https://doi.org/ 10.48047/AFJBS.6.5.2024. 8786-8798

African Journal of Biological Sciences

PHYTOCHEMISTRY, ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF *CYMBOPOGON CITRATES* STAPF. (LEMONGRASS)

[1]Rinchi Bora, [2]Syeda Fakrunnisa Begum, [3]Anil Kumar, [4]Mathews T Thelly, [5]Hemant Tawale, [6]Pratichi Mandal, [7]S. N. Suresh**

[1]Assistant Professor, Faculty of Pharmaceutical Science, Assam down town University**

[2]Assistant Professor in Zoology, GDC for Men, Cluster University, Kurnool.

[3]Department of Botany, DDU Gorakhpur University Gorakhpur-273009

[4]Associate Professor, Head and Research Guide, Department of Botany, Kuriakose Elias College Mannanam, 686561

[5]Associate Professor and Head of the Department of Pharmacognosy, Konkan Gyanpeeth Rahul Dharkar College of Pharmacy and Research Institute, Dahivali - Parade Vengaon Road, Post -Tiware, Tal: Karjat, Dist: Raigad, Navi Mumbai, Maharashtra, India- 410201.

[6]School of Pharmacy, Sister Nivedita University, New Town, West Bengal 700156

[7]Associate Professor of Biotechnology, Rathinam College of Arts and Science, Coimbatore

Corresponding Author**

Rinchi Bora

Assistant Professor, Faculty of Pharmaceutical Science, Assam down town University

Article History
Volume 6, Issue 5, 2024
Received: 15 May 2024
Accepted: 02 Jun 2024
doi: ***10.48047/AFJBS.6.5.2024. 8786-8798***

Abstract

Phytochemicals are secondary metabolites found in plants that have medicinal and other useful uses. We discuss the effects on the gut microbiota and the phytochemistry and pharmacological possibilities of lemongrass (Cymbopogon) in this review. The anti-diabetic and antioxidant properties of lemongrass are well-known. As a result, it finds extensive application in the cosmetics, pharmaceutical, food, and feed industries. Geraniol, alkaloids, terpenoids, and phenolic metabolites (such as phenolic acids, flavonoids, stilbenes, and lignans) are powerful bioactive components found in lemongrass. There is no more valuable therapeutic herb than lemongrass. In addition, the phytochemicals included in lemongrass have the ability to promote health by restoring harmony to the microbiome. Because it does not produce any harmful byproducts or residue, lemongrass is ideal for use as an ingredient in industrial food and feed. Optimized nutrient absorption in the gastrointestinal system is enhanced by modulating the gut ecology using lemongrass powder and essential oils, which provide anti-microbial, anti-inflammatory, and antioxidant reactions. In this review, we will go deeper into the phytochemical, pharmacological, and medicinal properties of lemon grass.

Keywords: Pharmacological Activity, Phytochemical, Lemon grass, DPPH

Introduction

In developing countries like India, where a wide variety of ailments are treated using aromatic and medicinal plants, these plants play a crucial role in the healthcare system. When compared to the potentially harmful effects of allopathic treatment, the use of herbal remedies within the traditional medical system is clearly superior [Oladeji, O. S., Adelowo, F. E., Ayodele, D. T., & Odelade, K. A. 2019]. To put it simply, plants are like little chemical factories; they secrete a wide variety of phytochemicals, each with the potential to affect animal and human physiology in its own special way. The search for novel medications can benefit from studies of the phytochemical and pharmacological characteristics of medicinal plant extracts [Mukarram, M., Choudhary, S., Khan, M. A., Poltronieri, P., Khan, M. M. A., Ali, J., ... & Shahid, M. 2021]. When used as a medicine, single components can have unexpected side effects; however, plant extracts contain a synergistic blend of components that mitigate the negative effects of each other

and produce a more positive overall effect. Because of this, modern scientific study focuses on learning more about aromatic and medicinal herbs so that we can create new medications with better physiological functions and fewer side effects [Natrajan, D., Srinivasan, S., Sundar, K., & Ravindran, A. 2015]. Isolation of bioactive molecules with large health consequences has been made possible by recent breakthroughs in biotechnology and novel, robust, and less labor-intensive extraction methods, which have expedited the research of natural chemicals [Gogoi, R., Lying, R., Sarma, N., Begum, T., Pandey, S. K., & Lal, M. 2020]. The procedure has been sped up, making this possible. This review study looks at the phytochemical and pharmacological properties of lemongrass. Lemon grass, or Cymbopogon citratus Stapf., is a tall perennial grass with tufted fibrous roots and rhizomes. Being a member of the Poaceae family, this plant is famous for the abundance of oil found in its tissues. Clusters of green, leathery leaves grow on short underground stems. Despite the herb's widespread cultivation in the tropics and subtropics now, it may have originated in India [Maleš, I., Pedisić, S., Zorić, Z., Elez-Garofulić, I., Repajić, M., You, L., 2022]. The rise of novel dietary methods that aim to improve health by combining many functional components has contributed to the rising popularity of nutraceuticals and designer foods. Nutrification is an attractive method of improving one's diet's health because many of plants' non-nutritive components, sometimes called phytoconstituents, have been linked to substantial health advantages. There has been encouraging progress in the use of plant-based therapies for the treatment of metabolic and immunological disorders. Both formal systems, such as Unani and Ayurveda, and more informal ones, such native, folk, and tribal medicine, have made use of them as therapeutic agents since the dawn of time. Plants have a variety of components that do not provide sustenance. Some of these components include fibre and active phytochemicals such terpenoids, flavonoids, lignans, sulphide, plant sterols, polyphenolics, coumarins, carotenoids, and saponins... It has long been recognized that these phytochemicals, which are active, may have medicinal uses. Diets rich in plant-based polyphenolic and phenolic combinations are essential for good health and should be maintained at all costs [Al Kury, L. T., Abdoh, A., Ikbariah, K., Sadek, B., & Mahgoub, M. 2021]. Plant leaves are a good source of phenolics, and fruit seeds and peel are good sources of flavonoids and polyphenols. When handled properly, lemongrass has the potential to be an antioxidant powerhouse. Alkaloids, flavonoids, terpenoids, saponins, phenols, and tannins are only a few of the bioactive compounds found in lemongrass leaves [Santos, P. L., Matos, J. P. S., Picot, L., Almeida, J. R., Quintans, J. S., & Quintans-Júnior, L. J. 2019]. Lemongrass gets its unique flavour from these compounds. Reportedly, the leaves have the highest amount of antioxidant effect against hepatitis virus

expression. [M. N., Jabbar, S., Mahmood, S., ... & Ibrahim, S. A. 2022] Phenolic chemicals increase a substance's antioxidant capacity; these compounds can scavenge chelating pro-oxidants, free radicals, and metal ions, and they may also function as reducing agents. Irrespective of how antioxidants are typically used to justify the medicinal value of polyphenolic and phenolic compounds, this remains true. The tropical grass known as lemongrass has a strong scent and is rich in nutrients; it is native to those areas. Research has shown that lemongrass, both the herb and its essential oil, can effectively cure Helicobacter pylori ulcers, and the herb itself has antiparasitic properties. [A., Fournier-Level, A., Dunshea, F., & Jusuf, P. R. 2022] In addition to relieving stomach cramps, lemongrass tea is highly recommended for people with constipation, diarrhoea, or both. While its antibacterial action is modest, it can benefit gut health by decreasing colon bacterial populations.

Material & Methods

Identification of Plants

The identification of Lemongrass as a therapeutic plant was confirmed with the assistance of Prof. Tahira Israr, director of the Botany Department at ICAR's Pusa Campus in New Delhi.

Drying and Grinding of the Plant

Following a thorough washing, the collected plants were then roughly chopped with shears and blades. To speed up the drying process and shield them from dust and other airborne pollutants, they were relocated to a shady, cool spot. [Samarth, R. M., Samarth, M., & Matsumoto, Y. 2017] About three weeks passed in a completely dark room as the material dried. An evenly sized powder should be obtained once the plants have dried fully, and the surface area should be maximized for the most efficient extraction procedure.

DPPH Assay

An alcoholic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used in a spectrophotometric experiment to evaluate the radical scavenging activities of the extracts, as reported by [Boeira, C. P., Piovesan, N., Flores, D. C. B., Soquetta, M. B., Lucas, B. N., Heck, R. T., ... & Terra, N. N. 2020] For your reference, the following steps are taken: first, a 200 mM DPPH solution in 100% ethanol is mixed with extracts-DMSO solutions ranging from 15.6-250 g/mL. After that, the combination is exposed to a dark incubator set at 25.1 degrees Celsius for

30 minutes. [Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. 2011]. In order to compare each sample to a reference solution made up of DPPH and DMSO, spectrophotometric values were taken at 517 nm after the incubation period. The extent to which the chrometric state of DPPH changed from purple to yellow after decrease was assessed in this evaluation. Ascorbic acid was considered to be the gold standard in this specific study. As a proportion of the radical reduction in the extracts' capacity to scavenge free radicals was evaluated. Each extract's IC50 value was calculated by fitting the data to a calibration curve; to guarantee precision, the tests were performed three times.

$$\text{Radical scavenging activity \%} = \left(1 - \left(\frac{\text{Abs}_{517}\ \text{control}}{\text{Abs}_{517}\ \text{sample or standared control}}\right)\right) \times 100.$$

Nitric Oxide

It can be challenging to detect NO in tissue because of its short half-life and the likelihood of interference from other metabolites. This means that NO detection methods must be extremely sensitive. [Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. 2011] Furthermore, it is advised to employ a minimum of two methods to precisely measure NO, as each approach has its own benefits and drawbacks. Amperometric methods with NO-specific electrodes, fluorescent probes, EPR, chemiluminescence, the oxyhaemoglobin (Hb-O2) test, laser photoacoustics, membrane inlet-mass spectrometry, and the Griess reaction are among the most common ways to quantify NO in plant and animal systems. The use of fluorescent probes is another method. Both of these techniques can measure concentrations of free NO and NOx molecules like NO2, respectively.

$$\text{NO scavenging \%} = \left(\frac{[\text{Abs}_{546}\ \text{control} - \text{Abs}_{546}\ \text{sample}]}{\text{Abs}_{546}\ \text{control}}\right) \times 100.$$

Alpha-amylase assay

To summarise, an enzyme-containing pig pancreas solution combined with 15 ul of plant extract diluted in phosphate buffer (50 g/ml - 200 g/ml) was mixed with 5 um of the same. Stirring the mixture was the next step. Twenty litres of starch solution was added to the mixture after ten [ML, S. F., Lodder, H. M., Gianotti Filho, O., Ferreira, T. M., & Carlini, E. A. 1986] minutes of incubation at 37 degrees Celsius. The reaction was initiated by leaving the mixture undisturbed for another thirty minutes at the same temperature. By incorporating 10 ul of 1M HCl and 75 ul of iodine reagent into each well, the process was halted. The combined volume of all the wells was thus 100 ul. A phosphate buffer with a pH of 6.9 and acarbose at 64 g/ml were also prepared so that a comparison could be made with the extract. [Pirog, T. P., Shevchuk, T. A., Voloshina, I. N., & Karpenko, E. V. 2004]. All of the samples were analysed without the use of a starch control or an enzyme control. To determine the inhibitory impact percentage, we first measured absorbance at 580 nm and then applied the following formula.

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Absorbance of the untreated (Control)}}{\text{Absorbance of the test well}}\right) \times 100$$

Statically Analysis:

A version of GraphPad Prism, 5.0.3, was used for the statistical analysis. Every test was repeated three times to ensure accuracy. The data are shown as means plus standard errors of the mean, where n is the number of experiments. A p value of 0.05 was considered to indicate a statistically significant difference between the two groups. [Tayeboon, G. S., Tavakoli, F., Hassani, S., Khanavi, M., Sabzevari, O., & Ostad, S. N. 2013]. To demonstrate statistical significance, we used analysis of variance (with Bonferroni post hoc testing) and paired t-tests.

RESULTS & DISCUSSION

DPPH Assay

It was shown that the hydrogen-donating capacity may be used as a measure of the antioxidant activity of C. citratus leaf extracts in terms of their ability to scavenge DPPH radicals. Both extracts showed nearly identical radical scavenging efficacy at low doses (100-200 g/mL). Figure 1 shows a comparison of the DPPH radical scavenging activities of Ew and E50 extracts with a standard control, ascorbic acid. Figure 1 shows that the Ew extract had an IC50 value of

about 278 and a maximal inhibition of about 45%. It seems that the maximum inhibitory concentration increases in direct proportion to the extract concentration. However, a noticeable rise in the percentage of inhibition was seen when the concentration of the E50 extract was increased to 750 g/mL. An IC50 value of 258.90 was associated with the highest percentage of inhibition, which reached 48.3%, at even higher concentrations of the extract (Figure 1). It seemed like the inhibitory proportion will level out when this amount was surpassed. Different amounts of terpenes were extracted, which could explain the difference. To obtain the same amount of inhibition as the standard control when it is present at a concentration of around 20 g/mL, it requires about 1500 g/mL of the extract, which is comparable to an IC50 of 6.21 g/mL. These results were in line with previous research that had shown similar results for C. citratus extracts having increased IC50 values.

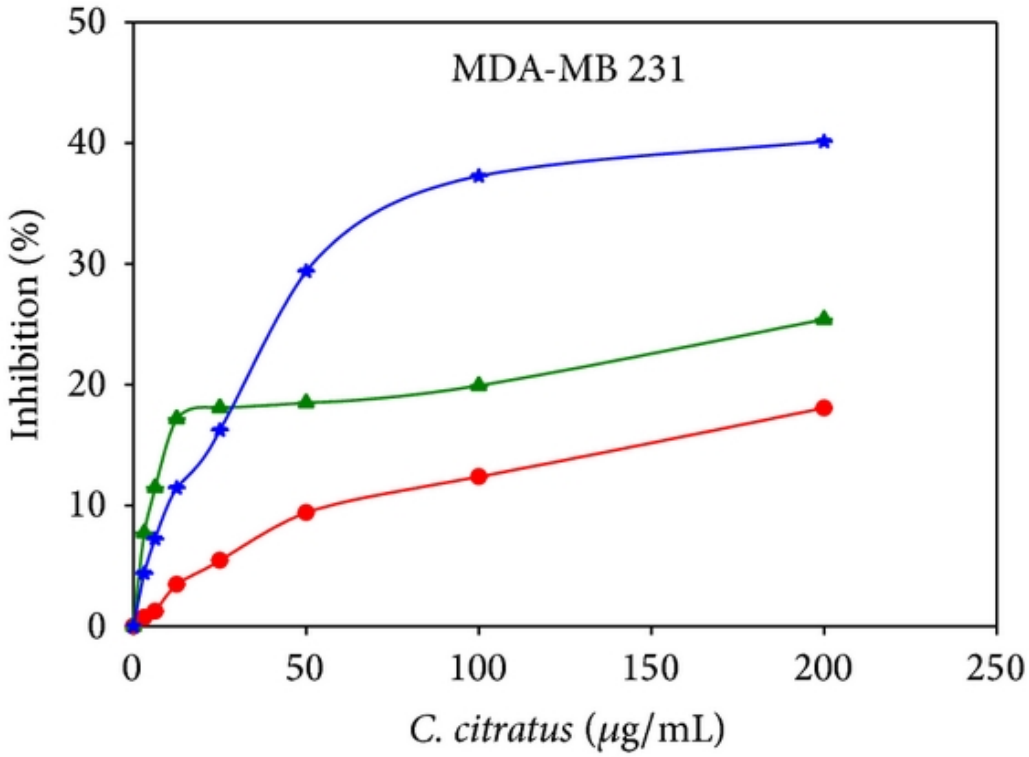

Fig: 1 The data thus far suggest that the primary antioxidant activity of phytochemical extracts derived from *C. citratus* leaves is due to their ability to donate both protons and electrons. Secondary antioxidants, on the other hand, limit the production of hydroxyl radicals made by Fenton's reaction because they operate as effective ligands in chelating metal ions. Among the studied extracts, the Ew one showed the most activity, with an IC50

value of 172.2 ±31 g/mL and a metal ion inhibition of 71.2%. The extract from E50, on the other hand, had an IC50 value of 456.5±30 g/mL, indicating that its inhibitory effect on metal ions was only 40% effective.

Nitric Oxide

Figure 2 shows that, in contrast to the positive control, aminoguanidine (0.96 ±0.2), the crude extract of lemon grass reduced the NO accumulation in LPS-activated RAW macrophage cells in a concentration-dependent manner. Both the absolute and relative values of NO generation were significantly reduced at 50 and 100 g/ml of extract concentration when compared to the untreated control (6.0 ±0.3). Further evidence that the observed inhibition of NO generation was not due to cell death was provided by MTT viability cell assays, which demonstrated that the extract had no influence on the number of active macrophages.

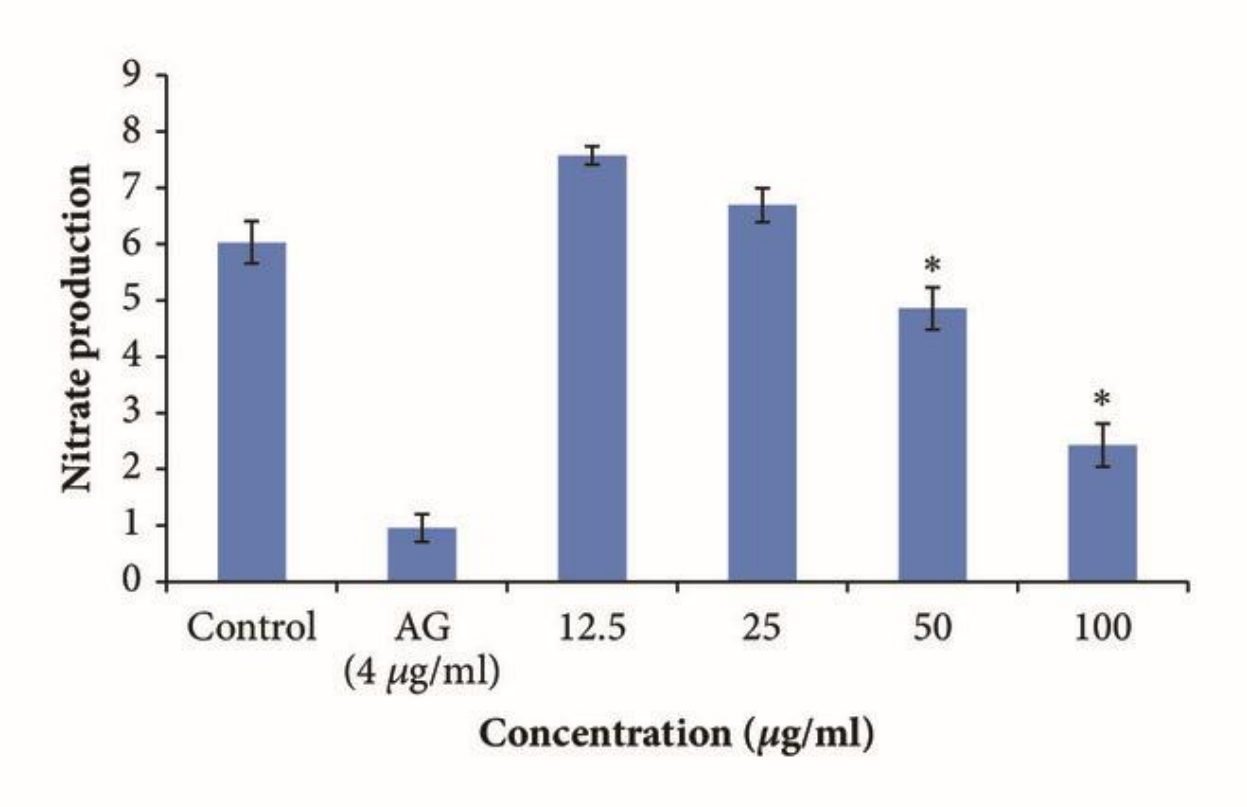

Fig. 2: Whether LPS-induced NO production in RAW macrophages is affected by lemon grass aqueous extract. When the MTT assay was run simultaneously, no significant damage was found (data not shown). Data are presented as the mean with standard

deviation (n = 4). shows a decrease that is statistically significant ($p<0.05$) when contrasted with the control group that did not get any treatment. Nothing changed when compared to the aminoguanidine active control.

Alpha amylase Assay

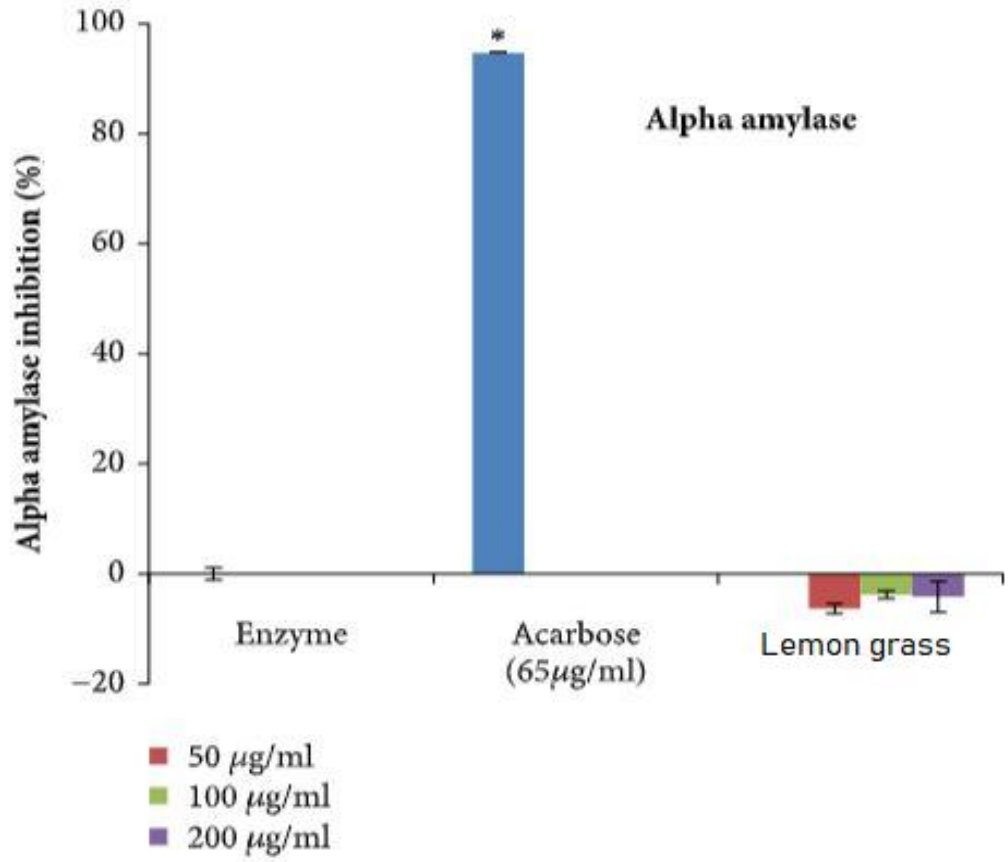

Fig: 3 How an aqueous lemon grass extract affects alpha-amylase activity. With 4 observations, the data are shown as the mean with standard deviation. shows that the increase was significantly different from the enzyme control group ($p>0.05$). Concentration did not increase noticeably in comparison to the acarbose-treated positive controls.

The results showed that the alpha-amylase enzyme was unaffected by the Lemon grass extract at any of the concentrations tested, but the alpha-glucosidase enzyme was mildly but noticeably affected (Figure 3). The extract showed a 32.6% improvement in its capacity to inhibit alpha-glucosidase activity at the maximal concentration (200 g/ml). Despite the fact that the extract and the control shown moderate levels of activity against alpha-amylase and alpha-glucosidase, the

positive controls, acarbose and EGCG, outperformed both the extract and the control by a significant margin.

Conclusion

Researcher examined the antioxidant and cancer-preventive properties of *C. citratus* extracts. There is evidence that Ew and E50 extracts can protect cells from DPPH. Additionally, the extracts included a high concentration of compounds that chelated iron and scavenged nitric oxide. Thus, our findings provide credence to *C. citratus* usage in the standard management of diabetes. Caution is required while using the plant extract because of fears that it may be poisonous.

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