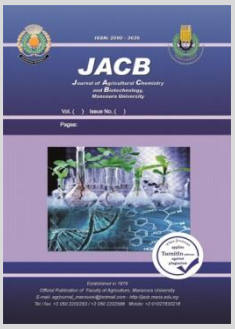
Journal of Agricultural Chemistry and Biotechnology

Journal homepage: www.jacb.mans.edu.eg
Available online at: www.jacb.journals.ekb.eg

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

Hanaa M. Hassan[1]; M. A. Aboel-Ainin[2] ; Shimaa K. Ali[3] and A. G. G. Darwish[1*]

[1]Department of Biochemistry, Faculty of Agriculture, Minia University, Minia 61519, Egypt
[2]Department of Biochemistry, Faculty of Agriculture, Beni-Suef University, Beni-Suef 62521, Egypt.
[3]Department of Agricultural Microbiology, Faculty of Agriculture, Beni-Suef University, Beni-Suef 62521, Egypt.



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 candiasis, body ache, head ache, digestive problems etc. This study deals with the antioxidant activities such as DPPH and FRAP of mehanol 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 : citrates

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

* **Corresponding author.**
E-mail address: ahmed.darwish@mu.edu.eg
DOI: 10.21608/jacb.2021.149473

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 N_2 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 **(Siriwoharn *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 Folin was added and left for half an hour in darkness at ambient temperature. Then, 15 µl of Na_2CO_3 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 = $[1-(A_{sample}-A_{background})/ (A_{DMSO}-A_{background})]$ *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 (Fe^{3+}) to ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/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 37ºC 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 Cº 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) [(10/1/1 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 $AlCl_3$ method.

Table 1. Total phenolic and flavonoid contents of methanolic extract of *C. citratus*.

Extract	Total phenolic content (mg/g)	Total flavonoid content (µg/g)
C. citratus	130.33±1.23	193.63±4.63

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. The data are presented as the mean ± SD of technical replicates (*n*=9).

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*.

Extract	DPPH activity (%)			FRAP (µM trolox)
	(12.5 uM)	(25 uM)	(50 uM)	
C. citratus	10.12±2.86	14.94±2.20	26.03±1.60	3496.77±20.55

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.p.* zone 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.

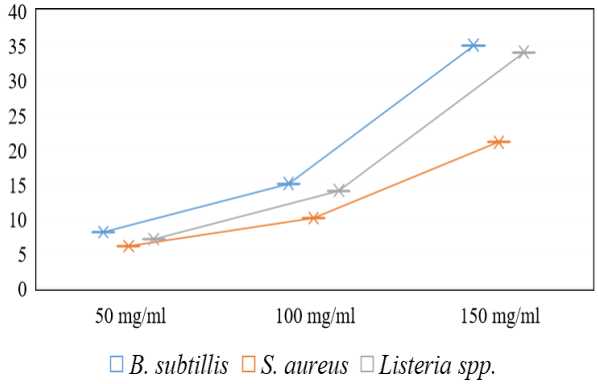


Fig. 2. The antimicrobial activity of lemongrass extract

Our results in the same line with Rozynska and Gwiazdowska, (2020) who showed that the highest TPC, TFC, DPPH radical scavenging activity and FRAP value in lemongrass extract. (Lambert, 2002 and Hindumathy, 2011) reported that the lemongrass extract is effective against growth *Listeria spp.p.* becteria.

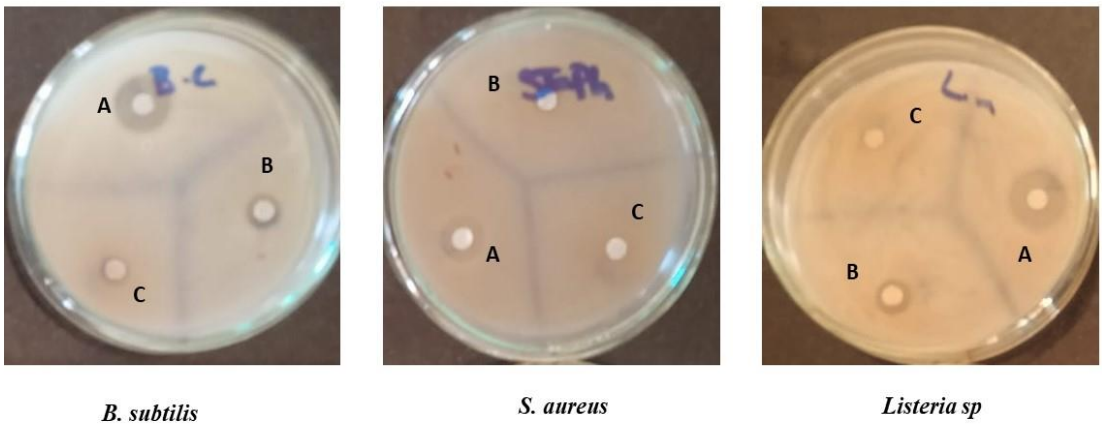


Fig. 3. Showed the antimicrobial activity of *C. citratus* against three different gram positive strains *B. subtilis, S. aureus* and *Listeria spp.* using three different concentrations of Lemongrass MeOH extract A:150mg/ml; B:100mg/ml; C:50mg/ml.

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

Organisms	(Zone of Inhibition (mm		
	٥٠ ١٠٠mg	١٥٠mg	mg
Bacillus. Subtilis	8	15	35
Staphelococcus. Aureus	6	10	21
Listeria spp.	7	14	34

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.p.* 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

The support of Beni suef University and Florida A&M University is greatly appreciated.

REFERENCE

Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*. 239(1), 70- 76.

Benzie IFF, Strain JJ (1999). Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Oxidants and Antioxidants*. Pt A, 299, 15-27.

Chang CC, Yang MH, Wen HM, Chern JC (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal*. 10(3): 178-182.

Darwish AGG, Samy MN, Sugimoto S, Otsuka H, Abdel-Salam H, Matsunami K (2016). Effects of hepatoprotective compounds from the leaves of *Lumnitzera racemosa* on acetaminophen-induced liver damage *in vitro*. *Chem Pharm Bull (Tokyo)*. 64(4): 360-365, 2016. DOI: 10.1248/cpb.c15-00830.

Firuzi O, Lacanna A, Petrucci R, Marrosu G, Saso L (2005). Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. *Biochimica Et Biophysica Acta-General Subjects*. 1721(1-3), 174-184.

Geetha TS, Geetha N (2014). Phytochemical screening, quantitative analysis of primary and secondary metabolites of Cymbopogon citratus (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. *International Journal of pharmtech research*. 6(2):521-529.

Hindumathy CK (2011). *Invitro* study of antibacterial activity of *Cymbopogon citratus*. *World Acad Sci Eng Technol*. 50: 173-177.

Jimenez-Alvarez D, Giuffrida F, Vanrobaeys F, Golay PA, Cotring C, Lardeau A, Keely BJ (2008). High-throughput methods to assess lipophilic and hydrophilic antioxidant capacity of food extracts *in vitro*. *Journal of Agricultural and Food Chemistry*. 56(10), 3470-3477.

Joshi M, Gaonkar K, Mangoankar S, Satarkar S (2012). Pharmacological investigation of Areca catechu extracts for evaluation of learning, memory and behavior in rats. *Journal of Pharmacognosy and Phytochemistry International Current Pharmaceutical Journal*. 1(6):128-132.

Lambert PA (2002). Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. *J. Appl Microbiol.* 92(Suppl): 46S-54S.

Ohno T, Kita M, Yamaoka Y, Imamura S, Yamamoto T, Mitsufuji S (2003). Antimicrobial activity of essential oils against *Helicobacter pylori*. *Helicobacter*. 8(3): 207-215.

Praveen Kumar Gupta, BS Rithu, Shruthi A, Anushree Vinayak Lokur and Raksha M (2019). Phytochemical screening and qualitative analysis of Cymbopogon citratus. *Journal of Pharmacognosy and Phytochemistry*. 8(4): 3338-3343.

Różyńska M. S and Gwiazdowska D (2020). Antioxidant and antibacterial properties of lemon, sweet, and cereal grasses.*Journal of food processing and preservation* ,44(12), pp.14984.

Sandhya Madhan Mohan, Bhavana Pandey (2014). Phytochemical analysis of *Centella asiatica* L. World journal of Pharmacy and pharmaceutical sciences. 5(8):1342-1347.

Shruti Sunil Ranade, Padma Thiagarajan (2015). Lemon Grass, Int. *J Pharm. Sci. Rev. Res.* 35(2):162-167.

Siriwoharn T, Wrolstad RE, Finn CE, Pereira CB (2004). Influence of cultivar, maturity, and sampling on blackberry (Rubus L. Hybrids) anthocyanins, polyphenolics, and antioxidant properties. *J Agric Food Chem*. 52(26): 8021-8030,. PMID: 15612791. DOI: 10.1021/jf048619y

Tsao R, Yang R, Young JC (2003). Antioxidant isoflavones in Osage orange, Maclura pomifera (Raf.) Schneid. *Journal of Agricultural and Food Chemistry*,.51(22), 6445-6451.

Umar M, Mohammed IB, Oko JO, Tafinta IY, Aliko AA, Jobbi DY (2016). Phytochemical Analysis and Antimicrobial Effect of Lemon Grass (*Cymbopogon citratus*) Obtained From Zaria, Kaduna State, Nigeria. *Journal of Complementary and Alternative Medical Research*. 1-8.

النشاط المضاد للأكسدة والمضادات لنمو الميكروبات للمستخلص الميثانولى لحشيشة الليمون

هناء محمد حسن[١]، مصطفى عبدالمنعم أبوالعينين[٢] ، شيماء كامل على[٣] و أحمد جمعة جمعة درويش[١]

[١] قسم الكيمياء الحيوية – كلية الزراعة - جامعة المنيا - مصر

[٢] قسم الكيمياء الحيوية – كلية الزراعة - جامعة بنى سويف - مصر

[٣] قسم الميكروبيولوجيا الزراعية – كلية الزراعة - جامعة بنى سويف - مصر

Cymbopogon citratus ، المعروف عالمياً باسم Lemongrass ، هو نبات عشبي صغير من عائلة Poaceae. يتم استخدامه كدواء تقليدي لعلاج العديد من الأمراض مثل الحمى وألتهاب الحلق والسعال وألتهاب الحنجرة وألتهاب الشعب الهوائية وآلام الجسم وآلام الرأس ومشاكل الجهاز الهضمي وغيرها. تتناول هذه الدراسة الأنشطة المضادة للأكسدة مثل DPPH و FRAP من مستخلص الميثانولى لأوراق حشيشة الليمون متبوعًا بالتقدير الكمي للمركبات الفينولية الكلية (TPCs) والفلافينويدات الكلية (TFCs). تم أيضاً إختبار النشاط المضاد للميكروبات للمستخلص لإبراز القيمة الطبية له. أظهر مستخلص الميثانول نشاطاً معنوياً كمضاد للأكسدة بأستخدام DPPH و FRAP وكانت النتائج كالتالى (٢٦,٠٣ ± ١,٦٠ ميكرومتر ؛ ٩٢٢,٤٣ ميكرومتر trolox / 100 جم وزن جاف ، على التوالي) مقارنة مع Trolox كمادة مضادة للأكسدة قياسية. يحتوي مستخلص *C. citratus* على محتوى المركبات الفينولية الكلية والفلافونويدات الكلية ١٣٠,٣٣ ± ١,٢٣ ؛ ١٩٣,٦٣ ± ٤,٦٣ على التوالي كما أظهرت النتائج أن مستخلص *C. citratus* له نشاطاً كبيراً فى تثبيط نمو بكتريا *Bacillus. Subtilis* عند أستخدامه بتركيز ١٥٠ مجم / مل من المستخلص الميثانول وهو أعلى تركيز مستخدم فى التجربة.