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Bioactivity analysis of lemongrass (*Cymbopogan citratus*) essential oil

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Abstract: Diseases such as diabetes mellitus and gout are among the chronic diseases affecting worldwide population. Investigation is required to find the alternative approaches to treat these chronic diseases, such as plant based medicine. In this study, lemongrass (*Cymbopogan citratus*) was chosen and examined on the basis of their usage in traditional medicines throughout Southeast Asia. GCMS analysis revealed the major constituents of the lemongrass essential oil which compromise 67.769% and 67.328% of the total oil respectively. Total phenolic content of the essential oil was analyzed by Folin Ciocalteau method and the results indicated that highest amount of phenolic content was obtained from essential oil extracted from lemongrasses stalk, with phenolic concentration of 2100.769 mg/l GAE. Anti oxidant activity was examined by DPPH scavenging test and the highest inhibition was obtained by essential oil extracted from lemongrass stalk (89.5%). β-glucosidase inhibition assay was carried out using an *in-vitro* model for anti diabetic test and lemongrass stalk essential oil showed highest degree of inhibitory activity (89.63%). Anti gout test was examined by xanthine oxidase inhibition (XOI) assay with the maximum percentage of xanthine oxidase inhibition of 81.34% obtained from lemongrass stalk essential oil.

Keywords: Anti-diabetic, anti-gout, anti-oxidant, gas chromatography-mass spectrophotometer (GCMS), lemongrass essential oil, total phenolic content

Introduction

Medicinal plants are source of a great economic value. Plant herbs are naturally gifted at the synthesis of medicinal compounds. The extraction and characterization of bioactive compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value. Treatment using medicines of natural origin is gaining momentum nowadays on account of increasing concern about potentially harmful synthetic additives (Reische, 1998). *Cymbopogan citratus* (commonly named, lemongrass), is a great interest due to its commercially valuable essential oils and widely used in food technology as well as in traditional medicine. Owing to the new attraction for natural products like essential oils, despite their wide use and being familiar to us as fragrances, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the environment.

People nowadays are more aware on health issue due to the emergence of new diseases. Treatment using plant-based medicine appears to be an alternative approach due to the adverse effects associated with the use of synthetic drugs. The genetic diversity of plants has provided us not only survival, but a high degree of comfort and the most important thing of all is the potential treatment for various diseases (Payne *et al*., 1991). The anti-oxidant properties of essential oils might be encouraging to consider them as natural oxidant in nutraceuticals and pharmaceutical preparations. In recent years, there is an increasing interest in finding antioxidant photochemical, because they can inhibit the propagation of free radical reactions, protect the human body from diseases (Kinsella, 1993; Terao *et al*., 1997; Reische, 1998) and retard lipid oxidative rancidity in foods (Duthie, 1993).

One of the common chronic disease infected many Malaysian citizen nowadays is diabetes mellitus which is a metabolic disorder affecting carbohydrate, fat and protein metabolism. The worldwide survey reported that the percentage of people being affected by this disease is increasing yearly. The increase in demand in industrially developed countries to use alternative approaches to treat diabetes, such as plant-based medicines, is due to the side effects associated with the use of insulin and oral hypoglycaemic agents (Marles *et al*., 1994). Therefore, it is crucial to search for newer anti-diabetic agents that possess less side effects and safe to be used on human bodies.

Gout is another common chronic disease nowadays which occurs in individuals who have high serum uric acid levels. The treatment of gout entails the use of therapeutic agents such as xanthine oxidase inhibitors (XOI). The prototypical of allopurinol as XOI has

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been the cornerstone of the clinical management of gout and conditions associated with hyperuricemia for several decades (Fields *et al*., 1996; Pal Pacher *et al*., 2006). However, it is important to note that the current XOI, including allopurinol possess some side effects where some people develop rash as they are allergic to allopurinol (Kong *et al*., 2000). Furthermore, severe reactions also occur including liver function abnormalities (Fields *et al*., 1996). The most apprehension complication with regard to allopurinol is called "allopurinol hypersensitivity syndrome" which can sometimes be fatal and life-threatening (Kong *et al*., 2000; Berry *et al*., 2004). Hence, introducing natural remedy as new source of gout medication is highly granted. Therefore, this present study aimed to find the potentiality of lemongrass essential oil which could be the alternative approach for the treatment of chronic diseases such as diabetes, gout and other related diseases to substitute the conventional drugs which posesses some adverse effects.

Materials and Methods

Raw material and sample preparations

Lemongrass after harvesting was separated from its stalks and leaves. The air-dried method was applied for drying. Water used in steam distillation process was distillation water. In these experiments the mass of lemongrass (both leaves and stalks) are fixed at 200 g for each load. After drying, the lemongrass was cut into small pieces at the length of 4 to 8 mm.

Extraction of essential oil

The essential oils tested were isolated by the hydro-distillation method using Clevenger-type apparatus (Kawther *et al*., 2007). They were extracted separately from leaves and stalks of lemongrass at certain temperature and extraction time based on the optimization range to give a mixture of water/essential oil. Dichloromethane was used to separate the essential oil from the water layer. The recovered oils were dried over anhydrous sodium sulphate and stored in darkness between 4 and 6°C for further analysis.

GCMS analysis

The analysis of the essential oil was performed using a Hewlett Packard 5890 II GC, equipped with a HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm) and a HP 5972 mass selective detector. For GC-MS detection, an electron ionization system was used with ionization energy of 70 eV. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. Column temperature was initially at 50°C, then gradually increased to 150°C at a 3°C/min rate, held for 10 min and finally increased to 250°C at 10°C/min. Diluted samples (1/100 in acetone) of 1.0 µl were injected manually and splitless. The components were identified based on the comparison of their relative retention time and mass spectra with those of NBS75K library data of the GC-MS system, literature data and standards of the main components.

Determination of total phenolic content

The total phenolic contents of the extracted samples were determined by Folin-Ciocalteau method (Perry *et al*., 1999). In a 100 mL volumetric flask, 0.5 g of dry gallic acid was dissolved in 10 mL of ethanol and diluted to volume with water. For the preparation of standard curve 0, 1, 2, 3, 5, and 10 mL of the phenol stock solution was taken into 100 mL volumetric flasks and diluted to required volume with water. These solutions had concentrations of 0, 50, 100, 150, 250 and 500 mg/L gallic acid. In 15 mL Falcon tube, 2370 µL of distilled water, 30 µL of sample and 150 µL Folin-Ciocalteu reagent were added and vortexes. After 1 minute, 450 µL of aqueous sodium carbonate (20%) was added, and then the mixture was vortexes and allowed to stand at 40°C for 30 min before reading the absorbance. The absorbance was taken at 750 nm. All measurements were carried out in triplicate. The total phenolic acids concentration was calculated from the calibration curve (Figure 1), using Gallic acid as the standard and the results were expressed as mg/L of Gallic acid equivalents (GAE mg/L).

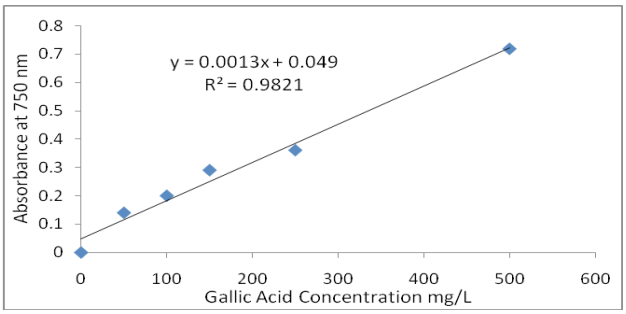

Figure 1. Folin-Ciocalteau gallic acid standard curve

DPPH radical scavenging (anti-oxidant assay)

DPPH radical-scavenging activity was determined by the suggested method (Berry *et al*., 2004) with slight modification. Briefly, 0.025 ml of sample extract of various concentrations, 0.075 ml of distilled water and 0.1 ml methanol were put into a 96-well micro plate. Then, 0.025 ml of 1mM DPPH in methanol solution was added. The sample mixture

was incubated at 37°C for 30 min. The absorbance was measured at 517 nm. A DPPH radical solution without sample extract was used as a control. All analyses were run in triplicates. The scavenging activity was expressed as percentage of inhibition, which was calculated according to the below equation:

Scavenging ability (%) = [(ΔA_{517} of control - ΔA_{517} of sample) /ΔA_{517} of control] X100 (1)

β-Glucosidase inhibition (anti-diabetic assay)

The β-glucosidase inhibition assay was performed using modification method. Sweets almond β-glucosidase was dissolved in 1 ml ice-cold of 0.05 M Tris-HCl buffer (pH 7.8) and then diluted with 0.2% bovive serum albumin (BSA) solution in 0.01 M phosphate buffer (pH 7.0) to give concentration of 0.022 unit/ml solution. This assay was based on the reaction of p-Nitrophenol formed during the reaction which determined spectrophotometrically at 400 nm. First, 1 ml of acetate buffer (0.1 M, pH 5.0) and 0.5 ml of p-nitrophenyl β-D-glucopyranoside (PNPG) solution (0.02 M) were mixed in a test tube and equilibrated in the water bath at 37°C for 15 min. The reaction was started by addition of 200 µl of enzyme solution and 300 µl of plant extract (10 mg/ml in dimethyl sulfoxide, DMSO). The tubes were incubated for a total of 15 min in the same condition. Then 2 ml of sodium carbonate, Na_2CO_3 solution (0.2 M) were added to stop the reaction. At the same time, control incubations which represent 100% of enzyme activity were conducted in identical fashion replacing plant extract with DMSO (300 µl). For blank incubations, which allow for absorbance produced by the plant extract, the enzyme solution was replaced with BSA solution (200 µl) and the same procedure was carried out as above. β-glucosidase activity was determined by measuring the absorbance of the mixture at 400 nm. The inhibition activity was calculated as equation below, where A = Control, B = OD test and C = OD blank.

$$\% \text{ inhibition} = \frac{A-(B-C)}{A} \text{ X } 100 \quad (2)$$

Xanthine oxidase inhibition (anti-gout assay)

The xanthine oxidase inhibition (XOI) activity was assayed spectrophotometrically under aerobic condition. Allopurinol at 100 µg/ml (Umamaheswari *et al*., 2007), a known XOI was used as a positive control for the inhibition test. The assay mixture comprises of 0.1 ml of test solution (100 µg / ml of extracts), 0.3 ml of 0.1M phosphate buffer at pH 7.5, 0.1 ml of dH_2O, and 0.1 ml of enzyme solution (0.12 units / ml of xanthine oxidase in 0.1M phosphate buffer at pH 7.5). The mixture was pre-incubated at 25°C for 15 min. Then, 0.2 ml of substrate solution (150 mM of xanthine in 0.1M phosphate buffer at pH 7.5) was added into the mixture. The mixture was incubated at 25°C for 30 min. After that, the reaction was stopped with the addition of 0.2 ml of 1M HCl. The absorbance was measured using a UV/VIS spectrophotometer at 290 nm. The same components were applied in the preparation of a blank; however, the enzyme solution was replaced with the phosphate buffer. Another reaction mixture was prepared (control) having 100 µl of DMSO instead of test compounds in order to have maximum uric acid formation. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid per minute at 25°C. The data collected from the assay were used to determine the inhibition activity. XOI activity was assessed as the percentage of XO inhibition according to the below equation,

$$\% \, XO \, Inhibition = \left(1 - {}^{\beta}/_{\alpha}\right) X \, 100 \quad (3)$$

where; β = Activity of the Xanthine Oxidase with test extract.

α = Activity of the Xanthine Oxidase without test extract.

Results and Discussion

Chemical composition of essential oils

A total of 68 compounds were characterized in lemongrass' stalk essential oil while the essential oil of lemongrass' leaves consists of 72 compounds. GCMS analysis revealed that geranial (32.10% and 29.64%), neral (22.36% and 21.73%), geraniol (5.40% and 7.75%), limonene (5.71% and 5.92%) and β-myrcene (2.20% and 2.28%), were the major constituents of the stalks and leaves' lemongrass essential oil respectively, comprising 67.77% and 67.33% of the total oil. These compositions of essentials oil from lemongrass' stalks and leaves which been extracted using steam distillation extraction are compared to the standard of pure lemongrass essential oil, which was bought from Sigma – Aldrich Chemistry. From the GCMS analysis on the standard essential oil, it revealed that the major constituents of the essential oil are higher compared to the essential oil extracted in this study, with the total of geranial (44.29%), neral (31.36), geraniol (10.01%), limonene (6.09%) and β-myrcene (3.56%), comprising 95.31% of the total oil. Most of the studies and literature found on lemongrass were focused only on the leaves part.

Based on literature data, it appears that geranial, neral, geraniol, limonene and β-myrcene have been found as major compounds in many other

Cymbopogan species with the main chemical component of lemongrass oil is citral (Luiz *et al*., 2001; Huynh, 2008). Citral or 3,7-dimethyl-2,6-octadienal is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (*trans*citral, citral A) and neral (*cis*-citral, citral B) (Huynh, 2008). Citral is an important raw material used in the pharmaceutical, perfumery and cosmetic industries, especially for the synthesis of Vitamin A and ionone (Efraim *et al*., 1998). Chemically, citral is a mixture of two aldehydes that have the same molecular formula, $C_{10}H_{16}O$, but different structures. However, it is noteworthy that the composition of any plant essential oil studies is influenced by several factors, such as local, climatic, seasonal and experimental conditions (Perry *et al*., 1999; Daferera *et al*., 2000).

Total phenolic content

Total phenolic constituents of the standard and extracted lemongrass essential oil were determined by experimental methods involving Folin-Ciocalteau test (Singleton *et al*., 1999). The total phenolic concentration was calculated from the calibration curve, using Gallic acid as a standard (Figure 1), and the total phenolic content were expressed as mg/l Gallic acid equivalents (GAE). The distribution of phenolic content of all three essential oil samples is presented in Figure 2. The results indicated that all the essential oil samples gave positive results with the highest amount was obtained from the essential oil extracted from stalk which has phenolic content of 2100.769 mg/l GAE, followed by standard essential brought from Sigma with 1962.308 mg/l GAE, and the lowest concentration of 1846.920 mg/l GAE was obtained from the essential oil extracted from lemongrass leaves.

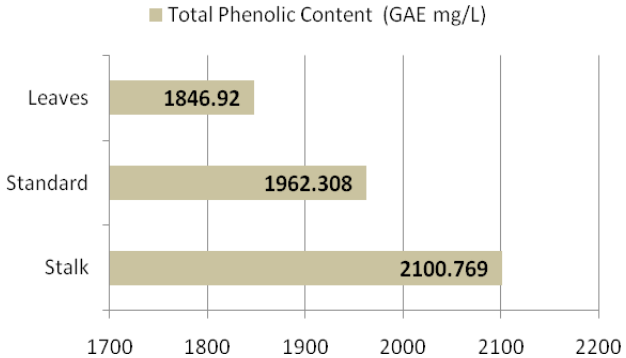

Figure 2. Total phenolic content (mg/l GAE) for different parts of lemongrass essential oil

Phenolic compounds constitute a large group of secondary metabolites derived from phenylalanine and are widely distributed throughout the Plant Kingdom. Phenolic compounds serve diverse functions such as imparting colour to leaves and fruits, attracting or repelling insects, antimicrobial action, antiviral activity, protection from harmful ultraviolet radiation and protection from herbivores (Harborne *et al*., 2000). A great number of reports have established that plant phenolic compounds including flavonoids are potent antioxidants with reported antimutagenic and anticarcinogenic effects (Middleton *et al*., 1994; Rice-Evans, 1997). Thus, the characterization of the chemical composition and phenolic profile of the lemongrass essential oil would help in contributing to further development of natural health products industry.

Anti-oxidant activity

The three sample of lemongrass essential oil were subjected for the anti-oxidant activity test namely DPPH free radical scavenging assay and the results are shown in Figure 3. From Figure 3, it can be summarized that the essential oil extracted from the lemongrass stalk has highest anti-oxidant activity with up to 89% inhibition at the ratio of 1:2 for the volume concentration of essential oil per volume of solvent. As illustrated in the figure, we can see that lemongrass stalk' essential oil inhibit almost the same rate with the standard essential oil bought from Sigma – Aldrich Chemistry which is used as the control. The leaves essential oil showed slightly lower inhibition with the highest anti-oxidant activity of 78.89% at the ratio of 1:2 (volume essential oil/ volume solvent).

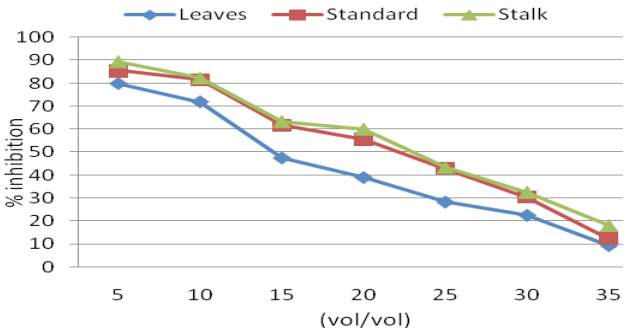

Figure 3. Free radical scavenging activities of the extracted essential oils and standard essential oil from Sigma (as positive control) measured by DPPH assay

The anti-oxidative effectiveness in natural sources was reported to be mostly due to phenolic compounds (Moller *et al*., 1999). The high amount of the phenolic content will lead to higher anti oxidative efficiency. Recent studies have shown that phenolic phytochemicals have high antioxidant activity and certain therapeutic properties (Shetty *et al*., 2005), including anti-diabetic and anti-hypertension activity (Kwon *et al*., 2005). The key role of phenolic compounds as scavengers of free radicals is

emphasized in several reports (Madsen *et al*., 1996; Moller *et al*., 1999). Moreover, radical-scavenging activity is one of various mechanisms contributing to overall activity, thereby creating synergistic effects. Thus this explains the high percentage activity of free radical scavenging activity in the essential oil extracted from stalk due to its high amount of total phenolic content.

As can be seen from the graph patterns, the essential oil exhibits high DPPH scavenging activities at high concentration of essential oil. The anti oxidant inhibition tend to decrease as the concentration decreases. However, for pure samples of essential oil (without any dilution), there is no result of inhibition value were observed. At high concentration, some compounds tend to increase DNA damage. For example, research done by Fan and Lou (2004), found that some polyphenols were good antioxidants at low concentration but, at higher concentration, they induced cellular DNA damage. At too high concentration, it could damage and permeabilize mitochondria, the antioxidant was oxidized and could react as prooxidant damaging DNA and proteins. Thus this might explains the zero inhibition results inhibited in this research. This DPPH scavenging assay is crucial since anti oxidant properties play important role which protects human body against free radicals that may lead to chronic and degenerative diseases.

Anti-diabetic activity

The anti diabetic activity for the three sample of lemongrass essential oils were analyzed by the β-Glucosidase inhibition. As illustrated in the Figure 4, all the essential oil samples showed positive results with certain level of inhibition. As can be seen from Figure 4, it can be concluded that the essential oil extracted from lemongrass' stalk was found to have highest inhibition of β-glucosidase activity among those three samples with up to 89.63% inhibition at the ratio of 1:2 for the volume concentration of essential oil per volume of solvent. The leaves and standard essential oil showed small percentage difference in their anti diabetic activity with the highest β-glucosidase activity of 79.26% and 73.8% respectively at the same ratio value of 1:2. From the graph pattern, it can be conclude that the β-glucosidase activities tend to deceases as the concentration of lemongrass essential oil decreases. The anti diabetic activity is increases at the high level of essential oil concentration. As the β-glucosidase inhibition increases, it indicated that the rate of carbohydrate digestion is higher and this scenario could help in reducing the level of blood sugar in human body thus decreases the risks of diabetes. However, zero inhibition values were observed when the crude samples were used without any dilution. These zero values could indicate that the β-glucosidase was activated or there was no inhibition effect in these samples. If this were to occur *in vivo*, it would aggravate, rather than improve the diabetic condition since the rate of glucose production would be increased and thereby the serum levels rise more rapidly (Ali *et al*., 2006).

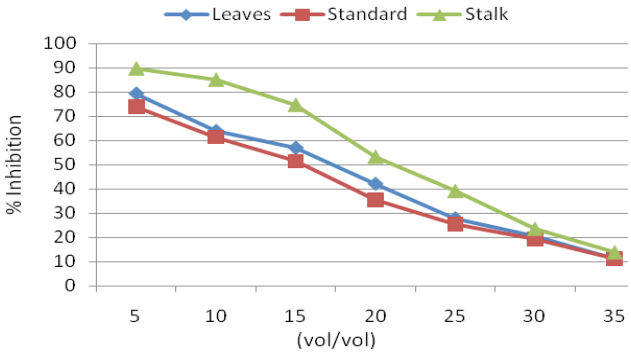

Figure 4. Anti-diabetic activity of the extracted essential oils and standard essential oil from Sigma (as positive control) measured by β-Glucosidase assay

Anti-gout activity

In addition, the anti gout activity of the lemongrass essential oil were analyzed by the Xanthine Oxidase Inhibition (XOI) assay and the results was shown in Figure 5. As illustrated in Figure 5, it can be concluded that the essential oil extracted from the stalk and the standard essential oil obtained from Sigma showed higher xanthine oxidase inhibition compared to essential oil extracted from the leaves. Essential oil which scored the highest percentage inhibition was the one extracted from the stalks with 81.34% inhibition activity at the ratio of volume concentration of essential oil per volume of solvent of 1:2, followed by the standard essential oil (80.02%) and essential extracted from leaves with 74.51% inhibition at the same volume ratio. Both essential oil extracted from leaves and stalks were showing positive results where they possessed an inhibition rate higher than 50% at high sample concentration, same goes to the standard essential oil. From the graph pattern, the inhibition activity decreases as the essential oil concentration decreases.

As the xanthine oxidase inhibition increases, it indicated that the production of uric acid is lesser thus this condition decreases the risks of gout. From the graph portrayed, it can be seen that the IC_{50} value where the inhibition activity possesses at least 50% activity was achievable at the ratio of volume concentration of essential oil per volume of solvent of 1:6 dilution onwards. However zero xanthine oxidase inhibition was observed when the pure samples were

analyzed. This might indicate that the xanthine oxidase was activated where the production of uric acid might increases or there was no inhibition effect in this sample at all. As for the overall assessment, it can be conclude that lemongrass essential oil has high potentiality to be an alternative source of anti gout drugs as it has potential compounds containing xanthine oxidase inhibitor (XOI).

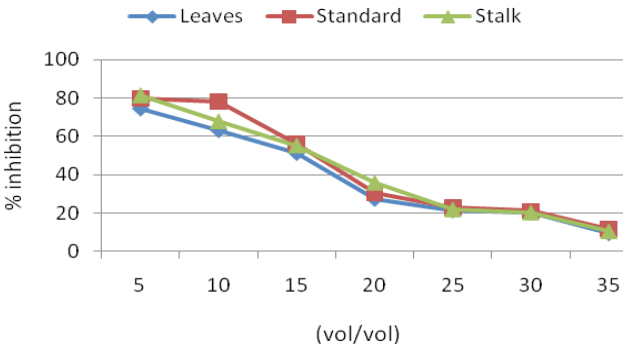

Figure 5. Anti-gout activity of the extracted essential oils and standard essential oil from Sigma (as positive control) measured by Xanthine Oxidase Inhibition (XOI) assay

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