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ANTIOXIDANT ACTIVITIES OF LEMONGRASS WITH SOLVENT MULTI-STEP EXTRACTION MICROWAVE-ASSISTED EXTRACTION AS NATURAL FOOD PRESERVATIVE

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ABSTRACT

The use of synthetic preservatives is considered to have an adverse effect (carcinogenic) upon prolong consumption. Lemongrass *(Cymbopogon citratus)* is a plant that has bioactive components to act as antioxidants and potential to use as a natural food preservative. Bioactive components can be non-polar, semi-polar and polar; therefore, to determine the dominant bioactive components, a solvent multi-step extraction carried out. This study aimed to determine the specific bioactive components of lemongrass (antioxidant activity, total phenolic content and total flavonoids) suitable of polarity in the leaves and stem of lemongrass extract obtained from solvent multi-step extraction with Microwave-Assisted Extraction. The solvent used is ethanol (polar), ethyl acetate (semi-polar) and n-hexane (non-polar). The result showed that the highest bioactive components obtained from the polar stem lemongrass with total phenolic content of 19.31 mg GAE/g, flavonoids of 3.31 mg GAE/g. This result related to antioxidant activity of the extract of 79.96 %. The high antioxidant activity showed that lemongrass has potential to be used as a natural food preservative, especially in high fat food products.

Keywords: *Antioxidants, lemongrass, multi-step extraction, natural food preservative*

ABSTRAK

Penggunaan pengawet sintesis dinilai dapat memberikan efek buruk (karsinogenik) jika dikosumsi dalam jangka panjang. Serai dapur (*Cymbopogon citratus*) merupakan tanaman yang memiliki komponen bioaktif yang berperan sebagai antioksidan berpotensi digunakan sebagai pengawet alami pangan. Komponen bioaktif dapat bersifat non polar, semi-polar dan polar sehingga untuk mengetahui komponen bioaktif dominan pada serai dapur dilakukan ekstraksi bertingkat. Penelitian ini bertujuan untuk mengetahui komponen bioaktif spesifik serai dapur (aktivitas antioksidan, total fenol dan total flavonoid) sesuai tingkat kepolarannya pada bagian tanaman daun dan batang serai dapur hasil ekstraksi pelarut bertingkat dengan metode Microwave-Assisted Extraction (MAE). Pelarut yang digunakan pada penelitian ini adalah polar (etanol), semi polar (etil asetat), dan non polar (n-hexana). Metode penelitian antara lain: 1) ekstraksi pelarut bertingkat; 2) pengujian meliputi penangkapan radikal radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH), pengukuran total fenolik (Follin Ciocalteu) dan total flavonoid. Hasil penelitian menunjukan bahwa komponen bioaktif tertinggi pada bagian batang serai dapur bersifat polar meliputi total fenolik (19,31 mg GAE/g), flavonoid (3,31 mg GAE/g) hasil ini berbanding lurus dengan kapasitas penangkapan radikal (DPPH) sebesar (79,96%). Tingginya antioksidan pada serai dapur membuktikan bahwa serai dapur potesial digunakan sebagai pengawet alami pangan terutama pada produk tinggi lemak.

Kata kunci: *Antioksidan, ekstraksi pelarut bertingkat, pengawet alami pangan, serai dapur*

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INTRODUCTION

Antioxidants is important in preventing changes or damage in foods containing lipids (Naufalin, 2019). Synthetic antioxidants such as Butylated Hydroxytoluen (BHT), Tertbutylhydroxyquinone (TBHQ) and Butylated Hydroxyanisole (BHA), is commonly used in compliance to regulations (Sukardi, 2001). However, the use of synthetic preservatives was suggested to possess risk to the body if consumed for prolonged time. According to Namiki (1990), BHT utilisation is limited due to the carcinogenic properties. Research conducted by Aprilia *et al.* (2018) showed that the use of BHT can significantly damage the kidneys in Wistar rats. The finding has led into efforts in the uses of natural food preservatives in a yearning concern for healthier ingredient.

Several common compounds from natural ingredients possessing antioxidant activities are vitamin C, vitamin E, carotenoids, phenolic compounds, and polyphenols. There are about eight thousand plants that contain phenolic compounds and half of them are in the class of flavonoids. Phenolic compounds are included in the largest phytochemical group in plants that have antioxidant activity (Sulaeman *et al.*, 2011). The flavonoids that have antioxidant activity include flavones, flavonols, isoflavones, catechins, flavonols, and chalcones. This is because the -OH group and the double bond (> C = C) are owned by these compounds.

Cholifah *et al* (2017) observed that addition of garlic and pandan leaves was able to increase the shelf life of tofu products, compared to those without. The research suggested the flavonoid content in garlic and pandan leaves work as antioxidant that suppress oxidation reactions and also as antibacterial agent. The components of thymol, carvarol, zingerone, gingerol, hydroxytyrosol can inhibit peroxidation in capturing peroxyl radicals. Another study by Susilowati and Harningsih (2015) showed that flavonols in shallots act as a strong antioxidant and was able to inhibit the process of rancidity of coconut oil. Ginger extract in soybean oil and sunflower oil has also shown protection against auto-oxidation in oil (Sukandi, 2001).

The use of antioxidants as natural preservatives is still minimal, especially in the food industry. Despite of the abundant availability, they are considered to have low effectiveness and practicality, unstable in processing conditions and, quite importantly, affect sensory properties in food products. Appropriate method is required to produce effective and efficient natural preservatives for several utilization in food, such as edible coating on fresh meat or high-fat products, as well as BHA, BHQ substitute.

Indonesia, that was long known for its wealthiness of biodiversity, is also known as a herb and spices producer, including *Cymbopogan citratus*. *C. citratus* not only enrich the taste of food, but may also act as antioxidants and antimicrobials; putting the herb into potential ingredient to be utilised as a natural preservative in food. Lemongrass or *C. citratus* is easy to find because it is easy to cultivate on various types of soil. Research by Hamza *et al.* (2009) stated that lemongrass extract contains antioxidants in the form of phytochemical compounds, including; saponins, tannins, alkaloids, flavonoids. Lemongrass leaves has bioactive compounds, such as; phenols, flavonoid, tannins, and compounds which has many sulfide and alkaloid groups. Kanopa *et al*. (2012) states that the presence of these compounds is potential as antioxidants. Furthermore, Pujawati *et al.* (2009) stated that the simplicia extract of lemongrass inhibit the growth of *Candida albicans* at concentration of 0.4 %. Lemongrass essential oil inhibit the growth of *Eshericia coli*, *Staphylococcus aureus* (Apriliani *et al.*, 2014) and inhibit the growth of caterpillars on cabbage leaves (*Plutella xylostella*) at a concentration of 1.5 % (Prasetyo *et al.*, 2013). The antioxidant activities that are complemented with antimicrobial activities supported further exploration of lemongrass as potential natural preservative.

Extraction is a common method in acquiring bioactive components (Erminawati *et al.*, 2019). Multi-step extraction is an extraction that can carried out in stages to produce certain compounds that specifically extracted in each solvent used by two or more solvents. The multi-step extraction can be carried out starting by dissolving the material in the solvent with the lowest polarity level, namely

non-polar, then semi-polar and finally the polar solvent. Various bioactive components in lemongrass might have different level of polarities (non-polar, semi-polar or polar), and therefore multi-step extraction could be carried out to determine the dominant and specific bioactive components according to the polarity level.

Solvent is an important consideration in extraction, as it highly correlates to the affinity of the compound targeted, polarity and polar groups. A material will dissolve in a solvent of the same polarity (Sudarmadji *et al.*, 1989; Septiana and Asnani, 2012). N-hexane is a solvent that can extract semi-polar components such as fat and oil; which is volatile, with boiling point between 65-70 ℃ (Susanti, 2012). Its volatile properties, is practical in preventing n-hexane to come with the extract. One of the semi-polar solvents that are often used in the extraction of essential oils is ethyl acetate, the boiling point is almost the same as n-hexane, which is 77 ℃. Ethanol solvent is a relatively harmless polar solvent with high absorption of electromagnetic energy (Yulianti, 2014). Research conducted by Aliyudin (2019) stated that 70% ethanol with ingredients to solvents ratio of 1:10 w/v, produced the highest total phenolic content and antioxidants in lemongrass stem concentrate.

The principle of multi-step extraction is to dissolve materials in several solvents. One of the methods that can optimally produce bioactive components according to polarity is the microwave-assisted extraction (MAE) method. The study of Romadanu et al., (2018), used multi step extraction of non-polar semi-polar and polar solvents, which is specific bioactive components of the lotus flower; obtained that the polar solvent (methanol) produced higher antioxidants compare to other solvents. Harianingsih (2018), states that MAE method application resulted in higher yield of citronellal bioactive component, compared to other extraction methods observed in the study. MAE advantages come from its short extraction time, less solvent and electrical energy requirement. In MAE, temperature control can be easily done and therefore may benefit extraction of thermos-labile materials or compounds.

Based on the above background; there is currently no research that combines these two methods a multi-step extraction research carried out using the microwave-assisted extraction of lemongrass to identify specific bioactive components, namely; total flavonoids, total phenolic content and antioxidant activity according to the polarity level in order to increase the functional and economic value of lemongrass.

MATERIALS AND METHOD

Materials

Materials used in this research are; stems and leaves of lemongrass obtained from Sumbang, Banyumas, Central Java (with specification: fresh, light green to dark green color, age 8-10 months), aquades, Folin-Ciocalteu, Sodium carbonate (Na_2CO_3) (2 %), HCL, Methanol (PA), DPPH, Ethanol (90 %), N-Hexane, Ethanol (70%), Ethyl Acetate, $AlCl_3$, Potassium acetate (CH_3COOK).

Method

Sample preparation

The lemongrass plant is cut as follows; 15 cm from the rot considered as the stem and the rest considered as the leaves. Lemongrass leaves and stems sorted, washed using clean running water. Furthermore, reduced the size to ± 0.5 cm. Then arranged on a stainless steel pan and dried using a cabinet dryer at 55 °C. The dry lemongrass blended for ± 2-5 min. to powder.

Multi-step extraction using the Microwave-Assisted Extraction (MAE)

In this research, the type of extraction carried out is a multi-step extraction. The process of making lemongrass extract based on modified in previous studies (Erminawati *et al.*, 2019). The Lemongrass (150 g) is put into 500 mL erlenmeyer then dissolved using a multi-step starting with non-polar n-hexane (1: 6 w/v), the sample is extracted using a microwave at 225 watts for 5 min. The filtrate is filtered using filter paper, evaporated to remove the solvent using a vacuum rotary evaporator at 70 °C to form lemongrass filtrate. Meanwhile, the pulp

extracted using semi-polar solvent ethyl acetate (1: 4 w/v) using microwave. The same operation the polar ethanol (70 %) solvent (1:10 w/v). A summary of the multi-step extraction showed in Figure 1.

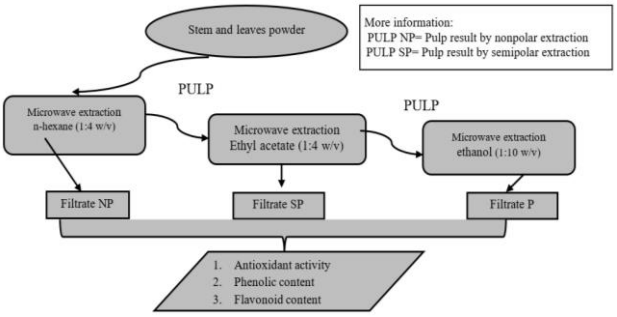

Figure 1. Summary of the multi-step extraction lemongrass

Antioxidant activity (Gadow *et al.*, 1997)

The total antioxidant activity determined based on the difference absorbance of the solution. A 0.0063 g DPPH powder diluted in 100 ml of pure methanol. The samples were made in a 20.000 ppm dilution series. A total of 2 ml extract solution mixed with 2 ml of 0.16 mM DPPH solution, then vortex for 1 min, left for 30 min, measured the absorbance using a UV-VIS spectrophotometer at 517 nm. Calculate the corrected absorbance using the formula:

$$\% \text{ inhibition} = \frac{Ao - As}{Ao} \times 100\%$$

Description: A_0: blank absorbance, As : sample absorbance

Total phenol content (Chew *et al.*, 2009 modified)

Natrium carbonate (Na_2CO_3) (2 g) diluted in 100 ml of distilled water. Dilute 0.1 ml of Follin by adding 0.1 ml of distilled water (1:1 v/v). The sample diluted 100 x with ethanol. Add 2 ml Na_2CO_3 to 0.1 ml sample solution, then let stand for 2 min, then add 0.1 ml Follin reagent. Vortex for 1 min, incubated for 30 min. at room temperature and dark room. Measure the absorbance using a UV-VIS spectrophotometer at 765 nm. Gallic acid used as standard.

Total flavonoid content (Ipandi *et al.*, 2016).

A total of 1 ml sample was reacted with 0.1 ml $AlCl_3$ 2%, vortex for 1 min. Add 1 ml of potassium acetate (CH_3COOK) (1.76 %); Votex again for 1 min, then incubated for 60 min in the dark. Measure the absorbance using a UV-VIS spectrophotometer at 435 nm.

Data analysis

Parametric variable data analyzed using variance test or ANOVA (Analysis of Variance) at the 5% level and if it shows a real effect on the treatment then followed by the DMRT (Duncan's Multiple Range Test).

RESULTS AND DISCUSSION

Total phenolic content

Total phenolic analysis determines the total phenolic content in the extract from the standard curve equation for gallic acid. The compounds were determined spectrophotometrically using the method of Chew *et al.* (2009) with Follin-Ciocalteu reagent. This reagent oxidizes the phenolics (alkaline salts) and reduces to a molybdenum-tungsten complex reaction. Where phenolics are found in alkaline solutions, while the Follin-Ciocalteu reagent and its products are unstable in alkaline conditions to form a blue complex so that it detected by a spectrophotometer. The resulting color is highly dependent on the hydroxyl group and the location of these groups in the molecular structure. The increase in color indicates in the concentration of phenolic compounds.

The total phenolic content is calculated by entering the absorbance value data sample into the linear regression line equation y = ax + b, which is obtained from the gallic acid calibration curve. The

results of the total phenolic content in GAE (gallic acid equivalent) units, mg of extract concentration per gram of sample (mg GAE/g sample) (Lestari *et al.*, 2018). The results of phenol analysis presented in Figure 2.

Solvent polarity determined by the dielectric constant, along with the dielectric constant of hexane, ethyl acetate, ethanol, methanol and water, were 1.89; 6.02; 24.30; 33.60; and 80.40, respectively. The greater dielectric constant the more polar the solvent (Sudarmadji *et al.*, 1989). As shown in Figure 3, higher total phenolic content was observed in the lemongrass extracted with polar solvent (ethanol), with three highest content observed in the lemongrass stem extracted using ethanol (19.13 mg GAE/g), followed by lemongrass leaves extracted using ethanol (15.80 mg GAE/g) and then lemongrass stem extracted using ethyl acetate (14.74 mg GAE/g). This data indicates that the phenolic components found in lemongrass distributed in the polar to semi-polar range. The highest total phenolic in stem with ethanol is thought to be due to the phenol structure found in lemongrass stem which more polar (containing many hydroxyl groups) than semi-polar leaves so that they will dissolve more easily if dissolved by a polar solvent with a polarity degree of 33.60 (Sanga and Katja, 2011). Ethanol considered very appropriate for extracting materials that contain polar components.

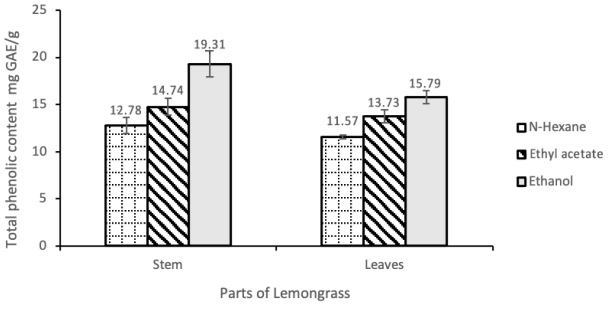

Figure 2. Total phenolic content of lemongrass stem and leaves

Previous research, conducted by Aliyudin (2019), found that phenol content of lemongrass stem with ethanol at the same concentration and extraction method was 12.07 mg GAE / g, smaller than the results of this study. This proves that multi-step extraction can maximize extract bioactive components such as total phenol by using the right solvent, ethanol. In conjunction, Septiana and Asnani (2012) reported that the polyphenol content obtained in multi-step extraction was higher than single extraction. In accordance with the purpose of multi-step combine with microwave assisted obtain a more specific or pure component. Extraction process employing microwave energy, which was absorbed by the compound within the cell. The absorbed energy increased the cell temperature and triggered pressure from within, allowing cell lysis and extraction process due to damage of the cell structure. Research by Kusnady et al. (2017) showed that the optimal time of microwave extraction to produce the total phenolic was five minutes, as yield for the phenolic content decrease after 5 minutes.

Phenolic compounds have a structural diversity ranging from simple phenols to complexes components. The potential of phenolic compounds as antioxidants caused by the presence of hydroxyl groups in phenol compounds. The hydroxyl group can function as a hydrogen atom contributor when it reacts with radical compounds via electrons, which can prevent the oxidation process. Phenolic compounds can react with peroxyl radicals to end the chain reaction (Wright *et al.*, 2001; Budhiyanti, 2013). Phenolic antioxidant activity depends on the number and position of the hydroxyl group in relation to the carboxyl functional group in the aromatic ring. Andiana, et al. (2019) found that encapsulated extract of rice husks has a total phenolic content of 3,125 mg GAE/g that may act as antioxidant, and also shown to inhibit the activity of gram-negative and gram-positive bacteria in tofu, therefore had the potential to be utilized a natural food preservative.

Not only act in food preservation of easily oxidized food , phenolic compounds also play role in health maintenance and disease prevention. Alshikh *et al.* (2015) reported that the phenolic compounds present in nuts not only contribute to organoleptic

properties but also benefit health. (Yusmiati *et al.*, 2012) reported that red tea extract or green tea at the given doses (150 mg and 300 mg) can reduce the risk of atherosclerosis in mice on an atherogenic diet as evidenced by a significant reduction in the number of foam cells. Antioxidant activity from the polyphenol compounds of red and green teas reduced the oxidation stresses, especially the lipoprotein components and reducing the reactivity of lipid peroxides which have behaved as free radicals. The antioxidant action was associated with the existence of double bonds and hydroxyl groups in these polyphenol compounds that play role as oxidants and free radicals scavenger (Yusmiati *et al.*, 2012). The oxidation stress is commonly associated with atherosclerosis and following coronary heart disease, a blockage of the arteries due to the build-up of cholesterol plaque which blocks blood flow to the body's organs.

Total flavonoids

Determination of flavonoid levels was using Chang (2002); this method compared with the quercetin standard at maximum wavelength at 435 nm. During the test, the flavonoids in the sample will react with $AlCl_3$ and form a yellow complex. Flavonoids are one of the bioactive components that almost found in all plants, based on Figure 3. The flavonoid framework consists of one aromatic ring A, one aromatic ring B, and a heterocyclic middle ring containing oxygen and this ring oxidized form is used as the basis for dividing flavonoids into its sub-groups (Dewi, 2018). Flavonoids are classified based on the addition of the oxygen chain and distribution of the hydroxyl group between flavonols, isoflavones, flavanones, flavanonols and khalcones (Marby *et al*, 1970; Susilowati and Estingingrum, 2016). Flavonoids may act as natural antioxidants (Shahidi and Naczk, 1995) due to the presence of phenolic hydroxyl groups in their molecular structure. The total flavonoids in lemongrass, stem and leaves in this study is as presented in Figure 3.

The highest total flavonoids obtained in the ethanolic extract of lemongrass stem (3.31 mg GAE/g); followed by ethanolic extract of lemongrass leaves (2.53 mg GAE / g; and semi-polar solvent stem (2.38 mg GAE / g). The high content of lemongrass stem flavonoids is suggested to be caused by the presence of parenchyma cells which contain high number of bioactive components, including flavonoids.

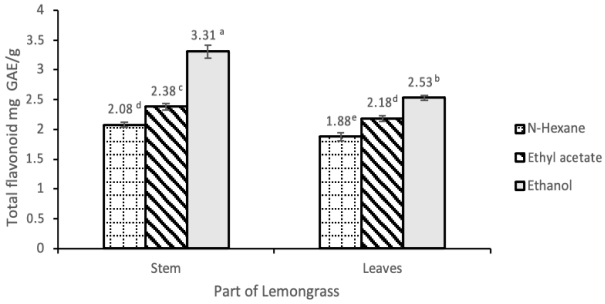

Figure 3. Flavonoid content of lemongrass stem and leaves

Flavonoid have different levels of polarity depending on their structure in plants, but it is known that flavonoid compounds are generally semi polar to polar. The structure of flavonoids, which are more dominant in polar, as seen in lemongrass leaves and stems, which is thought to affect the resulting flavonoids. The higher total flavonoid content in the polar solvent influenced by the multi-step extraction process, in which the extraction can extract specific bioactive components according to their solubility level. N-hexane is a non-polar solvent, that will extract non-polar compounds, suitable to extract lemongrass, because it is insoluble in water, and capable to dissolve nonpolar compounds at low boiling points (Gomarjoyo *et al.*, 2015). Likewise, ethyl acetate as semi-polar solvent extracts semi-polar compounds. Ethanol 70 % can easily extract polar compounds without any interference from being extracted from other group compounds, resulted highest yield of flavonoid content in both lemongrass stem and leaves.

Arbaayah and Kalsom (2013) reported the higher yield of flavonoid content in extracts using ethanol or distilled water as solvent. Solvent penetrates the plant cell wall, entering cell cavity containing

flavonoid. Efficiency of the extraction may differ due to flavonoid content, plant pat, nature/type of flavonoids especially its polarity and therefore its affinity to polar or less polar solvents. Similarity of flavonoid characters with polar and semi-polar solvents enhance the extraction of flavonoid content in lemongrass.

Flavonoids are phenolic compounds that are widely isolated from plants because of their antioxidant, anti-microbial and anticancer benefits. Flavonoids contribute to its antioxidant activity in vitro by binding (chelating) metal ions such as Fe and Cu. Metal ions such as Cu and Fe, can catalyze reactions that eventually produce free radicals (Mira *et al.*, 2002; Muchtadi, 2012). As antioxidants, flavonoid not only can prevent oxidation but also possess an anticancer activity. Several prenylated flavonoids that have been isolated from plants show cytotoxicity activity against several cancer cells, such as artelastin, artelastochromene, artelasticin in MCF-7 cells (breast cancer), TK-10 (kidney cancer), UACC-62 (melanoma cancer); artelastoxanthone and artonol A in A549 (lung cancer), Hep3B (liver cancer), HT-29 (colon cancer), MCF-7 (breast cancer) (HH *et al.*, 2005). According to previous studies by Itoigawa *et al.*, 2002, prenylated flavonoids may induce cytotoxicity in some cancer cells.

The first mechanism of flavonoids as anticancer, is through activation of the apoptotic pathway of cancer cells correlation to the DNA fragmentation. This fragmentation begins with the release of the proximal DNA chain by reactive oxygen compounds such as hydroxyl radicals. This compound formed from the redox reaction of Cu (II). These copper compounds are mobilized by flavonoids; both from extra cells and intra-cell, especially from chromatin. Secondly, flavonoids as inhibitors of tumor / cancer proliferation, one of which by inhibiting protein kinase activity, so it blocks the signal transduction pathway from the cell membrane to the cell nucleus. Thirdly, by inhibiting the activity of tyrosine kinase receptor, to increase the activity of tyrosine kinase receptors in the growth of malignancy.

Antioxidant activity (DPPH)

DPPH Method to determine the ability of antioxidants to inhibit free radicals by donating hydrogen atoms. Compounds that are active as antioxidants reduce DPPH free radicals (2,2-diphenyl-1-picrylhydrazine) to diphenyl picryl hydrazine resulting in a color change in the DPPH solution in methanol from dark purple to yellow (Novianti, 2012; Romadanu *et al*, 2014) The antioxidant activity of lemongrass is expressed in the inhibition percentage. The results of the analysis of various treatments had a significant effect of DPPH radical scavenging. The value of DPPH radical scavenging capacity of lemongrass at 2000 ppm inhibition can be seen in Figure 4.

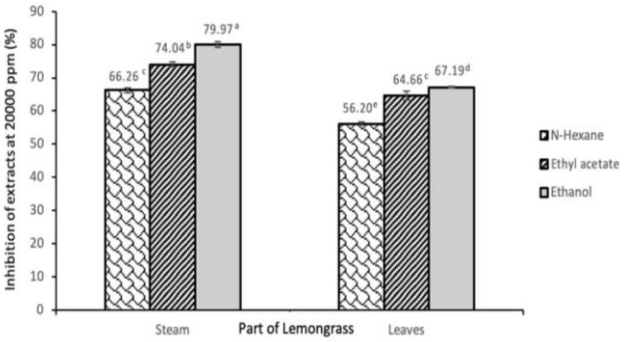

Figure 4. DPPH radical scavenging capacity of lemongrass stem and leaves extracts at 20000 ppm

As shown in Figure 4, the highest antioxidant activity found in the lemongrass stem using ethanol (79.967%). This result higher compare to the previous research by Aliyudin (2019) with the same concentration and solvent, which of (73.1%).

In conjunction to study of Suratmo (2009), the lower antioxidant activity of lemongrass stems and leaves using n-hexane solvent is suggested to be the impact of non-polar component with relatively lower strength of antioxidant activity. Suratmo (2009) observed that the hexane extract fraction, containing only nonpolar compounds, for example essential oils, fats and oils, did not show antioxidant activity. Multi-step extraction is therefore suggested to have more specific

performance in extraction according to the polarity degree of the compound. The multi-step extraction is effective in obtaining bioactive compound as the extraction was initiated using non-polar solvents to extract non-polar compounds such as fats and oils, and then followed by ethyl acetate and ethanol to extract semi-polar and polar compounds such as flavonoids, phenolic tannins.

Differences in the distribution of compounds in lemongrass stems, compared to leaves, affect the capacity for free radical scavenging. The content of bioactive components in lemongrass is different for each part, the stem has higher of type flavonoid are geranial, neral, and myrcene compounds than the lemongrass leaves. There were α-citral (geranial) and β-citral (neral) components which act as antioxidants and free radical scavengers (Mirghani *et al.,* 2012). The more hydroxyl groups, the greater is it antioxidant activity of a material because it will donate more hydrogen to radicals (Gazali *et al.*, 2019).

Research conducted by Mongkolsilp *et al.* (2004) about five medicinal plants from Thailand indicated that higher total phenolic content is associated with higher free radical scavenging activity; this is consistent with this study. One of the antioxidants found in lemongrass is phenolic. According to Winarsi (2007) phenolic compounds in the form of tannins, flavonoids are antioxidants that can quickly donate one hydrogen atom to DPPH radicals and produce derivatives in the form of antioxidant radicals; which are more stable because of the resonance in the benzene ring.

Beside flavonoids, phenolic components such as tannins are known to have antioxidant activity (Amarowicz, 2007; Saxena, *et al.,* 2013). Al Jaber *et al.* (2011) stated that the phenolic components (flavonoids and tannins), alkaloids, terpenoids, and organic sulfur components may act as natural antioxidants. Shah *et al.* (2011), identified several compound in *Cymbopogon citratus* including terpenes, alcohols, ketones, aldehyde and esters. Asaolu *et al.* (2009), analysed the phytochemical constituents of lemongrass leaves. The major phytoconstituents were essential oils (that contain α-citral, β-citral, nerol, geraniol, citronellal, terpinolene, geranyl acetate, myrecene and terpinol methylheptenone), flavonoids and phenolic compounds, which consist of luteolin, isoorientin 2'-O-rhamnoside, quercetin, kaempferol and apiginin.

Lemongrass high antioxidant activity not only inhibit oxidation reactions in food but also suggest potential health benefits. A study conducted by Oboh *et al.* (2010) in Nigeria showed that *C. citratus* contribution to the its health promotion potential as antioxidant actity. In that study, *C. citratus* that was extracted by hot water had significantly higher DPPH radical scavenging ability, Fe^{2+} chelating ability and OH* scavenging ability than those with cold water. Other research on animal studies was conducted by Gayathri *et al.* (2010), in which lemongrass decreased the toxic of lipid perroxidation (TBARS) on groups IV in both serum and heart tissue compatible with Vitamin E tested on male wistar albino into five different groups is group I and II rats were treated with vehicle. Groups III and IV rats treated with 100 and 200 mg/kg body weight. Group V with 100 mg/kg body weight of vitamin E. on 58 could replace it partially. Another study of lemongrass in human subject by Ray. (2010) using supplemented 250 ml of lemongrass decoction to 31 hypertensive individuals for the period of 16 weeks, showed that lemongrass decoction with a dose 2 times a day gave significant impact on the improvement of arterial pressure.

CONCLUSION

The multi-step extraction combined with the Microwave-Assisted Extraction (MAE) method starts with the use of a nonpolar solvent (n-hexane), following with a semi-polar solvent (ethyl acetate) and a polar solvent (ethanol) to produce specific bioactive components including total flavonoids and total phenolic; with high antioxidant activity. This method is more effective applied than the single extraction. The bioactive components in the specific part of the lemongrass *(Cymbopogon citratus)* stem are polar; extracted with polar solvents (ethanol); total phenolic content of 19.31 mg GAE/g, flavonoids of 3.31 mg GAE/g and antioxidant activity of 79.96%; which are higher than that of lemongrass leaves, and also higher than that of other solvents (ethyl acetate and n-hexane).

The high content of antioxidants proves that lemongrass has the potential to be used as a natural food antioxidant agent, especially in food products that contain high unsaturated fats.

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