Antioxidant and Antimicrobial activities of MEOH Extract of Lemongrass (Cymbopogon citratus)

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ABSTRACT

Cymbopogon citratus, universally known as Lemongrass is a small herbaceous plant of Poaceae family. It is used as traditional medicine for treatment of numerous diseases such as fever, sore throats, cough, laryngitis, bronchitis, oral candidiasis, body ache, head ache, digestive problems etc. This study deals with the antioxidant activities such as DPPH and FRAP of methanol extract of lemon grass leaves followed by quantitative analysis of total phenolics (TPCs) and flavonoids (TFCs) content. Antimicrobial activity of the extract was also been tested to highlight the medicinal values. The methanol extract showed significant antioxidant activity using DPPH and FRAP (26.03±1.60 μM; 922.43 μM trolox/100 g dry weight, respectively) compare to the standard Trolox. C. citratus extract has phenolic and flavonoid content as 130.33±1.23; 193.63±4.63, respectively. The results showed that extract of C. citratus exhibited maximum zone of inhibition (35mm) against Bacillus subtilis. at the highest concentration 150mg/ml methanol extract.

Keywords: Cymbopogon citratus, DPPH, FRAP, TPCs, TFCs, and Antimicrobial activity.

INTRODUCTION

Plants are important source of medicinal agents as they possess numerous active constituents of immense therapeutic value (Umar et al., 2016). Since ancient times, plants and herbs have been given a unique place in all the civilizations throughout the world (Joshi et al., 2012). Plant-based drugs are used worldwide for the treatment of various diseases because of their easy availability and less toxic effect to recipient compared to that of synthetic drugs (Umar et al., 2016). The use of herbal drugs increasing rapidly and it represents a substantial part world drug market (Sandhya and Bhavana., 2014). More than 75% of the world population depends upon medicinal plants for their basic health needs. Plant-based medicine has become a popular alternative for synthetic medicine because it does not cause any adverse effect (Shruti et al., 2015).

1. Secondary metabolites

Plants produce a huge variety of chemical compounds categorized as primary and secondary metabolites. Primary metabolites are engaged directly in growth and development whereas secondary metabolites have several medicinal value. There are broad range of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, cardiac glycosides etc. Each of these have certain functions and health advantages. Consequently they are used as fresh materials for pharmaceutical and cosmetic industries (Geetha and Geetha., 2014). Egypt has limited variety of plant sources and is origin of traditional system of medicines.

2. Lemongrass Plant Classification

Cymbopogon citratus, generally known as Lemongrass belongs to the poaceae family and genus Cymbopogon is a tall, monocotyledonous aromatic permanent plant with slim sharpedge green leaves with pointed apex. The origin of the plant is Asian countries.

Taxonomic details of the lemongrass:

Kingdom : Plantae
Division : Magnoliophyta
Class : Liliopsida
Order : Poales
Family : Poaceae
Genus : Cymbopogon
Species : citratus

The name lemongrass is due to typical lemon-like odour of the essential oil present in the shoot. The Cymbopogon genus members also known as aromatic grasses since they produce volatile oils (Shruti et al., 2015).

3. Therapeutic Properties of Lemongrass

Leaves of the Cymbopogon citratus plant (Figure 1) used for food, cosmetic as well as pharmaceutical applications. Lemongrass is one of the important medicinal plant and it has various applications in traditional medicines. Also it can be used for treatment of HIV complications, especially secondary bacterial infections (Umar et al., 2016).

Lemon grass has been traditionally used to treat various medical conditions due to various secondary metabolites present in it. It has been also used to treat fever, cough, elephantiasis flu, leprosy, malaria and other digestive problems. Antimicrobial activity of lemongrass against various bacteria, fungi, protozoa has also been reported. The scientific investigations and information on the therapeutic potentials of lemongrass is limited. The lack of scientific knowledge has restricted the use of lemongrass for clinical
applications (Praveen et al., 2019). The main target of this paper is to determine the antioxidant and antimicrobial activities of the methanolic extract of *C. citratus*. In addition to the total phenolic and flavonoid contents of MeOH extract.

Figure 1. Lemongrass plant (*C. citratus*).

MATERIALS AND METHODS

1. Plant Materials

Fresh *C. citratus* leaves were obtained from an agricultural farm in Minia university faculty of agriculture. The aerial part of the plant was washed and dried at room temperature and ground it into fine powder for extraction.

Preparation of plant extract

Samples were frozen in liquid N2 and grinded using Grinder (Metuchen, NJ, USA). The extraction process of 20 g/100 ml methanol was used and shake for 24 h at 25 °C, then filtered using Whatman paper (Thomas Scientific, USA), the supernatant was dried using rotary evaporator and speed vacuum. Then stored at 4°C. 10 mg/ml DMSO were dissolved for further analysis.

2. Phytochemical analysis

Total phenolic content (TPC)

Following the Folin-Ciocalteu colorimetric method, TPC of samples were measured (Siritwornthan et al., 2014) with minor modification for 96-well micro-plates. Briefly, 15 μl of diluted samples were placed into wells of 96-well micro-plates (GS, USA). Consequently, 240 μl of Folín was added and left for half an hour in darkness at ambient temperature. Then, 15 μl of Na2CO3 20% (wt/wt) were added to each well, adjust the micro-plate reader at shaken mode before start reading the TPC concentrations. The absorbance was measured at λ=755 nm with the micro-plate reader ACCURIS Smart Reader (Edison, NJ, USA). TPC was calculated using a standard curve set of serial dilutions of gallic acid (GAE). TPC values were performed in triplicate and expressed as [mg GAE/g (FM)].

Estimation of total flavonoid content (TFC)

Following previously described method (Chang et al., 2002). To determine the content of total flavonoid with minor modifications. 25 μl of samples were added to 75 μl of MeOH 96% (v/v). Then, 5 μl of 10% Aluminium Chloride and 5 μl of potassium acetate, then 140 μl with distilled water. Kept for half an hour in darkness at 25°C, the readings was measured at λ=415 nm. TFC content was calculated using a standard curve prepared using gradient dilutions of quercetin. The TFC was presented as mg QE/g (FM).

DPPH radical scavenging activity assay

The antioxidant activity was measured by DPPH radical scavenging activity (Darwish et al., 2016). The stock solution was prepared using 10 mg / 1 ml DMSO. Serial dilutions (a 96-well plate, achieving 100, 50, and 25 μg/ml final concentrations) for each extract was prepared. Readings was measured using at λ=515 nm. % DPPH inhibition = $\frac{[1-(A_{sample-\text{Background}})/(A_{DMSO-\text{Background}})]}{*100}$. Calibration curve was obtained using the inhibition rate values of the standard Trolox solution.

Ferric Reducing Antioxidant Potential FRAP

FRAP assay was performed for evaluating the total antioxidant activity. The assay is established on the reducing power of the antioxidant. A powerful antioxidant reduces the ferric ion (Fe³⁺) to ferrous ion (Fe²⁺); the latter forms a blue complex (Fe²⁺/TPTZ), which increases the absorption at 593 nm. Briefly, 20 μl of sample solution were added to the 96-well micro-plate followed by 280 μl of working FRAP solution. The mixtures were shaken, incubated at 37°C for 30 minutes in darkness, and then absorbance was measured using a 96 well micro-plate reader (Jimenez et al., 2008; Firuzi et al., 2005; Tsao et al., 2003). FRAP solutions were prepared as described previously (Benzie and Strain., 1996; Benzie and Strain, 1999). FRAP working solution was prepared daily and warmed at 37 °C for 10 minutes before use by mixing acetate buffer (300 mM, pH 3.6) ,TPTZ (2,4,6-tripyridyl-S-triazine) (40 mM dissolved with 40 mM HCl), and ferric chloride (20 mM in water) [(10/1/v/v)]. The FRAP working solution was prepared. The calibration curve was obtained using the inhibition rate values of Trolox.

3. Antimicrobial activity assay

Bacterial strains

In the present study, three bacterial strains *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*) and *Listeria spp.* were tested. The microorganisms were obtained from the Agricultural Microbiology department, Faculty of Agriculture, Beni-Suef University and Agricultural Microbiology department, Faculty of Agriculture, Minia University, Egypt.

Antibacterial activity

The sensitivity of the studied microbes to the methanolic extract of lemongrass was tested using three concentrations (50, 100, 150 mg/mL) by (Ohno et al., 2003).

4. Statistical analysis

Data obtained were subjected to analysis of variance method and the means were compared, using the Duncan's multiple range tests, through the procedures.

RESULTS AND DISCUSSION

1. Phytochemical content

Total Phenolic Compounds (TPCs) and Total Flavonoids (TFs)

The amount of total phenolic compounds and total flavonoids differed significantly in lemongrass sample (Table 1). The values of phenolics 130.33 mg GAE/100 g dry weight of plant material dry weight as measured by Folin’s reagent method. The total flavonoids value 193.63 mg quercetin/100 g dry weight as measured by the AlCl₃ method.
Table 1. Total phenolic and flavonoid contents of methanolic extract of C. citratus.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolic content (mg/g)</th>
<th>Total flavonoid content (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. citratus</td>
<td>130.33±1.23</td>
<td>193.63±4.63</td>
</tr>
</tbody>
</table>

TPCs expressed in mg Gallic acid equivalents/100 g dry weight of extract; TFs expressed in mg Quercetin equivalents/100 g dry weight of extract; Each value is the mean ± SD of triplicate measurements.

2. Free radical (DPPH) scavenging activity and Total antioxidant power of methanolic extract of C. citratus.

The free radical scavenging ability of the lemongrass extract found as 10.12 , 14.94 and 26.03 in concentrations 12.5, 25 and 50 uM Table (2).

Table 2. Percentage of DPPH inhibition and FRAP values for methanolic extract of C. citratus.

<table>
<thead>
<tr>
<th>Extract</th>
<th>DPPH activity (%)</th>
<th>FRAP (µM Trolox)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. citratus (12.5 uM)</td>
<td>10.12±2.86</td>
<td>3496.77±20.55</td>
</tr>
<tr>
<td>(25 uM)</td>
<td>14.94±2.20</td>
<td></td>
</tr>
<tr>
<td>(50 uM)</td>
<td>26.03±1.60</td>
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</table>

The data are presented as the mean ± SD of technical replicates (n=9). FRAP expressed in µM Trolox/100 g dry weight.

3. Antimicrobial activity

The methanolic extract has great activity and is effective as an antimicrobial pathogenic microbes were tested. The results showed that extract of C. citratus exhibited maximum zone of inhibition against B. subtilis, S. aureus and Listeria spp. zones of inhibitions are shown in Fig. (2), Fig. (3) and Table (3). It was observed that antibacterial activity of C. citratus plant leaves extract showed good results for Gram-positive micro-organisms.

![Fig. 2. The antimicrobial activity of lemongrass extract](image)

**Fig. 3.** Showed the antimicrobial activity of C. citratus against three different gram positive strains B. subtilis, S. aureus and Listeria spp. using three different concentrations of Lemongrass MeOH extract A:150mg/ml; B:100mg/ml; C:50mg/ml.

![B. subtilis](image) ![S. aureus](image) ![Listeria sp](image)

Table 3. Antibacterial activity of lemongrass extract against various selected pathogenic bacteria.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg</td>
</tr>
<tr>
<td>Bacillus. Subtilis</td>
<td>8</td>
</tr>
<tr>
<td>Staphelococcus. Aureus</td>
<td>6</td>
</tr>
<tr>
<td>Listeria spp.</td>
<td>7</td>
</tr>
</tbody>
</table>

CONCLUSION

The experimental study showed the high antioxidant activity of the methanolic extract of C. citratus may related to the high phenolic and flavonoid contents of C. citratus leaves. The antimicrobial activity was observed which inhibited the growth of Gram-positive micro-organisms such as B. subtilis, S. aureus and Listeria spp. The current study suggested that the daily used of lemongrass or its oil is very useful to protect our body from free radicals and oxidative stress. Also, a good source of antimicrobial components.

ACKNOWLEDGEMENTS

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REFERENCE


