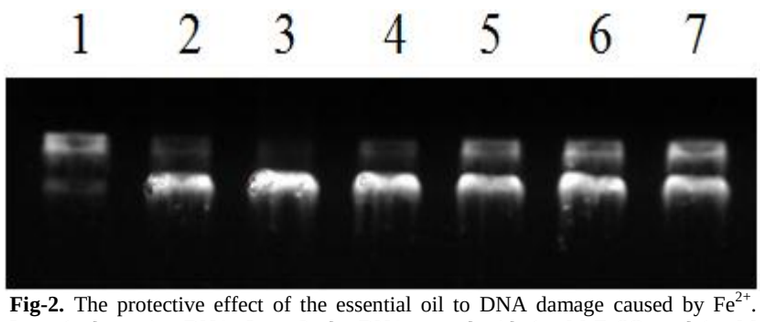

Fig-2. The protective effect of the essential oil to DNA damage caused by Fe^{2+}. Lane 1, the native DNA; Lane 2, the DNA treated with 1 mM $FeSO_4$ and 1 mM

H_2O_2; Lane 3-7, the DNA treated with 15.62, 31.25, 62.5, 125, and 250mg/mL essential oil, respectively.

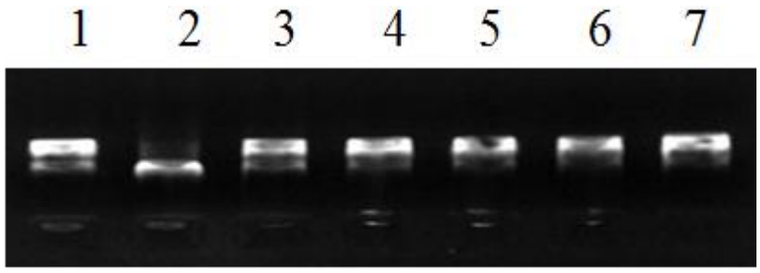

Fig-3. The protective effect of the essential oil to DNA damage caused by AAPH. Lane 1, the native DNA; Lane 2, the DNA treated with AAPH; Lane 3-7, the DNA treated with 3.9, 7.81, 15.62, 31.25, and 62.5 mg/mL essential oil, respectively.

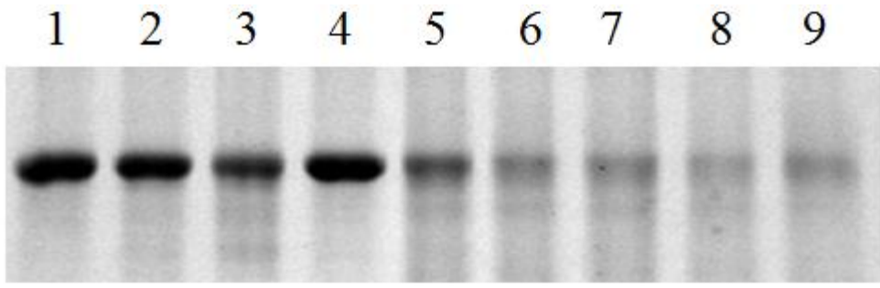

Fig-4. The inhibitions of the essential oil against BSA oxidative damage. Lane1, the native BSA; lane 2, the BSA and ethanol; lane 3, the BSA treated with 0.5 mM Cu^{2+}; lane 4, the BSA and essential oil; lane 5-9, the BSA treated with 0.5 mM Cu^{2+} and 0.625, 1.25, 2.5, 5, 10 mg/mL essential oil, respectively.