

Deodorizing Ability of *Houttuynia cordata* Thunb. (Dokudami) for Masking Garlic Odor

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Abstract

The deodorizing activity of dokudami (*Houttuyniacordata*Thunb.) for masking the odor of fresh garlic was investigated, and the volatility of the deodorizing compounds was examined. Two grams of garlic was placed in a beaker and heated for 5 min at 40 °C. Next, aliquots of 0.01 and 0.05 g of powdered samples of seven plants (dokudami, thyme, rosemary, coriander, dandelion, spiny sow thistle and green tea) were mixed with 1 ml of volatile or non-volatile dokudami extract and added to the garlic sample. The garlic odor intensity was subsequently evaluated by sniffing by 10 trained individuals. The deodorizing activity test was carried out at 0, 3 and 6 h after adding the dokudami extract. The deodorizing effect of dokudami on masking garlic odor was the highest of any plant and became higher with increased dose. In addition, the deodorizing activity of dokudami increased with time and persisted. Moreover, whether the deodorizing compounds of dokudami were volatile or non-volatile was investigated. The deodorizing activity of a volatile solution of dokudami against garlic was extremely higher than that of dokudami dry powder. Our findings revealed that the deodorizing compound of dokudami is volatile.

Keywords: Deodorizing activity, *Houttuyniacordata*Thunb., Garlic, Volatile, Porapak Q, Dokudami

1. Introduction

Recently, odors from garbage and excrement and body odors generated by pets and people are causing significant problems in Japan (Tominaga, 2009). To deal with such offensive odors, many deodorizers have been developed. However, there are a growing number of diverse sources of offensive odors, and consumers have demanded development of deodorizers that are pleasant smelling and safe with regard to hygiene and the environment.

The emission of a variety of molecules by people is influenced by genetics, diet, stress and disease. Halitosis (bad breath) frequently causes embarrassment, may affect interpersonal social communication (Bosy, 1997) and has become an important market for the pharmacological and cosmetic industries (Scully, 2008). Halitosis results from ingesting certain foods and drinks, such as spices, garlic, onion and durian, and from habits such as smoking tobacco and drinking alcohol. It is usually transient, often caused by sulfur-containing volatile agents and is considered to arise from both intra-oral and extra-oral origins (Suarez *et al.* 1999). In this study, we aimed to mask the garlic odor that is prevalent after eating garlic and which is generally perceived as unpleasant by many people (Shimizu *et al.*, 2004). Although garlic (*Allium sativum* L.) is used widely in various food recipes for its unique flavor and nutrition, garlic is also the cause of a malodorous smell after ingestion. Studies have shown that garlic's various sulfur-containing substances are the main cause of its malodor (McDowell and Kassebaum, 1993; Scully *et al.*, 1997). To our knowledge, however, no study has attempted to mask the garlic odor (Tamaki *et al.*, 2008), despite the fact that many studies have investigated the main compounds which contribute to garlic odor (Cai *et al.*, 1995).

Plants exhibit wide-ranging effects that include deodorizing, antibacterial and antioxidant activities. Green tea, which can deodorize bad breath, and ginger, wasabi and bamboo grass, which have antibacterial and bactericidal effects on the bacteria that cause food poisoning have been used successfully since ancient times. Tokita *et al.* (1984) reported that plant extracts of 40 species across 23 families have the strongest deodorizing effect of 167 species across 65 families of plants, and in particular, they reported that such deodorizing effects are notable in

the functionality of each plant.

Masuda *et al.* (2004) studied the deodorizing effect of different types of plant extracts and essential oils with respect to the deodorizing aspect of plant functionality. Catechin in green tea (Kida *et al.*, 2002) and biphenyl compounds in thyme (Nakatani *et al.*, 1989) were identified as deodorant compounds. Essential oil of herbs has many volatile compounds which have a deodorizing effect, and it was reported that essential oil of thyme, rosemary and sage suppressed the trimethylamine odor, which constitutes the main odor of fish-like smells (Kikuchi *et al.*, 1968, 1969). Furthermore, volatile compounds of coriander deodorized an offensive odor stemming from porcine large intestine (Kohara *et al.*, 2006; Ikeura *et al.*, 2010). Additionally, in Japan, many deodorizers utilize volatile aromatic compounds which are advantageous over water-soluble compounds for deodorizing widely diffused offensive odors.

Dokudami (*Houttuynia cordata* Thunb.) of Saururaceae is known as a medicinal plant in Japan and China (Meng, *et al.*, 2005a, 2005b). Its medicinal effects are antibacterial (Jong and Jean, 1993), anticancer (Kim *et al.*, 2001), antileukemic (Chang *et al.*, 2001) and antioxidant (Chen *et al.*, 2003) and can stimulate anti-platelet aggregation (Nishida *et al.*, 1988). In Japan, dokudami is called jyu-yaku according to the Japanese Pharmacopoeia. In some parts of Japan, dokudami is known to have a deodorizing effect. However, the deodorizing effect of dokudami has not been well described and its underlying mechanism and respective compounds remain largely unknown.

In this study, to mask the offensive odor of fresh garlic on bad breath, we investigated the deodorizing effect of rosemary, thyme, dandelion, spiny sow thistle and dokudami and examined the volatility of each deodorizing compound.

2. Materials and Methods

2.1 Materials

Dokudami (*Houttuynia cordata* Thunb.) was collected around Hiroshima Prefectural University from May to July 2001, and then cultured in a greenhouse. The stem and leaves were sampled at before flowering stage. Wild grasses such as dandelion (*Taraxacum grasses* Dahlst.), spiny sowthistle (*Sonchus asper* L.) and two herbs, thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.) were collected at the University farm, and green tea (*Camellia sinensis* L.) was purchased at a supermarket in Shobara city. The fresh leaves and stems of the plant samples, except for green tea, were dried for overnight at 40 °C and then homogenized using a blender (Wonder Blender, Osaka chemical Co.). All samples were stored at -80 °C until the deodorizing activity test or extraction of volatile compounds.

The fresh garlic sample was purchased at a supermarket in Shobara city, and then crashed by a mixer.

2.2 Porapak Q Column Extraction Method

Two gram of dried plant samples were added to 80 ml of 20% MeOH, and stirred for 24 hours at 25°C. The mixture was centrifuged at 12,000 × g for 20 min at 4°C, and the filtered supernatant was subjected to the Porapak Q (PQ) column concentration method. We followed the procedure described by Shimoda *et al.* (1987). The filtrate was passed through a column (20 cm length × 2 cm i.d.) packed with 10 mL of PQ (50–80 mesh, Waters Co., Milford, MA), and the filtrated solution was as non-volatile fraction. After the column was washed with 100 mL of deionized water, adsorbed volatile compounds were eluted with 100 mL of diethyl ether. The elute was dried over excess anhydrous sodium sulfate for overnight after the addition of an internal standard (10 µL of 1% cyclohexanol) in order to remove the residual moisture in the extracts. The volume of the extracts was reduced to 100 µL by evaporating the solvent under a gentle nitrogen stream.

2.3 The Deodorizing Activity Test

The offensive odor sample (2.0 g of the garlic) were placed in a 100 ml conical beaker and heated for 5 minutes at 40 °C, and then 0.01 and 0.05 g of the samples to dokudami or the wild grasses, herbs and green tea and 1 ml of volatile or non-volatile dokudami extract was added. The odor of the garlic was sniffed by the ten trained members (ten healthy adults, four men and six women, ranging in age from 21 to 47 years), and the deodorizing activity test was carried out after 0, 3 and 6 hours. The deodorizing activity against the offensive odor was measured using 5 grades (perfectly deodorized: 0, almost deodorized: 1, considerably deodorized: 2, a little deodorized: 3, completely not deodorized: 4).

2.4 Statistical Analysis

The significance of the experimental data was tested using the Turkey-Kramer test at $P < 0.05$.

3. Results

The deodorizing activity of dry powder made from various plants against fresh garlic is shown Figure 1. The odor intensity against 2.0 g of garlic (odor intensity 4.0) treated with 0.01 g of dokudami, thyme, rosemary, coriander, dandelion, spiny sow thistle and green tea was 1.5, 1.9, 1.9, 3.0, 3.2, 3.1 and 2.1, respectively. The odor intensity against 2.0 g of garlic treated with 0.05 g of dokudami, thyme, rosemary, coriander, dandelion, spiny sow thistle and green tea was 1.0, 1.4, 1.5, 2.2, 2.6, 2.2 and 2.1, respectively. The deodorizing effect of dokudami against garlic was the highest of all plants tested and increased with increasing dose. The deodorizing effect of thyme and rosemary was high and followed that of dokudami. Dandelion and spiny sow thistle did not produce a deodorizing effect against garlic, and the deodorizing activity of green tea was reported to be lower than that of dokudami.

Maintenance of the deodorizing effect of dry powder made from various plants on garlic is shown in Figure 2. The odor intensity of dokudami on garlic was 0.6 at both 3 and 6 h and persisted. The odor intensity of rosemary and thyme, on the other hand, decreased at 3 and 6 h, but not significantly, at the start of the treatment. The deodorizing effect also persisted, but less so than that of dokudami. Therefore, it is postulated that the deodorizing mechanism of these plants was masked (data not shown). The odor intensity of coriander, dandelion and spiny sow thistle tended to be high at 3 and 6 h but did not persist. Interestingly, the odor intensity of green tea was 1.5 at 3 h but 2.2 at 6 h, which was very similar to the intensity at the start of the treatment. Green tea exhibited a slight deodorizing effect, less than that of dokudami. These results showed that the deodorizing effect of dokudami was not only stronger than that of green tea, which is well known to have a strong deodorizing effect, but also more persistent.

To demonstrate whether the deodorizing compounds are volatile or non-volatile, volatile compounds of dokudami were extracted by Porapak Q column concentration extraction (PQ) methods, which are used for volatile extracts. We then evaluated the deodorizing effect of volatile and non-volatile fractions of dokudami on garlic (Fig. 3).

The odor intensity of the volatile and non-volatile fraction of dokudami was 0.3 and 3.5, respectively. The volatile fraction of dokudami was shown to have a significant deodorizing effect, which suggested that the deodorizing component of dokudami was volatile, because the component adsorbed strongly on the PQ resin due to polarity.

4. Discussion

The odor of garlic was found to arise from allicin (3-[(S)-2-propenylsulfanyl]-L-alanine) which is converted from alliin (2-propene-1-sulfinothionic acid S-2-propenyl ester) by the enzyme alliinase by destructed tissue (Lee *et al.*, 2003). Alliin is an unstable compound and is broken down into sulfur-containing compounds such as methyl mercaptan (Brodnitz *et al.*, 1971; Yu *et al.*, 1989). It is these sulfur-containing compounds that are directly responsible for the offensive odor of garlic (Block, 1992).

Studies on the deodorizing components and mechanism of deodorizing effects of plant extracts and dry materials have been previously reported (Tokita *et al.*, 1984; Nakatani *et al.*, 1989), and some plant extracts from these studies have been utilized as deodorizing materials (Tanaka and Sugiyama, 2001). In particular, there are many studies on methyl mercaptan causing bad breath. Tokita *et al.* (1984) reported that perilla plants have an effective deodorizing ability. Additionally, Negishi and Ozawa (1997) examined the deodorizing effect of polyphenol compounds and the reaction pathway of polyphenol oxidase in vegetables and mushrooms and described how these vegetables could be deodorized by the addition of polyphenol and diallyldisulfide. The flavonoid and catechin polyphenols in green tea did not have a deodorizing effect on garlic in this study. Moreover, the deodorizing effect of dandelion on methyl mercaptan was reported to have a deodorizing activity second to dokudami (Urabe *et al.*, 1999), but its deodorizing effect on garlic was not shown at all in the present study. Nakatani *et al.* (1989) reported that 4'-hydroxy-5,5'-diisopropyl-2,2'-dimethylbiphenyl-3,4-dione in thyme has a deodorizing effect on methyl mercaptan.

Urabe *et al.* (1994) previously reported that methanol extract from dokudami exhibited a deodorizing effect on methyl mercaptan, but they did not study whether the volatile compounds of dokudami could in fact deodorize. Therefore, we have shown for the first time that the volatile compounds of dokudami do have a deodorizing capability against garlic.

In conclusion, we have demonstrated deodorizing activity of dokudami against fresh garlic and clarified that the deodorant compounds were volatile. The deodorizing effect of dokudami against garlic was the highest of all the studied plants and increased in line with increased dose and time. Moreover, the deodorizing activity of the

volatile solution of dokudami against garlic was extremely higher than that of dokudami dry powder. Results revealed that the deodorizing compound of dokudami is volatile. In near future, we need to identify the deodorizing compounds and elucidate the deodorizing mechanism.

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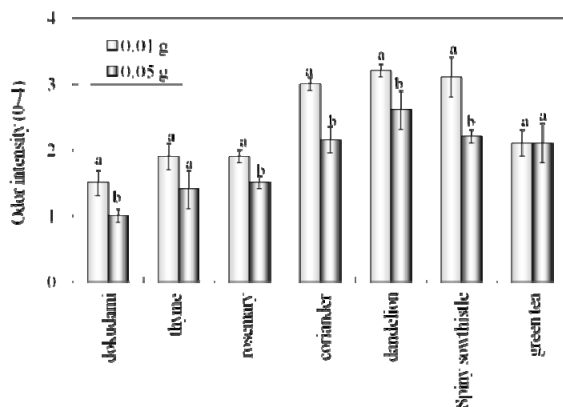


Figure 1. Deodorizing activity of various dry plant materials against garlic

Garlic odor intensity was determined as follows: 0, perfectly deodorized; 1, almost deodorized; 2, considerably deodorized; 3, slightly deodorized; 4, completely not deodorized.

The deodorizing activity of dry plant material samples (0.01 g, white bars; 0.05 g, gray bars) was carried out against 2.0 g garlic. Values per plant with the same letter do not significantly differ at $p < 0.05$ by the Turkey-Kramer test. Vertical bars represent the standard error of the mean value ($n=3$).

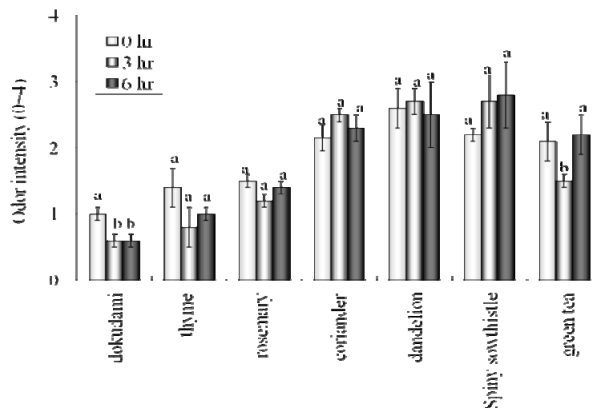


Figure 2. Deodorizing activity of various dry plant materials against garlic after treatment

Garlic odor intensity was determined as follows: 0, perfectly deodorized; 1, almost deodorized; 2, considerably deodorized; 3, slightly deodorized; 4, completely not deodorized.

The deodorizing activity of dry plant material samples (0.05 g) was carried out against 2.0 g garlic and the deodorizing intensity was measured at initial time (white bars) and after 3 h (gray bars) and 6 h (black bars). Values per plant with the same letter do not significantly differ at $p < 0.05$ by the Turkey-Kramer test. Vertical bars represent the standard error of the mean value ($n=3$).

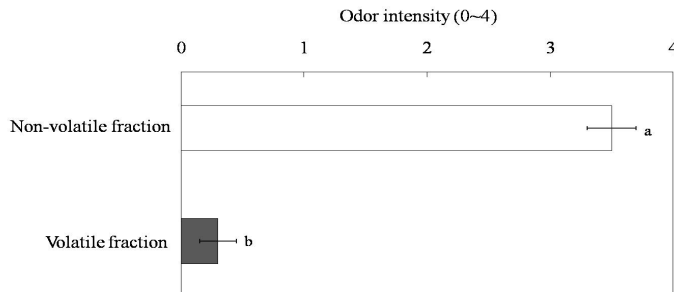


Figure 3. Deodorizing activity of volatile and non-volatile fractions of dokudami against garlic

Garlic odor intensity was determined as follows: 0, perfectly deodorized; 1, almost deodorized; 2, considerably deodorized; 3, slightly deodorized; 4, completely not deodorized.

The deodorizing activity was carried out against 2.0 g garlic with a 0.5-g sample pellet with 10 ml supernatant and 1 ml of volatile or non-volatile fraction. Values per plant with the same letter do not significantly differ at $p < 0.05$ by the Turkey-Kramer test. Vertical bars represent the standard error of the mean value ($n=3$).