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Evaluation of the lemongrass plant (*Cymbopogon citratus*) extracted in different solvents for antioxidant and antibacterial activity against human pathogens

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PEER REVIEW

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Comments

This was a good study that the authors has determined the antibacterial activity, analyzed the chemical antioxidant property, and DNA protective activity against free radicals by lemongrass plant extracts. The results are interesting, and the research suggested that the lemongrass plant (*C. citratus*) extract has antimicrobial activity, antioxidant activity and DNA damage protectively, which might be helpful in preventing various human pathogenic organisms and oxidative stress induced disease.

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ABSTRACT

Objective: To test antibacterial and antioxidant activity of the lemongrass plant *Cymbopogon citratus* (*C. citratus*) leaves extracted serially by the solvents (chloroform, methanol and water).

Methods: The plant leaves extracts were used for antibacterial activity on *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Nocardia* sp., *Serratia* sp., and *Enterobacter aeruginosa* microorganisms by the Kirby Bauer agar disc diffusion method. This study was carried out on lemongrass plant leaf extracts in different concentration of all solvents. The leaf extracts from different solvents were tested for their scavenging activity against the stable free radical DPPH in quantization using a spectrophotometric assay. Oxidative damage was induced *in vitro* by treating blood DNA and analyzing the effects of the leaf extracts.

Results: The results showed that *C. citratus* extracts exhibited maximum zones of inhibition in chloroform, methanol and water extracts. It was Observed that the *C. citratus* extracts exhibited maximum zone of inhibition against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. Analyzed data in the present work suggested that antibacterial activity of *C. citratus* plant leaf extracts showed good results for Gram-positive and Gram-negative organisms. DPPH scavenging activity was highly elicited by the extract of *C. citratus*. Chloroform, methanol and water extracts of *C. citratus* leaves effectively decreased the extent of DNA damage.

Conclusions: The present study suggested that the lemongrass plant extracts could offer various health benefits.

KEYWORDS

Lemongrass plant, *Cymbopogon citratus*, Antibacterial, Antioxidant, Human pathogens**1. Introduction**

Medicinal plants constitute the base of health care systems in many societies. The recovery of the knowledge and practices associated with these plant resources are part of an important strategy linked to the conservation of biodiversity, discovery of new medicines, and the bettering of the quality of life of poor rural communities[1].

The increasing prevalence of multidrug resistance in pathogenic microorganisms and undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin. Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, anti-mutagenic effects and

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anti-carcinogenic potentials^[2]. Essential oils are extracted from a variety of plants, including trees, shrubs, flowers, grasses, herbs and fruits. The oils extracted are concentrated in different parts of the plant. Many essential oils are known to exert antimicrobial activity, but the mechanism of action is often not entirely understood. Membrane disruption by the lipophilic constituents may be involved in this mechanism. An important characteristic of essential oils and their constituents is the hydrophobicity, which enables them to partition in the lipids of bacterial cell membrane and mitochondria thus disturbing the structures and rendering them more permeable. The chemical components of essential oils are analyzed by gas chromatography mass spectrometry^[3].

Lemongrass is a tropical perennial plant which yields the Cochin oil of commerce. It is known as *Bhustarah* in Sanskrit, *Gandhatran* in Hindi, *Injippullu* in Malayalam, *Vasanapullu* in Tamil, *Majjigehallu* in Kannada and *Nimmagaddi* in Telugu (Figure 1). The name of lemongrass is derived from the typical lemon-like odour of the essential oil present in the shoot. *Cymbopogon citratus* (*C. citratus*) flourishes in sunny, warm, humid conditions of the tropics. In Kerala short periods above 30 °C have little general effect on plants, but severely reduce oil content. Lemongrass flourishes in a wide variety of soil ranging from rich loam to poor laterite. Calcareous and water-logged soils are unsuitable for its cultivation^[4]. Plants growing in sandy soils have higher leaf oil yield and citral content. Lemongrass will grow and produce average herbage and oil yields on highly saline soils.



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

In Nepal and India, lemongrass is traditionally used as a sedative, in addition to a treatment for fever, and an indigenous cure for infectious diseases. The herb has also been used as an external treatment for skin complaints like ringworm, athlete's foot and scabies. Because lemongrass

has been known to control overactive oil glands, it can also be used as a toning astringent to cleanse oily skin and tighten pores. In some countries, it is used to reduce acne, pimples and blackheads. Lemongrass is also used as a treatment for lice and dandruff^[5]. One of the most important applications of lemongrass is in the field of cancer research. Studies conducted on animals show that lemongrass oil may prevent colon cancer, as well as other types of cancer. Lemongrass (*C. citratus*) caused apoptosis (programmed cell death) in cancer cells. Through *in vitro* studies, the researchers examined the effect of citral, a molecule found in lemongrass, on both normal and cancerous cells. Citral, which is also found in lemon peels, is the substance that gives lemongrass its distinctive aroma and flavor. Compared to all other plants, lemongrass contains the highest amount of citral^[6].

The composition of plant oils and extracts is known to vary according to local climatic and environmental conditions. Furthermore, some oils with the same common name may be derived from different plant species. Secondly, the method used to assess antimicrobial activity, and the choice of test organism(s) varies between publications. A method frequently used to screen plant extracts for antimicrobial activity is the agar disc diffusion technique^[7]. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols^[8].

All living organisms have endogenous defense systems against oxidative damage, such lipid peroxidation, DNA damage and inhibition of cell communication due to reactive oxygen species. There are two main antioxidant defense mechanisms. The first one is antioxidant defense with enzymes such as superoxide dismutase which catalyses dismutation of superoxide anions to hydrogen peroxide, and catalase which converts hydrogen peroxide (H₂O₂) into molecular oxygen and water. The second one is antioxidant defense with non-enzymatic components, such as polyphenols, ascorbic acid, and carotenoids^[9]. Ayurvedic medicine preaches that using natural plants can promote self-healing attain good health and longevity, and provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases or conditions. Leaves of lemongrass plant are known to have various biological activities, including hypolipidemic, anti-atherosclerotic and anti-oxidant^[10], immune boosting agent, hypotensive and tumor suppressive effect^[11,12]. Lemongrass leaves are extensively used for treating tissues inflammation, cardiovascular and liver diseases and regulate blood sugar and cholesterol^[13]. The main target of this paper is to determine the antibacterial

activity, analyze the chemical antioxidant property and DNA protective activity against free radicals by lemongrass plant extracts with various solvents like chloroform, methanol and water.

2. Materials and methods

2.1. Sample collection

The *C. citratus* plant leaves were used for this study. The lemongrass plant leaves were collected from Sourashtra College Park in Sourashtra College campus, Madurai, Tamil Nadu.

2.2. Bacterial strains

In the present study, four Gram-negative bacteria were used, namely, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus vulgaris* (*P. vulgaris*), *Serratia* sp. and *Enterobacter aerogenes* (*E. aerogenes*) and three Gram-positive bacterial such as *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*) and *Nocardia* sp. were tested. The microorganisms were obtained from the Biotechnology department in Sourashtra College, Madurai Kamaraj University, Madurai, Tamilnadu, India.

2.3. Sample preparation

The collected plant leaves were shade dried, cut into small pieces and grounded into coarse powder using a mixer. The coarse powder was then extracted in hot and cold conditions. A Soxhlet extractor apparatus was used for extraction with chloroform, methanol and water solvents^[14].

2.4. Solvent extraction

A total of 50 g lemongrass leaves powder was stirred with 500 mL of water at room temperature for 8 h. The extract was filtered and supernatant was collected. The extraction apparatus was mounted on a heating mantle using a clamp with water connection going in and out of condensers. The sample was kept in the thimble and dropped into the Soxhlet tube. The solvent was placed in the round bottom flask and the solvent evaporated and passed through the tube into Soxhlet extractor. The vapor was then condensed by flow under gravity and percolated through the beds of sample to extract the oil. At the end of extraction, the solvent was distilled off and the dried residues were weighed and dissolved in the appropriate solvent and stored in refrigerator at 4 °C until use. This process was repeated for 3 solvents chloroform, methanol, and water^[15].

2.5. Antibacterial activity

The residues of different solvents extraction were dissolved in dimethyl sulfoxide at different concentrations (25, 50, 100,

250, 500 mg/mL). Muller Hinton agar (Himedia, Mumbai) was prepared by dissolving 15.2 g in 400 mL of distilled water. Then the medium was sterilized by autoclaving at 121 °C and at 15 lb for 15 min. After sterilization, the medium and sterile Petri plates were transferred into laminar air flow chamber. Approximately 15–20 mL of the sterile medium was poured in each sterile Petri plates. The medium was allowed to solidify in laminar flow and inoculated with the overnight bacterial cultures. The sterile Whatman filter paper No. 1 disc was prepared and discs were soaked in the plant extracts and then placed over the inoculated plates. After 24 h of incubation at 37 °C, the zone of inhibition around the disc was measured^[7].

2.6. Radical scavenging activity–DPPH assay

Six different ethanol dilutions of lemongrass plant leaves extracts (10, 20, 30, 40, 50, 100 µg/mL) were mixed with 1 mL of 0.2 mmol/L ethanolic solutions of DPPH. Ethanol (1 mL) plus plant extract solution was used as blank. The absorbance was measured at 518 nm after 1 h of reaction at 37 °C. DPPH was prepared daily and protected from light. Scavenging capacity in percent (IC%) was calculated using the equation:

$$IC\% = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100\%$$

Where A_{sample} is the absorbance obtained in the presence of different extract concentrations and A_{blank} is that obtained in the absence of extracts. Tests were carried out in triplicate^[15].

2.7. Oxidative DNA damage protective activity

DNA was isolated from human blood serum. A total of 10 µL of DNA sample was taken in separate microfuge tubes covered with black paper. Then about 10 µL of plant extract samples was added into the DNA sample, and this was mixed gently and then incubated for 10 min. After 10 min, 10 µL of Fenton's reagent (ascorbic acid, FeCl₃, H₂O₂) were added to the above and mixed well and incubated at room temperature for 1 h in dark condition. Then 15 µL from each of the above was taken and added with 3 µL of the 6× loading dye. This was mixed well and loaded in 10 g/L agarose gel containing ethidium bromide. DNA with H₂O₂ was used for the negative control. The gel was electrophoresed at 50 V until the tracking dye has reached 1 cm from the bottom of the gel. The DNA was visualized and photographed using an Alpha DigiDoc digital gel documentation system (USA)^[16].

3. Results

The plant material was subjected to an extraction process with solvents like chloroform, methanol and water. The results showed that all solvent extracts of *C. citratus* exhibited maximum zone of inhibition against *B. subtilis*, *P. aeruginosa* and *P. vulgaris*. All solvent extracts of lemongrass plant exhibited minimum inhibition activity against *Nocardia* sp., *E. aerogenes*, *Serratia* sp., and *S.*

aureus. Zone of inhibitions are shown in Tables 1 to 3. It was observed that antibacterial activity of *C. citratus* plant leaf extracts showed good results for Gram-positive and Gram-negative organisms.

Table 1

Antimicrobial activity of chloroform extract.

Bacteria	Inhibition zone (mm)				
	500 mg/mL	250 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL
<i>B. subtilis</i>	21	19	18	17	16
<i>P. aeruginosa</i>	20	19	17	15	14
<i>P. vulgaris</i>	19	18	17	16	15
<i>S. aureus</i>	13	11	10	9	8
<i>Nocardia</i> sp.	9	8	7	6	5
<i>Serratia</i> sp.	7	6	5	4	3
<i>E. aerogenes</i>	8	7	6	5	4

Table 2

Antimicrobial activity of methanol extract.

Bacteria	Inhibition zone (mm)				
	500 mg/mL	250 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL
<i>B. subtilis</i>	18	17	16	15	13
<i>P. aeruginosa</i>	20	18	17	16	15
<i>P. vulgaris</i>	20	18	17	16	15
<i>S. aureus</i>	11	10	9	8	6
<i>Nocardia</i> sp.	7	6	5	4	3
<i>Serratia</i> sp.	5	4	3	2	2
<i>E. aerogenes</i>	7	6	5	4	3

Table 3

Antimicrobial activity of water extract.

Bacteria	Inhibition zone (mm)				
	500 mg/mL	250 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL
<i>B. subtilis</i>	20	17	16	15	14
<i>P. aeruginosa</i>	19	18	17	16	15
<i>P. vulgaris</i>	22	20	19	18	17
<i>S. aureus</i>	15	14	12	10	9
<i>Nocardia</i> sp.	10	9	8	7	6
<i>Serratia</i> sp.	9	8	7	5	4
<i>E. aerogenes</i>	7	6	5	4	3

The photometric quantification of the extent of DPPH scavenging in *C. citratus* plant leaf extracts was followed. The results, expressed as percent scavenging, are represented in Figure 2. The results revealed that the maximum extent of DPPH scavenging activity was in high concentration of plant extract in high level inhibition.

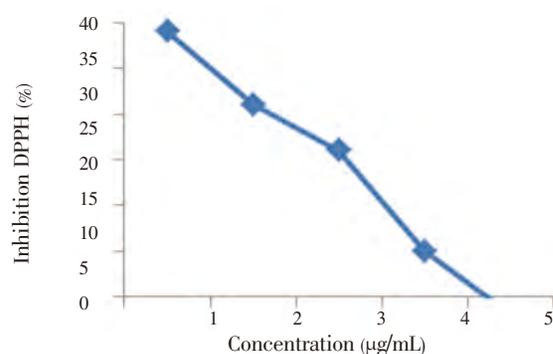


Figure 2. DPPH scavenging activity on extracts of *C. citratus* plant extract.

DNA protective activity by *C. citratus* was showed for isolated blood DNA against free radicals generated by H_2O_2 . This research was carried out with the chloroform, methanol and water extract. The results indicated that plant leaf extract had maximum DNA protective activity against free radicals at the all solvent extracts as shown in Figure 3. It was observed that the *C. citratus* plant leaves extract had maximum DNA protective effect.

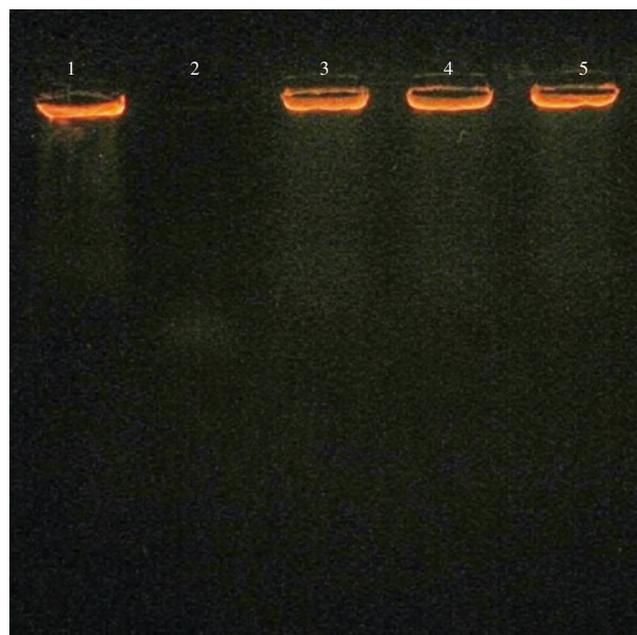


Figure 3. DNA protective activity in *C. citratus* plant extract in Gel Doc.

1: Human blood DNA (control), 2: Human blood DNA+ H_2O_2 (Damaged DNA), 3: Human blood DNA+ H_2O_2 +*C. citratus* leaves extract on chloroform, 4: Human blood DNA+ H_2O_2 +*C. citratus* leaves extract on methanol, 5: Human blood DNA+ H_2O_2 +*C. citratus* leaves extract on water.

4. Discussion

Lemongrass plant oils and extracts have been used for a wide variety of purposes for many thousands of years, and the lemongrass oil is used for preservation of food crops. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies[17]. Differences in sensitivity between Gram-positive and Gram-negative bacteria to the extract can probably be attributed to the structural and compositional differences in membranes between the two groups[18]. The Gram-negative bacteria have an outer membrane that serves as an impermeable barrier for many small molecules. Our research is consistent with findings of other researchers so that most plant extracts have inhibition effect on Gram-positive and Gram-negative bacterial[19–25]. Several investigators studied the antimicrobial activity of essential oil of lemongrass plant against pathogenic bacterial strains and found that *Enterococcus*

fecalis was the most sensitive microorganism, while *P. aeruginosa* was the most resistant^[25–27]. The present study showed that an methanol extract of lemongrass leaf had an antibacterial action against all the four Gram-positive bacteria. The leaf extract has a strong antibacterial activity. All solvent (chloroform, methanol and water) extracts showed good activities against human pathogens.

DPPH radical has been widely used to test the free radical scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids. This test aims at measuring the capacity of the extracts to scavenge the stable radical DPPH formed in the solution by donation of hydrogen atom or an electron^[28]. If the extracts have the capacity to scavenge the DPPH free radical, the initial blue/purple solution will change into yellow color due to the formation of diphenyl picryl hydrazine. The reaction is used as a measure of the ability of the extracts to scavenge any free radical. Since IC₅₀ represents the concentration of the extract that is able to scavenge half of the DPPH free radical presents in the test solution, the lower this value is, the higher the antioxidant activity of the extract has^[29]. The antioxidant activity supports the medicinal use of lemongrass plant by local population. The flavonoid compounds of lemongrass plant leaves aqueous extract are responsible for its antioxidant activity. Flavonoid compounds in several aqueous extracts have strong antioxidant and free radical scavenging activities and are more effective in protecting cells from free radical damage. The phenolic compounds of lemongrass plant leaves extract contribute directly to antioxidant activity. These metabolites also act as radical scavengers, reducing agents, hydrogen donors and singlet oxygen quenchers^[30].

Hydrogen peroxide is believed to cause DNA strand breakage by generation of hydroxyl radicals close to the DNA molecule. It is an extremely reactive species formed in biological systems and is capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. The presence of flavanoid compounds in lemongrass leaves extracts showed DNA protective activity against free radicals generated by H₂O₂^[16].

This research suggested that the lemongrass plant (*C. citratus*) extract has antimicrobial activity, antioxidant activity and DNA damage protectively, which could be helpful in preventing various human pathogenic organisms and oxidative stress induced disease. The results of the present study also indicate that the plant leaves possess many phytochemical which could be beneficial for human health.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Different parts of the lemongrass plants have been shown to have various uses, however, its antimicrobial properties and protective effects have not been widely studied.

Research frontiers

The investigation is conducted for the antibacterial activity, analysis of the chemical antioxidant properties, and the DNA protective activity against free radicals by lemongrass plant extracts with chloroform, methanol, and water solvents.

Related reports

Similar results were found in other studies with respect to the antibacterial properties of lemongrass.

Innovations & breakthroughs

The testing of antioxidant properties and activity against free radicals is a very good idea.

Applications

The lemongrass plant shows good antioxidant activity and its use as a medicinal plant together with the findings from this study shows that it would be very useful.

Peer review

This was a good study that the authors has determined the antibacterial activity, analyzed the chemical antioxidant property, and DNA protective activity against free radicals by lemongrass plant extracts. The results are interesting, and the research suggested that the lemongrass plant (*C. citratus*) extract has antimicrobial activity, antioxidant activity and DNA damage protectively, which might be helpful in preventing various human pathogenic organisms and oxidative stress induced disease.

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