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Determination of antioxidant capacity and α-amylase inhibitory activity of the essential oils from citronella grass and lemongrass

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Abstract

The objective of the present study was to determine the antioxidant capacity of and *in vitro* α-amylase inhibitory activity of the essential oils extracted from citronella grass and lemongrass. The chemical composition of the extracted essential oils was determined by GC-MS. The antioxidant capacity of the essential oils was determine by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, and the α-amylase inhibitory activity was evaluated by 3,5-dinitrosalicylic acid (DNSA) method. Both oils showed antioxidant and α-amylase inhibitory properties. IC_{50} values of the antioxidant capacity of both citronella grass and lemongrass oils were 0.46±0.012 and 4.73±0.15 µL/mL, respectively. While the α-amylase inhibitory activities, also expressed as IC_{50} = 6.59±0.20 and 6.97±0.12 µL/mL for the two oils were very similar. The results showed that both oils exhibited low antioxidant activity as compared to α-tocopherol standard. However, the α-amylase inhibitory activity was significantly higher as compared to acarbose drug currently used for controlling glucose levels in diabetic patients. Thus both these oils need to be further evaluated for antidiabetic potential.

Introduction

Citronella grass (*Cymbopogon nardus* Rendle) and lemongrass (*Cymbopogon citratus* (DC) Stapf.), are native herbal plants of Thailand. Both these herbs and their essential oils are known for their biological activities such as antimicrobial, antiacetylcholinesterase, and antioxidant activities (Cheel *et al*., 2005; Mata *et al*., 2007; Shaaban *et al*., 2010). In addition, *Cymbopogon* species plant has also long been used for acne, althea's foot, excessive perspiration, and flatulence (Julia, 1995; Aziz and Abbass, 2010).

In recent studies on plant materials such as eucalyptus, Lebanon cedar, peppers, cinnamon, curry leaves, etc. (Loizzo *et al*., 2007; Ranilla *et al*., 2010; Sahin and Candan, 2010; Ponnusamy *et al*., 2011), it was shown that the essential oils and plant extracts show significant α-amylase inhibitory and antioxidant activity. The α-amylase is the one of the main enzymes in human that catalyses the hydrolysis of 1,4-glucosidic linkage of complex carbohydrates like starch into simple sugars namely, maltose. Inhibition of the α-amylase activity is one of the possible six mechanisms that can be potentially used for controlling diabetes.

Controlling the glucose production from complex carbohydrates is considered to be effective in controlling diabetes. Although the inhibitory drug like acarbose is which inhibits α-amylase and α-glucosidase used in controlling glucose level in type 2 diabetic patients, the acarbose has undesirable side effects, especially flatulence and diarrhea (Chakrabarti and Rajagopalan, 2002). Natural products extracted from plants are known to controlling hyperglycemia (Ranilla *et al*., 2010; Ponnusamy *et al*., 2011). The monoterpenes, sesquiterpenes, and oxygenic derivatives in herbal plant extracts are known to inhibit the activity of carbohydrate hydrolyzing enzymes (Sahin and Candan, 2010; Tamil *et al*., 2010). In addition, phenolic compounds are commonly known for their antioxidant activity health beneficial promotion properties (Nickavar *et al*., 2008). Thus, it is of significant interest to evaluate the antioxidant capacity and α-amylase inhibitory activity of the essential oil extracted from the commonly consumed

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natural herbs.

The aim of this study was to characterize the VACs of citronella grass and lemongrass leaves, and then evaluate their antioxidant capacity and α-amylase inhibitory activity.

Materials and Methods

Chemicals

All chemicals used were of analytical reagent grade. Tapioca powder was purchased from local market in Thailand. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH●) and α-amylase from *Bacillus megaterium* bacteria were purchased from Fluka (Switzerland). α-Tocopherol was obtained from Sigma (USA). Dimethylsulfoxide (DMSO) was purchased from Merck (USA) and 3,5-dinitrosalicylicacid (DNSA) was procured from Sigma (Germany). Acarbose was purchased from PT Bayer (Indonesia) and eugenol standard (99%) was obtained from Acros organics (Australia).

Plant materials

Fresh citronella grass and lemongrass leaves were collected from the Agriculture farm of Khon Kaen University, Thailand. All samples were collected in the morning during the months of June-July, 2011. The plant material was immediately transferred to icebox. The leaves were dried at ambient temperature (~30°C) for 3 days in the laboratory. The dried leaves were ground using a kitchen grinder (HR2067, Philips, Netherlands). The dried ground plant was used for the extraction of essential oils.

Extraction of the essential oils

The ground leaves of citronella grass and lemongrass were accurately weighted (0.2 kg) in a 250-mL round bottom flask. Deionised water (100 mL) was added to the dried powder leaves. The plant material was immersed thoroughly in water and distilled by hydrodistillation method for 3 hours. These essential oils evaporated together with water vapour were collected as the oily drop after condensation into a closed conical flask. The essential oil was separated from an aqueous phase using a separatory funnel. The essential oil was hydrated by passing over small amount of anhydrous sodium sulphate. The dried oil sample was stored in dark at 4°C until used.

GC-MS analysis

The analysis of chemical composition of citronella grass and lemongrass essential oils was performed by gas chromatography-mass spectrometry (GC-MS) using a Trace GC chromatograph equipped with a Finnigan Polaris Q mass spectrometer (Thermo Finnigan, USA) under electron ionization (EI) mode. The optimum separation conditions for identification of the components in the essential oils was achieved with ZB-5 column (30 m x 0.25 mm, 0.25 µm film thickness, Phenomenex USA), using split injection mode at 200°C, (1:50), with helium as carrier gas at a flow rate of 1.0 mL/min. The oven was programmed with step temperature gradients starting at 40°C for 2 min, then increased to 120°C (with a ramp rate of 8°C/min), to 140°C (3°C/min), and the oven temperature was raised to 220°C (10°C/min) and then held on at this temperature for 2 min. The mass spectral scans were collected from 40-450 m/z.

Free radical scavenging activity

Free radical scavenging activity of the citronella grass and lemongrass essential oils was measured by DPPH assay (Gulluce *et al*., 2007). The oil sample was dissolved in methanol to give concentration from 0.2 – 0.8 µL/mL for citronella grass oil and 1.0 – 5.0 µL/mL for lemongrass oil. Then 1 mL of the essential oil solution and added into 2 mL methanolic solution of 100 µM DPPH. For control reaction, essential oil was replaced with 100% methanol. The mixture was incubated for 2 hours in dark at ambient temperature. Finally, the absorbance was measured at 517 nm using an ultraviolet-visible spectrophotometer (Agilent 8453 UV-Vis Spectroscopy System, Germany). For positive standard control α-tocopherol was used. The antiradical scavenging activity for pure eugenol standard solutions (1.0-5.0 µL/mL) was also evaluated. Antioxidant activity was calculated using the following equation:

$$\%\ \text{Antioxidant activity} = \frac{[A_{control} - A_{sample}]}{A_{control}} \times 100$$

Where is the absorbance of the control reaction (containing all reagents with methanol), and is the absorbance of the essential oil sample in the DPPH solution. The % DPPH radical inhibition was plotted against the sample concentrations and regression curve was established for calculation of the IC_{50} value.

α-Amylase inhibition assay

The α-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method (Miller, 1959). A starch solution (0.25% w/v) was prepared by stirring 0.125 g of tapioca powder in 50 mL of 20 mM sodium phosphate buffer containing 6.7 mM sodium chloride at pH 6.9. One unit of α-amylase enzyme solution was prepared by mixing 0.0253 g of α-amylase in 100 mL of cold distillation water. Citronella grass and lemongrass oils were dissolved in DMSO to give concentrations from 1.0 – 9.0 µL/mL. The color reagent was prepared by mixing sodium potassium tartrate solution (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH) and 96 mM of 3,5-dinitrosalicylic acid solution (0.4381 g of 3,5-dinitrosalicylic acid in 20 mL of deionized water). One unit of α-amylase solution and citronella grass and lemongrass oils were mixed thoroughly in a tube and incubated for 15 min. Then 500 µL of the starch solution was added into each tube and incubated for 15 min. The reaction was terminated by addition of 500 µL DNSA reagent, placed in boiling water bath for 5 min. The mixture was cooled to ambient temperature, diluted with 5 mL distilled water, and the absorbance was measured at 540 nm using a visible spectrophotometer (Spectronic 15, Thermo Fisher Scientific, India). The blank control of reaction showing 100% enzyme activity was conducted by replacing the essential oil with DMSO (1.0 mL). To eliminate the absorbance effect of essential oil, a blank solution was also used and the reaction was terminated by DNSA before adding the starch solution. Acarbose solution (diluted in DMSO to 80 – 400 µL/mL) was used as a positive control. The production of maltose will decrease with α-amylase inhibitory activity which will result in reduced absorbance intensity. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the following equation:

$$\%\ \alpha\text{-amylase Inhibitory activity} = 100 - \left[\frac{[\text{Maltose}]_{\text{sample}} \times 100}{[\text{Maltose}]_{\text{control}}}\right]$$

The % α-amylase inhibition was plotted against the sample concentration and regression curve established for calculation of the IC_{50} value.

Statistical Analysis

For the essential oils, standard compounds and positive control, three samples were prepared for each assay. The data was presented as mean ± standard deviation of three experiments.

Results and Discussion

The VACs composition of the essential oils

The VACs composition of these essential oil samples from both citronella grass and lemongrass leaves was analyzed by GC-MS. Table 1 shows that fifteen compounds were identified in the essential oils from citronella grass and only twelve compounds were detected in lemongrass oil. The composition analyses obtained in the current study agrees with previously published report (Heiba and Rizk, 1986). The major components in the citronella oil were geraniol (55.57%), geranial (10.18%), and neral (8.34%) and other minor ones composed of α-copaene (6.61%), citronellol (4.33%), γ- and δ-cardinene (4.26%), eugenol (2.51%), β-caryophylene (2.32%), humulene (1.63%), α-cubebene (1.23%), α-pinene (1.14%), citronellal (0.63%), δ-cardinol (0.58%), limonene oxide (0.47%), and germacrene-D (0.20%).

Table 1. VACs identified in citronella grass and lemongrass leaves by GC-MS

Component Name (MW, g/mol)	Retention Time (min)	MS Fragments, m/z	% Abundance*	
			Citronella grass	Lemongrass
Myrcene (136.2)	7.66	77.15,91.06,93.02,107.08	N.D.	9.73
α-Thujene (136.2)	7.83	77.13,91.05,93.02,105.01,121.07	N.D.	0.90
Cis-β-Ocimene (136.2)	8.57	77.11,91.04,93.02,105.02,121.06,122.05	N.D.	1.22
α-Pinene (136.2)	8.82	77.13,91.09,93.07,121.08,135.07	1.14	N.D.
Trans-β-Ocimene (136.2)	8.83	79.11,91.06,93.03,121.07,134.99	N.D.	1.03
Limonene oxide (152.2)	11.06	79.09,81.08,91.06,109.01,119.07, 137.03,152.01	0.47	1.50
Citronellal (154.2)	11.43	81.11, 93.06,121.02,123.02,139.04	0.63	2.72
Citronellol (156.3)	12.43	67.09,81.05,95.02,109.02,137.96	4.33	0.59
Cis-citral (neral) (152.2)	12.76	79.16,95.07,109.04,119.04,136.99, 152.93	8.34	27.48
Geraniol (154.2)	13.18	81.09,93.05,121.03,136.99,139.04	55.57	3.23
Trans-citral (geranial) (152.2)	13.50	83.11,95.10,109.07,137.00,152.86, 153.91	10.18	49.40
Eugenol (164.2)	15.74	77.12,103.12,149.05,164.02,164.93, 165.99	2.51	N.D.
Humulene (204.4)	16.35	67.12,93.03,121.01,135.97,153.93	1.63	1.81
α-Copaene (204.4)	17.59	79.16,91.11,105.08,133.03,161.07, 189.05,190.05	6.61	0.38
α-Cubebene (204.4)	18.46	77.13,91.10,105.08,119.04,133.02, 161.07,162.06,204.02	1.23	N.D.
Germacrene-D (204.4)	18.68	105.09,133.05,161.01,162.07,164.03, 203.93,205.00	0.20	N.D.
δ-Cadinol (222.4)	19.10	105.09,108.07,133.07,161.02,189.02, 204.03,205.04,207.03	0.58	N.D.
γ-Cadinene (204.4)**	19.79	77.15,91.16,105.14,119.11,133.01, 161.14,162.12,198.16,204.09,205.12	4.26	N.D.
δ-Cadinene (204.4)**		79.13,91.13,105.11,119.07,133.06, 161.01,162.06,189.09,204.01,205.00		
β-Caryophyllene (204.4)	21.12	77.12,91.07,105.05,107.06,146.98, 161.06,189.02,205.08	2.32	N.D.

*as calculated from normalized relative peak area
**expressed as two isomers
m/z with under line is found as base fragment (100%).

N.D. : not detectable

For lemongrass essential oil, the major components were geranial (49.40%), neral (27.48%), and myrcene (9.73%) and other minor ones were geraniol (3.23%), citronellal (2.72%), humulene (1.81%), limonene oxide (1.50%), cis-β-ocimene (1.22%), trans-β-ocimene (1.03%), α-thujene (0.90%), citronellol (0.59%), and α-copaene (0.38%).

The monoterpenes, sesquiterpenes, and their oxygenated derivatives are the major component of the essential oils from herbal plants. These compounds are known to exhibit diverse biological. It is documented in an article by Sahin and Candan (2010) that essential oils are known to possess

Table 2. DPPH free radical scavenging and α-amylase inhibitory activity of citronella and lemongrass oils compared with α-tocopherol (positive control), acarbose (positive control) and eugenol standard solution (average ± SD, n=3)

Sample	DPPH IC_{50} (μL mL^{-1})	α-amylase inhibitory activity IC_{50} (μL mL^{-1})
Citronella oil	0.46±0.012	6.59±0.20
Lemongrass oil	4.73±0.15	6.97±0.12
α-Tocopherol	6.20 x 10^{-3}±0.05 x 10^{-3}	-
Eugenol	1.34±0.10	-
Acarbose	-	91.2±5.7

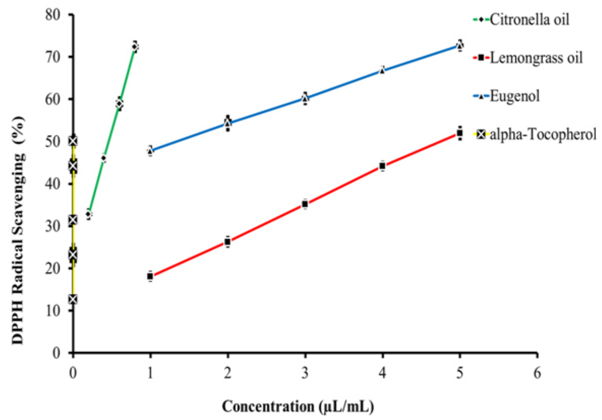

Figure 1. DPPH radical scavenging activity of different concentrations of citronella oil, lemongrass oil, eugenol, and α-tocopherol

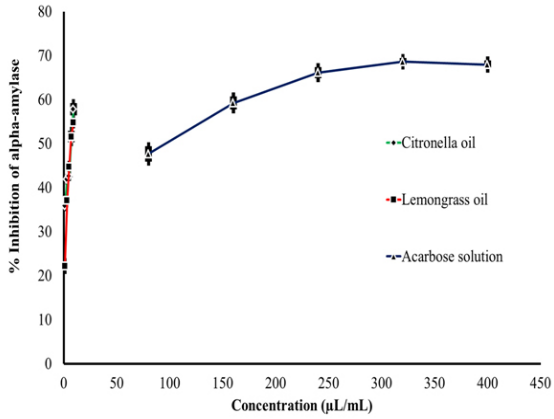

Figure 2. α-Amylase inhibitory activity of different concentrations of citronella oil, lemongrass oil, and acarbose solution

α-amylase inhibitory activity. In addition, the antioxidant properties of geraniol and eugenol has also been reported in literature (Mata *et al*., 2007; Politeo *et al*., 2007).

Antioxidant capacity and α–amylase inhibitory activity of the essential oils

The DPPH free radical scavenging activity is shown in Table 1. The results showed that positive control α-tocopherol showed maximum activity. The activity of citronella oil was almost ten times lower than α-tocopherol, whereas the activity of lemongrass oil was ~100 fold lower than α-tocopherol. Pure eugenol showed ~four times higher activity as compared to the lemongrass oil.

The IC_{50} values of DPPH free radical scavenging citronella and lemongrass oil were found to be 0.46 ±0.012 and 4.73 ±0.15 μL/mL, respectively (Figure 1). The higher antioxidant capacity of citronella oil as compared to lemongrass oil may be attributed to the present of eugenol. Eugenol being a phenolic compound reported that is in literature to be an antioxidant by donating a hydrogen atom to the free radicals (Politeo *et al*., 2007). The IC_{50} value of eugenol was determined to 1.34 ±0.10 μL/mL. The antioxidant activity of α-tocopherol was significantly higher as compared to the two essential oils and eugenol standard. However, in view of the potential application, the antioxidant capacity of the essential oil from citronella grass was greater than those of other essential oils extracted form *Eucalyptus camaldulensis* Dehnh (IC_{50} = 4.10 μL/mL) (Sahin and Candan, 2010). The antioxidant activity of lemongrass oil was found to be similar (IC_{50} = 4.73 μL/mL) to the oils extracted from *Eucalyptus camaldulensis* Dehnh.

The results of α-amylase inhibitory activities are shown in Table 2. The positive acarbose showed IC_{50} value as 91.2 μL/mL. However, the inhibitory activity of citronella and lemongrass oils were ~fifteen times more as compared to positive commercial standard drug acarbose that is used for reducing glucose level in blood. The inhibitory activity of citronella grass and lemongrass was very similar and showed a strong overlap as shown in Figure 2.

Figure 2 shows the α-amylase inhibitory activities of citronella, lemongrass oils and acarbose control (positive). It was found that the α-amylase inhibitory activities decreased with increasing essential oil concentrations. The IC_{50} values of citronella and lemongrass oils were found to be the similar (6.59 and 6.97 μL/mL, respectively), while their antioxidant activity was about 10-fold different as discussed earlier. As comparison, the α-amylase inhibitory activity between citronella and lemongrass oil were similar because the α-amylase inhibitory active components in citronella and lemongrass oil were not much different content. From literatures, α-pinene and himachalol would be a moderate inhibitor of α-amylase (Loizzo *et al*., 2007; Sahin and Candan, 2010). Sahin and Candan reported higher α-amylase inhibition activity of eucalyptus essential oil with IC_{50} at the concentration of 0.435 μL/mL. It might be a reason that eucalyptus oil has higher amount of α-pinene (3.45%) than citronella and lemongrass oils (Sahin and Candan, 2010). In addition, the acarbose is the well-known anti-diabetic drug, but it has major side effects of flatulence, diarrhea, and abdominal pain due to undigested carbohydrates. Both these essential oils showed significantly higher activity as compared to the acarbose and both oils have been traditional used in Thailand as flavoring agent. Thus, both these essential oils may play an importance role in carbohydrate catabolism and possibly used for controlling diabetes.

Conclusion

In this study, the chemical composition, antioxidant capacity and α-amylase inhibitory activity of both citronella grass and lemongrass essential oils is reported. The results indicated that both essential oils possess antioxidant property and can potentially play an important role in controlling diabetic. Additional studies are warranted with both these essential oils to further evaluate their potential in control of sugar levels in diabetic patients and as a free radicals scavenging. However, an in vivo study is further needed to confirm the efficiency of the essential oils for the treatment of diabetes.

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