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Determination of elemental, phenolic, antioxidant and flavonoid properties of Lemon grass (*Cymbopogon citratus* Stapf)

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Abstract

Commonly consumed Ghanaian lemon grass for tea making were analysed for contents of Potassium (K), Chlorine (Cl), Calcium (Ca), Magnesium (Mg) Manganese (Mn), Aluminium (Al), Copper (Cu) and Sodium (Na) across five locations using Instrumental Neutron Activation Analysis, in addition to total phenolic, antioxidant and flavonoid activities using standard methods. Potassium was the most abundance element and Cu was the least. Concentrations of these eight elements differed from location to location. Total phenolic activity in cold and hot percolations ranged from 1.3 to 4.7 mg and 2.6 to 7.3 mg of garlic acid equivalents (GAE)/g dw respectively; total antioxidant activity in cold and hot percolations ranged from 65.4 to 81.3 % and 65.4 to 81.3% respectively; and total flavonoid concentration ranged from 6.9 to 11.3 µg/g Quercetin Equivalent (QE) and 6.9 to 12.9 µg/g QE dry weight basis for cold and hot percolations respectively. The temperature of percolations therefore had pronounced effect on total phenol, antioxidant and flavonoid activities, which underscores the importance of boiling of lemongrass herbal teas to allow both simple and complex phenols to percolate faster and provide more antioxidant activities.

Introduction

Lemon grass (*Cymbopogon citratus* Stapf) is a C4 tropical and sub-tropical grass. The leaves contain up to 1±5% dw essential oils, mainly citral with a typical lemonade aroma (Tovar *et al*., 2010). Citral is a mixture of isomeric acyclic monoterpene aldehydes, i.e., neral (*cis*-citral) and geranial (*trans*-citral) (Rauber *et al*., 2005). The oil is used in fragrance formulations into numerous consumer products including geranial (39.0%), neral (29.4%). It myrcene (18.0%), geraniol (1.7%), and linalol (1.3%) (Chisowa *et al*., 1998). The grass possesses antifungal, mosquito repellent, insecticidal, anti-diabetic, anti-septic, anti-carcinogenic in humans and anti-mutagenic properties towards chemical-induced mutation in some *Salmonella typhimurium* strains. Japanese and Brazilians use tea prepared from *C. lunatus* to protect food from spoilage (Tiwari *et al.*, 2010). In North Africa the lemon grass tea is considered a refreshing beverage due to its pleasant aroma and taste (IUCN, 2005). The inner core of the rhizome is consumes as an aphrodisiac. Furthermore, the herbal infusion is used as food and medicine to treat fever conditions in Togo and Ghana, nervous and gastrointestinal disturbances (Carlini *et al*., 1986). In Nigeria, smoke from the burning grass is said to dispel temporary maniacal symptoms (Ayda *et al*., 2010). In North Africa, it is used as spices for seasoning food, fine fragrance for flavouring custard, traditional meat recipes, used as greens, salads, spices or condiments (Rivera *et al*., 2006).

Oxidations in living organisms produce energy to fuel biological processes in addition to free radicals. The uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing. Phenolic compounds are important secondary metabolites that are responsible for important properties in foods and drinks, such as color, aroma, bitterness and astringency (Brossaud *et al.*, 2001). Lemon grass contains natural antioxidants and anti-inflammatory agents which scavenge free radicals from the human body; lemon grass tea is an important source of bioactive compounds that could play a role in preventing oxidative damage and human diseases. Through this study, we expect to gain insights into different kinds of elements found in lemon grass and their concentrations across five locations in addition to total phenolic, antioxidant and flavonoid compositions under hot and cold

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percolations.

Materials and Methods

Source of materials

Lemon grass leaves were collected in July 2011 from five locations in Southern Ghana. Sample PSI was collected from the Botanical Garden of the University of Ghana which is a mountainous area in the Greater Accra Region. Samples VVSA and VVSB were obtained from Valley View University Site A and B respectively: which is also a mountainous area within the same region as PSI. The two sites are about 1.5 km apart. Samples AK1 and AK2 were collected from Akatsi training College which is a plane lowland area in the Volta Region. AK1 and AK2 are about 1.5 km apart and Akatsi is about 160km from Accra.

Chemicals

A 1,1-diphenyl1-2-picrylhydrazyl (DPPH), methanol, ethanol, Folin-Ciocalteau (FC) reagent, Sodium Carbonate (Na_2CO_3) and quercitin Gallic Acid (G.A) were acquired from Sigma-Aldrich in South Africa. Potassium acetate and aluminium chloride were obtained from BDH in England. All the chemicals were of analytical grade.

The elemental composition of Lemon grass

The elemental composition of the grass was determined by using Instrumental Neutron Activation Analysis (INAA) Method (Žukowska and Biziuk, 2008), which is an analytical technique based on the measurement of characteristic radiation from radionuclides formed directly or indirectly by neutron irradiation of samples (Parthasarathy, 1998). INAA method make it possible to simultaneous quantify many elements in a small amount of sample without chemical destruction.

Irradiation and counting

The irradiation of samples was done in a 30 kw GHARR-1 facility at 5 x 10^{11} n.cm^{-2}s^{-1} neutron flux at Ghana Atomic Energy Commission (GAEC). The samples were transported into and out of the reactor site by pneumatic transfer ("Rabbit") system. The duration of irradiation was chosen according to decay time (half-lives) of elements (Table 1). The duration of 2 minutes is for short-lived nuclides with half-lives in seconds and minutes; an hour with 24 hours delay time for medium-lived nuclides with half-lives in hours but not greater than 48 hours; 4 hours with 2 to 3 weeks delay time for long-lived nuclides with half-lives greater than 2 days. Irradiation activated elements in the samples to radioactive nuclei state, which decays by emitting characteristic γ photons

Table 1. contains nuclear data of radio-nuclides used for instrumental neutron analysis of minor and trace elements

Element	Nuclear reaction	Decay time*	Used γ-ray energy (keV)
Al	27Al(n, γ)28Al	2.24m	1778.99
Ca	48Ca(n,γ)49Ca	8.72min	3084.4
Cl	37Cl(n,γ)38Cl	37.24min	1642, 2167
Cu	65Cu(n,γ)66Cu	5.1min/12.7h	1039/1.354
K	41K(n,γ)42K	12.36 h	1524.58
Mg	26Mg(n,γ)27Mg	9.45min	1014.43
Mn	55Mn(n,γ)56Mn	2.579 h	846.7; 1810.7; 2112
Na	23Na(n,γ)24Na	14.96h/15.02h	1368.8, 2754.1

*y = years, d = days, h = hours, m = minutes. Adopted from Filby *et al.*, (1970).

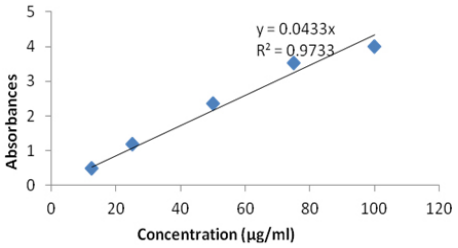

Figure 1. Standard curve of the Total Flavonoid content of samples

until returned to ground state with a characteristic half-life (Avino, 2009). The capsules were allowed to cool down until the level of activity is safe for handling (delay time). Counting was done in a high purity germanium well detector after 10 minutes of introduction of radioactive sample for short and medium nuclides and 10 hours for long lived radio nuclides measurements, respectively.

Qualitative and quantitative analysis of elements

The peak area is proportional to the radioisotope activity using a closed-end coaxial germanium (HPGe) semiconductor detector which operates at bias voltage of 1.5 kV with accompanying γ-spectroscopy accumulation software. The photo-peak energy value is used to identify elements and integration of area under photo-peak of irradiated samples and the standards under identical experimental conditions is used to determine the concentration of elements in the sample. Samples were analysed in triplicates.

Cold and hot percolations

Fifty-gram (50 g) of fresh lemon grass in triplicates were put in brown envelopes and dried at a temperature of 65°C to constant weight. Using mortar and pestle, the crispy leaves were homogenized into powder. Using cold (30°) temperature of percolation, a 0.7 g powder from each sample was added separately to 25 ml of double distilled deionised water and maintained at this temperature for 4 hrs with continuous shaking. The same procedure was followed for hot (60°C) percolation and extracts were filtered. Another 25 ml of double-distilled deionised water was added to the mark and the extraction process repeated. The filtrates were pooled to provide a total of 50 ml extract for each sample. In triplicates, the extract concentrations (extraction yields) were determined by pipetting 2

ml of filtered extract into pre-weighed petri-dish and evaporating off the solvents in an oven at 70°C to constant weight. The yield in 2 ml was used to standardize the remaining extracts which were stored at -20°C until required.

Determination of total phenolics content of C. citratus

The total phenolic contents in the cold and hot extracts were determined in triplicates following Folin-Ciocalteu method (Gao et al., 2000). A standard curve of absorbance (y) vs. Concentration(x) was drawn. A linear regression y = 1.736x + 0.159 (with $R^2 = 0.985$) was obtained. The total phenolic content was estimated according to the formula: C = cV/m (McDonald *et al.*, 2001), where C = Total phenolic content (GAE/g) dw, c = phenolic content (mg/ml) evaluated from the standard curve, V = volume of extract in ml (50 ml), and m = mass of the biomass in the extract (mg) in the reaction mixture.

Determination of total antioxidant activity of extracts of lemon grass

The total antioxidant activity of the grass was determined in triplicates using 1,1-diphenyl 1-2-picrylhydrazyl (DPPH) radical assay method (Botchway *et al*., 2007). The concentration of the test sample was determined by calculation based on the regression equation of the standard curve. Following Deore *et al.* (2009), the percentage scavenging inhibition (SI) of the test samples was calculated as:

$$\% \text{ scavenging inhibition (SI)} = \frac{\text{Absorbance of DPPH blank} - \text{Absorbance of sample}}{\text{Absorbance of DPPH blank}} \text{ X } 100$$

Determination of total flavonoids content of lemon grass

Total flavonoid content of the extracts was determined by employing aluminium chloride colorimetric assay method (Jia, 1999) using quercetin as the standard in triplicates. The linear regression equation (y = 0.0433x; $R^2 = 0.9733$) was deduced. Total flavonoid concentrations of samples were extrapolated from standard curve Figure 1 and values were expressed in terms of quercetin equivalents (QE) per gram of plant extract.

Statistical analysis

The bar chart facility in Microsoft Excel was used to illustrate the relative concentrations of the common elements present in the grass. In addition, the effect of the temperature of percolation on total phenollics, total antioxidants and total flavonoids were analysed using GenStat release 9.2 software. Means which were significant at $P < 0.05$ were separated by their respective least significant differences of values.

Results

Elements present in lemon grass and their relative concentrations

The elemental composition of lemon grass from five locations is presented in Figure 2. It is evident that all samples contained eight common elements: K, Cl, Ca, Mg, Na, Al, Mn and Cu, no matter their environment of collection. The first four elements (i.e., K, Cl, Ca and Mg) were higher in concentrations than remainders. K had a minimum content of 17130 mg/kg at PSI and a maximum of 28010 mg/kg at AK1, and an average of 24192 mg/kg. Chlorine had a minimum of 2116 mg/kg at PSI location to a maximum of 11040 in AK2. Cu had the lowest value of 1.0 at AK2 and a maximum value of 15 at VVB2 respectively and with a mean value of 9 mg/kg.

Phenolic activity of cold and hot percolations of lemon grass extracts

The total phenolics contents of the extracts are shown in Figure 3. The mean values of (i) lemon grass sources (average of hot plus cold percolations represented by AKI, AK2, PRI, VVSA and VVSB), (ii) hot percolation, (iii) cold percolation, and (iv) interactions between various samples sources and percolations were found to be statistically different at 5% significance level. Furthermore, the cold percolation extracts were significantly lower than those from hot percolations. Total phenolic contents in cold and hot percolations ranged from 1.3 to 4.7 mg of GAE/g dw and 2.6 to 7.3 mg of GAE/g dw respectively. The ranking of phenolic contents from lowest to highest in cold percolation treatment was 1.3 (VVSA), 1.8 (AK2), 2.0 (VVSB), 2.0 (PSI), 4.7 (AK1) while that of hot percolation treatment was 2 .6 (VVSA), 3.4 (VVSB), 5.4 (AK1), 7.4 (PSI) and 7.3 (AK2) mg of GAE/g dw.

Antioxidant activity of cold and hot percolations of lemon grass extracts

Figure 4 shows the total antioxidant activity of cold and hot extracts. The mean values of (i) lemon grass sources (average of hot plus cold percolations represented by AKI, AK2, PRI, VVSA and VVSB), (ii) hot percolation, (iii) cold percolation, and (iv) interactions between various samples sources and percolations were found to be statistically different at 5% significant level. Extracts obtained from the cold and hot percolations had similar total antioxidant activity. The total antioxidant activity in cold and hot percolations ranged from 65.4 to 81.3% and 65.4 to

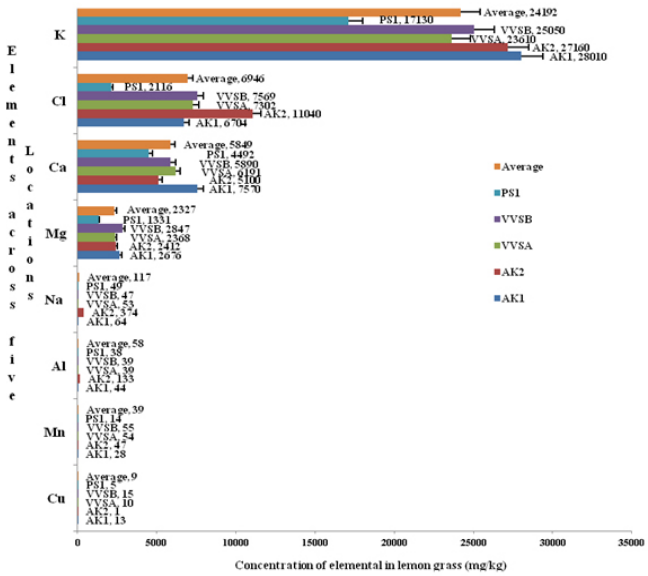

Figure 2. The concentration of elements across five sources of lemon grass

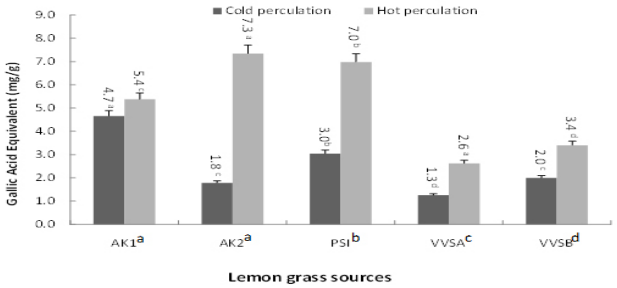

Figure 3. Total phenolic content of cold and hot percolations from lemon grass

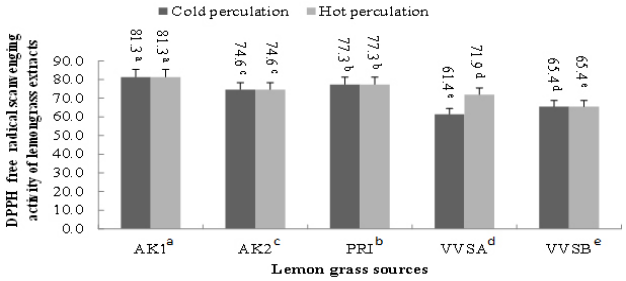

Figure 4. Total antioxidant activity of cold and hot extracts of lemon grass

81.3%, respectively. The ranking of total antioxidant activity of lemon grass from lowest to highest in cold percolation treatment was 61.4% (VVSA), 65.4% (VVSB), 74.6% (AK2), 77.3% (PSI), and 81.3% (AK1) and in hot percolation treatment was 65.4% (VVSB), 71.9% (VVSA), 74.6% (AK2), 77.3% (PSI) and 81.3% (AK1).

Flavonoid activity of cold and hot percolations of lemon grass extracts

Figure 5 shows the total flavonoid content of the *C. citratus* expressed in µg/g of Quercetin Equivalent (QE) in dw basis. The mean values of lemon grass sources (average of hot plus cold percolations represented by AKI, AK2, PRI, VVSA and VVSB),

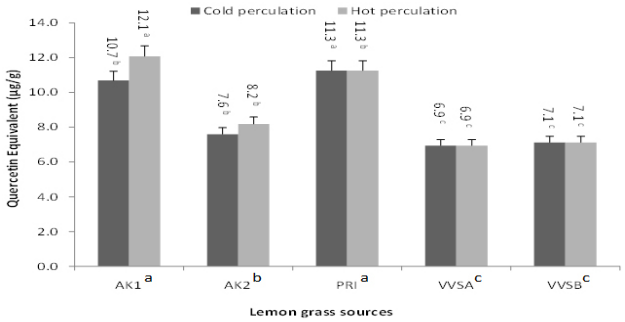

Figure 5. Total Flavonoid content of cold and hot extracts from lemon grass

(ii) hot percolation, (iii) cold percolation, and (iv) interactions between various sample sources and percolations were found to be statistically different at 5% significance level. The extracts obtained from cold and hot percolations had similar total flavonoid content. Total flavonoid content in cold and hot percolations ranged from 6.9 to 11.3 µg/g of QE dw basis and 6.9 to 12.9 µg/g of QE dw basis, respectively. The ranking of flavonoid contents from lowest to highest in cold percolation treatment was 6.9 (VVSA), 7.1 (VVSB), 7.6 (AK2), 10.7 (AK1), 11.3 (PSI) and in hot percolation treatment was 6.9 (VVSA), 7.1 (VVSB), 8.2 (AK2), 11.3 (PSI) and 12.1 (AK1) µg/g of QE dw basis.

Discussion

Elements present in lemon grass across five locations

Eight elements consisting of K, Cl, Ca, Mg, Mn, Al, Cu and Na were commonly found in five locations across both Greater Accra and Volta regions, although the sites of collection were over 160 km apart. This long distance of separation of sites implies the soil mineral compositions might have been different but lemon grass seemed to be undergoing selective absorption of similar minerals no matter how varied the soil and environmental conditions. Thus, differences in natural environments do not seem to have any influence on the quality of elements composed in the leaves of lemon grass. Therefore, elemental compositions of lemon grass are soil-type invariant.

The relative concentrations of elements in lemon grass

The relative concentration of each element in lemon grass varies enormously. It was observed that the first four elements (i.e., K, Cl, Ca and Mg) were consistently higher in concentrations than the remaining four (i.e., Mn, Al, Cu and Na). Potassium was the most abundance element and Cu was the least. It was also observed that the concentrations

of elements across locations were different. Thus, the relative abundance of elements in samples might depend on available elements in the soil moisture. In decreasing order of ranking of average concentration of elements observed in samples was 24192 mg/kg for K, followed by 6946 mg/kg for Cl, 5849 mg/kg for Ca, 2327 mg/kg for Mg, 117mg/kg for Na, 58 mg/kg Al, 39mg/kg for Mn, 9 mg/kg for Cu. Therefore, the quantitative elemental compositions differ from location to location whilst qualitative elemental composition remained the same across locations.

Potassiun (K) content

The importance of potassium is based on its influence on calcium homeostasis, particularly the urinary conservation and excretion of calcium. It also facilitates the transmission of nerve impulses and participates in the maintenance of the cardiac rhythm (Martin *et al*., 1985). The levels of potassium found in the samples ranged from 17130 to 28010 with an average of 24192 mg/kg. According to the United States Food and Drug Administration (FDA, 2000; Perkins-Veazie and Roberts, 2002), K provide health benefits if 1) a serving contains at least 10% of the recommended daily intake (RDI) of 3,500 mg K; 2) a serving contains no more than 140 mg sodium; 3) the food contains no more than 3 g saturated fat; 4) the food contains no more than 20 mg cholesterol; and 5) total saturated fatty acids contribute no more than 15% of the total caloric intake per serving. The average K level observed was higher than 1.56 g/kg found in other medicinal plants (Serfor-Armah, 2001). This implies lemongrass is a richer source of K for food supplements than some medicinal plants.

Chlorine (Cl) content

The chloride ion may play a more active and independent role in renal function (Toto *et al.*, 1984), neurophysiology (Sackmann and Neher, 1984) and nutrition (Honeyfield and Froseth, 1985). In addition highly mobile ions easily cross cell membranes and help in maintaining proper osmotic pressure, water balance and acid–base balance. The minimum and maximum values of chlorine observed in our five samples are 2116 and 11040, respectively. The average concentration observed was 6946 mg/kg. The daily intake of chloride in salted food is about 6 g (Meneely, 1973). The high level of Cl in lemon grass extract serves as the main extracellular anion in the body.

Calcium (Ca) content

Calcium (Ca) acts as the main structural element of bones and teeth in humans and is essential for the formation of fibrinogen which is vital for blood clotting. Low calcium intake causes deficiency in the body leading to osteoporosis and rickets in children. The range of 4492 - 7570 mg/kg and the average value of 5849 mg/kg Calcium were obtained in the five lemon grass samples. The RDI for calcium reported was 800 mg for males and 1000 mg for females (Miller *et al*., 2010). Therefore lemon grass could supply sufficient Ca to correct osteoporoses and blood clothing problems.

Magnesium (Mg) content

Magnesium is a co-factor for numerous body regulating enzymes and its functions include bone mineralization, protein building, nerve impulse transmission and teeth maintenance (Berdanier, 1994). Adequate levels of intracellular magnesium and potassium are important in ensuring sufficient blood flow to the muscles and internal organs. Magnesium helps to maintain normal acid-base balance and the control of nerves excitation level of the heart (Whang, 1998). It transmits nerve signals and minimises an amount of calcium that would enter a nerve cell to avoid muscle twitches, spasms and even convulsions. Because magnesium is involved in activating the body's main energy source (ATP), its absence results in general body weakness. The observed Mg composition of the lemon grass samples ranged from 1331 to 2847, with an average level of 2327 mg/kg. The Recommended Daily Allowance (RDA) are 350 mg/day for men and 280 mg/day for women, with no serious kidney problems. Therefore, Lemon grass tea could serve as alternative source of magnesium, similar to some medicinal plants reported to contain 0.14 – 0.23 g/kg of magnesium (Dim *et al.*, 2004).

Sodium (Na) content

Sodium together with Cl and K are electrolytes that maintain normal fluid balance inside and outside cells and a proper balance of acid and bases in the body. Deficiency of this element may result in muscle cramp and hypertension. When sodium intake in the form of sodium chloride is increased, it leads to increased renal calcium excretion to prevent gall stone formation, more than when sodium ingestion increased in the form of sodium bicarbonate or sodium acetate (Frassetto *et al*., 2001). The Na composition of the lemon grass sample ranged from 47 to 374, with an average of 117 mg/kg. These values observed were lower than the 500 mg/day minimum recommended daily allowance (RDA) for sodium in the United States (Mickleborough and Gotshall, 2004). Sodium intake will not be a problem in the face of adequate

calcium intake (Carbone *et al*., 2003) or potassium (Harrington and Cashman, 2003).

Aluminium (Al) content

Aluminium is naturally present in foods. The average Al concentration observed in lemon grass samples was 58 mg/kg and the range is 38-133 mg/kg. The average aluminium intake for humans is 10 mg/day (Cuciureanu *et al*., 2000). In case of drug administration, the normal average intake may reach 50-1000 mg/day. The lemon grass could therefore represent the minor source of aluminium for humans.

Manganese (Mn) content

Manganese (Mn) is known to play a key role in preventing diabetes, reducing symptoms of premenstrual syndromes and preventing epilepsy (Zhao *et al*., 2002). Adequate intake of Mn plays a vital role in preserving bone density against osteoporosis. The range of Mn obtained from the lemon grass sample was 15-55 mg/kg, and the average elemental composition was 39 mg/kg. The average level was higher than the RDI of 5 mg per day (Zhao *et al*., 2002).

Cupper (Cu) content

Copper (Cu) protects against oxygen radicals, enhances integrity of bones and lungs. It is a major component of collagen and elastin (Copper Development Association, 1984). The elemental composition of Cu from the lemon grass samples ranged from a minimum of 1.0 mg/kg to a maximum of 15 mg/kg, with a mean value of 9 mg/kg which was higher than 2 mg RDI of copper (Copper Development Association, 1984).

Phenolic activity of lemon grass extract

The total phenolic content was higher in hot percolation than cold percolation since higher kinetic energy (endothermic) was provided to molecules to break bonds and increase their mobility and solubility. The total phenolic contents in cold percolation ranged from 1.3 to 4.7 mg of gallic acid equivalents (GAE)/g dw whilst in a hot percolation, it ranged from 2.6 to 7.3 mg GAE/g dw. This shows that solubility increases with increase in temperature. Cold percolation might contain more simple and fast soluble phenols than complex and less soluble ones. Flavonoid is among simple sub-group of phenols and is expected to become more soluble than complex phenols. This finding underscores the importance of boiling herbal preparations prior to drinking to allow dissolution of both simple and complex water soluble phenolic compounds. The range of hot percolation observed (260-740 mg GAE/100 g dw) had compared favourably with the total phenolic content of 644.0 mg GAE/100 g reported for lemon grass (Chan *et al*., 2010).

Antioxidant activity of lemon grass extract

A single solvent was used for extraction but sufficient time was allowed for effective extraction, moreover the extraction was carried out twice with 20ml of water to ensure effective percolation. The total antioxidant activity for the lemon grass in cold and hot percolations ranged from 65.4 to 81.3% and 65.4 to 81.3%, respectively. These values were comparable to total antioxidant activity of various types of foods using DPPH in water method. It may however be lower than an extraction technique which allows simultaneous use of different solvents, because fat soluble antioxidants can dissolve in organic solvents to add up to total soluble antioxidants from aqueous solvents. It should be noted that the total antioxidant activity of fat soluble components could not be determined under DPPH extraction with aqueous media method because lemon grass has citral (77%) and limonene (8.5%), which induces activities of both lipophilic and hydrophilic antioxidants (Miron *et al*., 2010) but the lipophilic antioxidants activity is more effective than hydrophilic antioxidants (Tyagi and Malik, 2010). Therefore, these categories of un-available lipophilic anti-oxidants might not react with DPPH to reduce its radicals in aqueous medium.

Flavonoid activity of lemon grass extract

A significant effect of temperature of percolations on antioxidant properties of lemon grass extract was observed. Total flavonoid content of the extracts expressed in µg/g of Quercetin Equivalent (QE) dw basis shows that cold percolations at 30°C of lemon grass have similar total flavonoid content as hot percolations at 60°C. The total flavonoid content observed in cold and hot percolations ranged from 6.9 to 11.3 µg/g QE dw and 6.9 to 12.9 µg/g QE dw basis respectively. According to Kähkönen *et al.* (1999), the antioxidant activity does not necessarily correlate with high amounts of phenolics. This underscores the importance of examining both phenolic content and antioxidant activity when evaluating the antioxidant potential of lemon grass extracts. Likewise, high flavonoid content assayed with aluminium chloride colorimetric method (Jia, 1999) using Quercetin as standard does not necessarily mean a high antioxidant capacity of samples (Park *et al*., 1997). The $AlCl_3$ colorimetric test for flavonoids (Chang *et al.*, 2002) does not measure those flavonoids that do not bear

the characteristic chelating functional groups for Al binding. Essentially flavones (e.g., chrysin, apigenin, luteolin, etc.) and flavonols (e.g., quercetin, myricetin, morin, rutin, etc.) react with Al^{3+}, while flavanones and flavanols react with Al^{3+} slowly (Chang *et al.*, 2002). The flavonoid fractions of total phenolic was the most active against species involved in oxidative damage processes (Figueirinha, 2008). The flavonoids obtained in lemon grass could constitute favourably to human health.

Conclusion

Eight elements were common to five sources of lemon grass samples. The relative concentrations of these elements vary significantly. Potassium was the most abundant element found and Cu was the least. The relative abundance of elements in samples might depend on soil composition but the constancy of eight elements across potential variable environmental conditions attest to the fact that the crop might be selective in absorption of elements from the environment and therefore elemental types found in the leaves were not necessarily dependent on environmental variability. The total phenolic, antioxidant and flavonoid compositions had the following ranges: total phenolic contents in the cold percolation ranged from 1.3 to 4.7 mg of garlic acid equivalents (GAE)/g dw while hot percolations ranged from 2.6 to 7.3 mg GAE/g dw. The total antioxidant activity in cold and hot percolations ranged from 65.4 to 81.3% and 65.4 to 81.3%, respectively. Total flavonoid contents ranged from 6.9 to 11.3 µg/g QE dw and 6.9 to 12.9 µg/g QE dw basis for cold and hot percolations, respectively. The temperature of percolations was found to have a pronounced effect on antioxidant properties of the extract in the total phenolic total antioxidant and total flavonoids scavenging activities. The flavonoid fractions was the most active against species involved in oxidative damage processes and could constitute a potentially important source of compounds with favourable effects for human health even at cold percolation. These findings underscores the importance of boiling herbal preparations before drinking to allow both simple and complex soluble phenols to percolate more into solution and provide more antioxidant activities.

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