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Synthesis Characterization of Lemon Grass Nanoparticle and its Antioxidant, Anti-Bacterial and Anticancerious Activity

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Abstract - Lemon grass nanoparticles (LG-NPs) were synthesized using a green synthesis method, employing lemon grass extract and silver nitrate. The LG-NPs were characterized using UV-Vis spectroscopy, TEM, XRD, and FTIR spectroscopy. The antibacterial activity of LG-NPs was evaluated against E. coli, S. aureus, and P. aeruginosa, showing significant inhibition. The antioxidant activity of LG-NPs was assessed using DPPH and ABTS assays, exhibiting strong scavenging activity. Furthermore, LG-NPs demonstrated anti-proliferative activity against MCF-7 breast cancer cells. This study highlights the potential of LG-NPs as a natural, biocompatible, and multifunctional nanomaterial for biomedical applications.

Key Words: Lemon grass nanoparticles, green synthesis, antibacterial activity, antioxidant activity, anti-cancerous activity.

1. INTRODUCTION

The emergence of antibiotic-resistant bacteria and the growing demand for sustainable, eco-friendly solutions have sparked significant interest in the development of novel antimicrobial agents. In recent years, the use of plant extracts and nanoparticles has gained considerable attention due to their potential therapeutic applications. Lemon grass (Cymbopogon citratus), a perennial herb widely cultivated in tropical and subtropical regions, has been traditionally used for its medicinal properties, including antimicrobial and antioxidant activities.

Nanotechnology, the manipulation of matter at the nanoscale, has revolutionized various fields, including medicine, agriculture, and materials science. The synthesis of nanoparticles using plant extracts, also known as green synthesis, offers a promising approach for the development of biocompatible and sustainable nanomaterials. This method eliminates the need for harsh chemicals and high-energy processes, making it an attractive alternative to traditional synthesis methods.

In this study, we report the green synthesis of lemon grass nanoparticles (LG-NPs) using lemon grass extract and silver nitrate. The LG-NPs were characterized using various spectroscopic and microscopic techniques, including UV-Vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. The antibacterial activity of LG-NPs was evaluated against three common pathogens: Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), and Pseudomonas aeruginosa (P. aeruginosa). Additionally, the antioxidant activity of LG-NPs was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Furthermore, the anti-proliferative activity of LG-NPs was investigated against MCF-7 breast cancer cells.

2. LITERATURE SURVEY

1. Lemon grass: Lemon grass is an aromatic medicinal grass belonging to the genus Cymbopogon. It is prevalent in the semi-temperate and tropical regions of Asian, American and African continents. A strong lemon fragrance, a predominant feature of this grass, is due to the high citral content in its oil. The redolence of the oil enables its use in soaps, detergents and perfumes. It also finds an application in the pharmaceutical industry. A vast array of ethnopharmacological applications of lemon grass exist today. Apart from nutrients such as fats, proteins, fiber and minerals, it also contains various bioactive compounds which may be grouped into alkaloids, terpenoids, flavonoids, phenols, saponins and tannins. The health restorative capacity of lemon grass may be ascribed to the diverse secondary metabolites it produces. This review attempts to give an overall description of lemon grass, highlighting its medicinal properties which make it a potent herb for pharmacognostic applications.

2. A Study of the Antibacterial Activity of the Essential Oil of Lemon Grass: Lemon grass oil obtained by the steam distillation of the fresh aerial parts of Cymbopogon citratus (DC.) Stapf was investigated for antibacterial activity. The results obtained suggests that the oil possesses rapid bactericidal activity against Gram-negative and Gram-positive bacteria, the latter being more susceptible. Other factors that could affect the activity of the oil were also investigated. The dispersion of the oil in a dilution of dimethyl sulphoxide which does not have an effect on the microorganisms studied provided a convenient medium for the assay. It is being suggested that more information about the antibacterial activity of essential oils could be obtained using such a methodology.

3. Antiviral, Antibacterial, Antifungal, and Anticancer Activity of Plant Materials Derived from Cymbopogon citratus (DC.): The importance of natural plant materials in modern medicine is considerable, and raw materials with antiviral, antibacterial, antifungal, and anticancer properties are still sought because of microbe resistance and difficulties in anticancer therapy. This review focuses on the lemongrass Cymbopogon citratus (DC.) Stapf. and on the lemongrass oil properties and applications. Multiple applications of this plant were described in different latitudes and cultures, including cases of digestive disorders and anti-inflammatory, antipyretic, diaphoretic, stimulating, and antispasmodic conditions. Data from the literature on the composition of essential oil and extracts from C. citratus were analyzed, and the results of research on the antifungal, antibacterial, and antiviral effects were quoted. Essential oil inhibits the growth of fungi (Aspergillus niger, A. fumigatus, Candida spp.) and has an antibacterial effect (Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa). It also shows antiviral activity and deters insects. Lemongrass contains active substances with potential anticancer effects. This plant has apoptosis-stimulating properties, mainly through the activity of apigenin, which is the main active flavonoid in this plant. This active substance helps inhibit cell proliferation by stopping the cell cycle and directing cancer cells toward apoptosis.

4. Citral in lemon myrtle, lemongrass, litsea, and melissa essential oils suppress the growth and invasion of breast cancer cells: Although cancer therapy suppresses recurrence and prolongs life, it may be accompanied by strong side effects; thus, there is a strong demand for the development effective treatments with fewer side effects. Cancer therapy using plant-derived essential oils is attracting attention as one promising method. This study investigated the antitumor effects of essential oil volatiles on breast cancer cells and identifies four essential oils that display antitumor activity.

Phytochemical composition and pharmacological potential of lemongrass (Cymbopogon) and impact on gut microbiota: Phytochemicals are versatile plant secondary metabolites with therapeutic properties. In this review, we explore lemongrass's phytochemistry and pharmacological potential (Cymbopogon) as well as its impact on gut microbiota. Lemongrass is well-known for its antioxidant, anti-microbial, anti-inflammatory, anti-hypertensive, anti-diabetic, anti-mutagenicity, anxiolytic properties, and for its hypoglycemic and hypolipidemic activities. Therefore, it is widely used in pharmaceuticals, food, feed, and the cosmetics industry. Lemongrass contains phenolic metabolites (including phenolic acids, flavonoids, stilbenes, and lignans), terpenoids, and alkaloids, which are potent bioactive ingredients. Lemongrass is a precious medicinal plant. Furthermore, lemongrass phytochemicals are considered potential agents to improve health by establishing a balanced gut ecosystem. Lemongrass is considered a quintessential food and feed additive at the industrial level, since there are no issues with residue or toxins. Lemongrass powder and essential oils are used to modulate the gut ecosystem by generating anti-microbial, anti-inflammatory, and antioxidant responses, increasing the optimum nutrient absorption in the gut system. This review will further explore lemongrass's phytochemical, pharmacological, and therapeutic potential.

3. METHODLOGY

3.1 Materials:

Wet and dry lemon grass, water, distillation column, condenser, heating element, measuring cylinder, conical flasks, burette, glass bottles with stopper.

3.2 Plant Samples:

Cymbopogon citratus plants were collected from TNAU Nursery, Coimbatore. The plant sample was freshly cut, 10 cm from the root, in the morning of the day they were collected. Lemongrass (Cymbopogon Citratus), the percentage essential oil yield for the partially dried leaves was found to be higher than that of

the fresh leaves. Thus, once collected, the plant material was dried at room temperature for maximum 4 days, then kept in a sealed plastic bag at ambient temperature and protected from the light. Extraction yield increase by decreasing the particle size due to the higher amount of oil released as the leave cells are destroyed by milling. In order to improve the collection efficiency, the plant material was soaked in its distilled water for 30min before the extraction performed.

3.3 Extraction Process:

The lemongrass oil was obtained by hydrodistillation of fresh leaves from the plant, and was dried with anhydrous sodium sulfate. For hydrodistillation, 250g of fresh lemongrass leaves were cut into small pieces and placed in a 1L flask containing 400mL of distilled water. They were hydrodistilled for 5 h using a Clevenger-type apparatus according to the method described by Guenther (1950). The yield of essential oil was calculated using the following formula: Essential oils yield (%) = net weight of oils (g) / total weight of fresh leaves (g) × 100. The extraction yield was 0.4%. The oil was stored in dark glass bottles and kept at 4°C until use.

3.4 PHYTOCHEMICAL ANALYSIS

The qualitative phytochemical study was performed on the extracts by using below standard tests.

3.4.1 Test for Alkaloids (Wagner's test)

About few ml of plant extract was treated with 4-5 drops of Wagner's reagent. The formation of reddish brown precipitate confirms the presence of Alkaloids

3.4.2 Test for Phenol (Ferric chloride test)

About 2ml of the extract was treated with 10% ferric chloride solution and observed for the formation of deep blue / black colour.

3.4.3 Test for reducing sugars (Fehling's Test)

To 1 ml of the extract added few drops of Fehling's reagent and the mixture was boiled in a boiling water bath for 10 minutes and observed for the appearance of blue colour.

3.4.4. Test for Saponins (Foam test)

To 2 ml of the plant extract added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam for few seconds. The presence of foam confirms the presence of saponins.

3.4.5 Test for Flavonoids

To about 2ml of plant extract, few drops of 10% ferric chloride solution was added. The formation of green or blue colour indicates the presence of flavonoids.

3.4.6 Test for Phytosterols (Salkowski's Test)

One ml of the plant extract was treated with 2 ml of chloroform and few drops of acetic anhydride were added.To that mixture added equal amount of concentrated sulphuric acid was added. The formation of bluish green colour indicates the presence of phytosterols.

3.4.7 Test for Aminoacids and Proteins (Ninhydrin test)

To a few ml of plant extract added small amount of Ninhydrin reagent. A purple or violet colour formed indicates the presence of amino acids and proteins

3.4.8. Test for Steroids

About 2 ml of chloroform and 0.2 ml of concentrated sulphuric acid was added to 1ml of flower extract.The formation of red colour precipitate indicates the presence of steroids.

3.4.9 Test for Tannin

To about 1ml of plant extract added few drops of dilute ferric chloride solution. The presence of tannin is confirmed by the formation of dark green or blue color.

3.4.10 Test for glycosides

To 1ml of plant extract added few ml of concentrated sulphuric acid. Formation of red colour indicates the presence of glycoside.

3.5 Synthesis of silver Nanoparticles:

Add 10ml of silver nitrate solution and 90ml of extract in conical flask cover tightly keep it dark place . after 1 hour in measuring uv- spectrophotometer in 300-600nm range.the silver nanoparticals formation was monitored upto 48hr in uv- spectrophotometer measurements. FTIR measurements were obtained on a Nexus 670 FTIR instrument with the sample.

3.6 Antibacterial activity

- To perform antimicrobial activity using various bacterial and fungal species were selected viz., *Escherichia coli, staphylococcus aureus, klebsiella species*
- Media and culture condition
- Muller-Hinton Agar (MHA), Nutrient Broth (NB) and Luria Britani (LB) were used throughout the study for determining the antibacterial assay. The media was adjusted to the pH and autoclaved at 121°c for 15 minutes.

3.7 Preparation of the Bacterial Inoculum

Stock cultures were maintained at 4°c on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated with agitation for 24hours and at 37°c on shaking incubator and fungal cultures were incubated at 27°c for 3-5 days. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar. Bacterial cultures then incubated at 37°c for 24 hours and fungal incubated at 27°c for 3-5 days. A single colony was transferred to nutrient agar media slants were incubated at 37°c for 24 hours and potato dextrose slant were incubated at 27°c for 3-5 days. These stock cultures were kept at 4°c. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37°c for 18-20 hours for bacterial culture.

3.8 Disc Diffusion method

The antibacterial activity and antifungal activity of crude extract extracts was determined by Well Diffusion method (Bauer *et al*., 1996). MHA plates were prepared by pouring 20ml of molten media into sterile petriplates. After solidification of media, 20-25µl suspension of bacterial inoculums was swabbed uniformly. The sterile fabric discs were dipped into required solvents then placed in agar plates. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. Then plates were incubated at 37°c for about 24 hours and control was also maintained.

3.9 IN- VITRO ANTICANCER ACTIVITY

Cell line

The human Liver Cancer cell line-HepG-2 was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay:

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each

well and incubated at 37^{0}C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader.

The percentage cell viability was then calculated with respect to control as follows

% Cell viability = [A] Test / [A]control x 100

The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software.

4. FLOWCHART

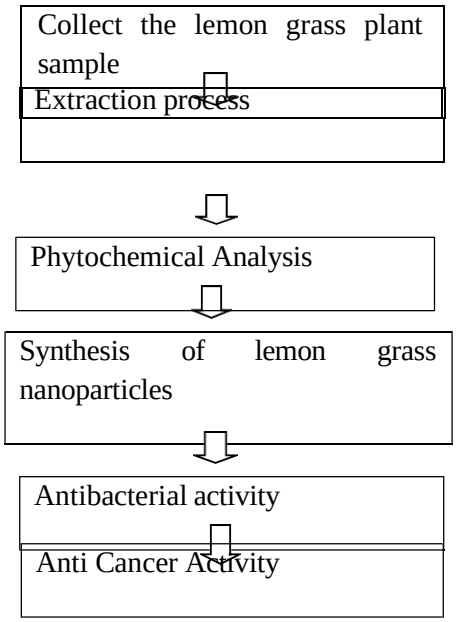
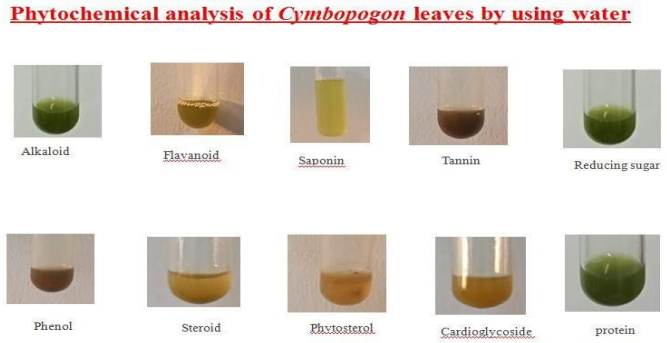


Fig:1 Flowchart

5. RESULTS AND DISSCUSSION

5.1 Phytochemical Analysis:



The qualitative phytochemical study was performed on the extracts by using below standard tests. The formation of reddish brown precipitate confirms the presence of Alkaloids for the formation of deep blue / black colour. Presence of reducing sugars observed for the appearance of blue colour. formation of persistent foam for few seconds. The presence of foam confirms the presence of saponins. The formation of green or blue colour indicates the presence of flavonoids. The formation of bluish green colour indicates the presence of phytosterols. A purple or violet colour formed indicates the presence of amino acids and proteins. The formation of red colour precipitate indicates the presence of steroids.The presence of tannin is confirmed by the formation of dark green or blue color. Formation of red colour indicates the presence of glycoside.

Fig 2: Phytochemical analysis of *Cymbopogon* leaves by using water

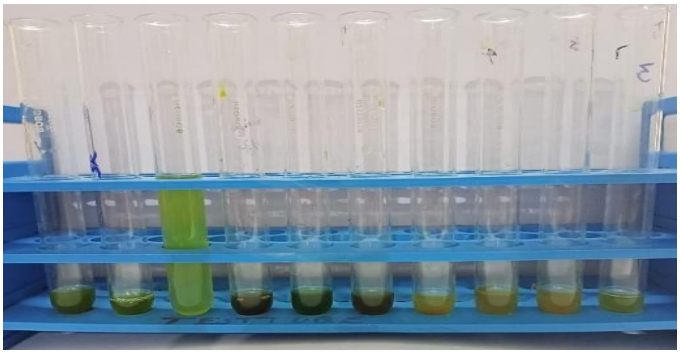


Figure: 3- Phytochemical Analysis of *Cymbopogon citratus* plant extract

S. no	Phytochemical constituents	Present / Absent	Observation
1	Alkaloid	-	No change
2	Flavanoid	+	Yellowish green colour formed
3	Saphonin	++	Foam formed
4	Tannin	+++	Brownish green colour formed
5	Reducing sugar	++	Dark green colour formed
6	Phenol	+++	Brown colour formed
7	Steroid	+++	Yellow colour formed
8	Phytosterol	+	Yellowish brown colour formed
9	Cardioglycoside	++	Yellowish brown colour formed
10	Protein	_	No change

Table: 1- Phytochemical Analysis of *Cymbopogon citratus* plant extract

5.2 Extraction and Synthesis of Lemon Grass Nanoparticle

Steam Distillation Method 150g of fresh lemongrass sample were placed into a 1 lit round bottom flask containing 250ml of distilled water. The flask was fitted with a rubber stopper connected to condenser and heated. Water at 0oC flowed counter currently through the condenser to condense the ensuring steam. When the water reached 100oC it started boiling ripping off the essential oil from the lemongrass. When the lemongrass got heated up, the essential oil that was extracted from the leaf mixed with the water vapor. Both passed through the condenser and the vapor was condensed into liquid. With the use of ice block, cooling was made possible and volatilization of the essential oil was avoided. The condensate was directly collected using a 500ml beaker and then poured into a separating funnel. This formed two layers of oil and water. The tap of the separating funnel was opened to let out the water while the oil was immediately collected into a 100 ml stoppered bottle. The bottle was closed tightly to prevent vaporization of the essential oil. The oil was collected and the volume of oil obtained was weighed. From the experiment carried out it was observed that the best method used in extraction is solvent extraction method because it gave more oil than any other method. This conforms to works done by other researchers. Steam distillation method yielded less oil ompared to the solvent extraction this is because most volatile content gets lost during the heating process.

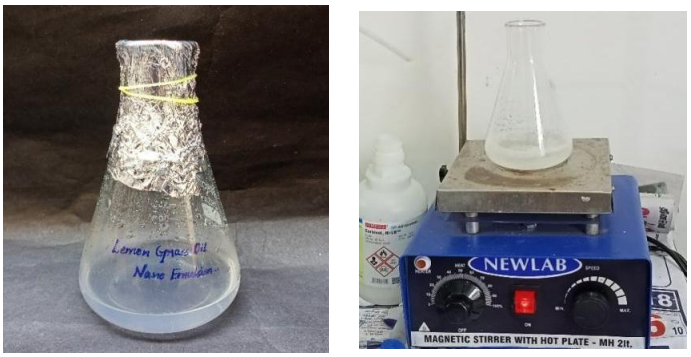
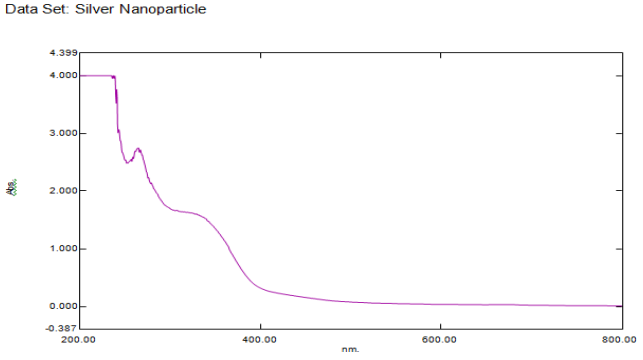


Figure: 4- Extraction and Synthesis of Lemon Grass Nanoparticle

Figure: 5 preparation of nanoparticle

5.3 UV Analysis Of Nano Hand Sanitizer



Graph- 1: UV Analysis of Nanoparticle

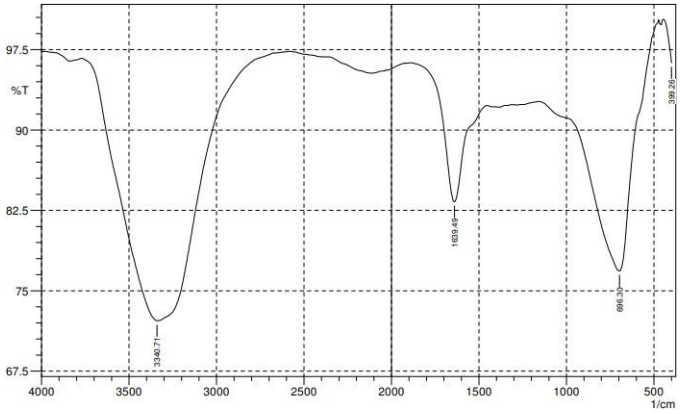
Characterization of the synthesized nanoparticles UV –Vis absorbance spectroscopy The bioreduction of the AUCL4 ions in solution was monitored by periodic sampling of aliquots (2ml) and measuring the UV- VIS spectra of the solution in 10-mm –optical –path- length quartz cuvettes with a systronics. UV –VIS spectrophotometer at a resolution of 1nm between 500 and 680 nm with a scanning speed of 1856 nm/min. After the OD values were taken upto 3 days at regular intervals. The sample was then centrifuged at 42,000 rpm for 10 minutes and pellet was dried and the nanopowder obtained was used for further analysis. The optical properties of AOT and SDS-capped gold nanoparticle solutions (samples 1–4) were monitored on a Hewlett-Packard diode array spectrophotometer (model HP- 8452) operated at a resolution of 2 nm.

5.3.1 FTIR Spectra Acquisition:

FTIR spectrometer (Alpha FTIR Spectrometer from Bruker optic), equipped with a deuterated triglycine sulphate (DTGS) as a detector and a germanium as beam splitter, interfaced to computer operating under Windows-based system, and connected to software of OPUS operating system (Version 7.0 Bruker optic), was used during FTIR spectra acquisition. A few drops of each sample were positioned in contact with attenuated total reflectance (ATR) plate. FTIR spectra were collected at frequency regions of 4000–800 cm^{-1} by coadding 32 scans and at resolution of 4 cm^{-1}. All spectra were substracted against a background of air spectra. After every scan, a new reference of air background spectra was taken. The ATR plate was carefully cleaned by scrubbing with isopropyl 70% twice followed by drying with soft tissue before being filled in with the next sample, making it possible to dry the ATR plate. These spectra were recorded as absorbance values at each data point in replicate two times.

5.3.2 FTIR RESULTS:

The results of FTIR analysis confirm the presence of totally 4 functional groups Which shows the major peak values that are 3340.71 (O-H), 2052.26(O-H), 2013.68(C=C), 1882.52 (C-Cl), 1639.49(=C-H), 606.30(O-H), 399.26(C-O), that functional groups such as non bonded, O-H stretch, carboxylic group, acidic, H bonded, C-H stretch, asymmetric stretching of –CH (CH2) vibration, C=N (stretch), carbon-carbon triple bond, multiple bonding, carbonyl compound frequency, C=O stretch, C=C stretch, O-H bend, alcoholic group, C-N stretch, C-O stretch, PO3 stretch, =C-H bending and C-Cl respectively.



Graph- 2: FTIR Results

5.3.3 SEM Analysis for nanoparticles

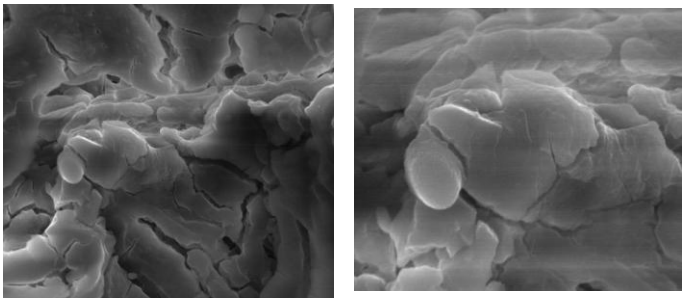


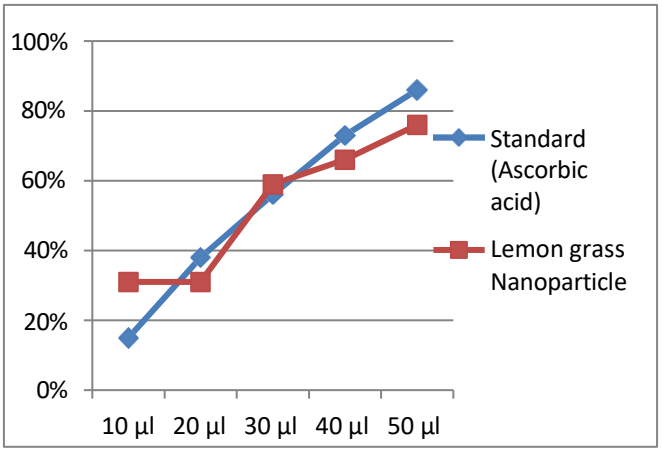
Figure: 6 SEM Analysis for nanoparticles

SEM Details: MIRA3 TASCAN: HV: 15 KV; View field: 20.7 µM; SEM magnification: 10.KX; Width: 6.58 mm

5.3.4 DPPH Assay:

Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes.

Table: 2 DPPH Assay



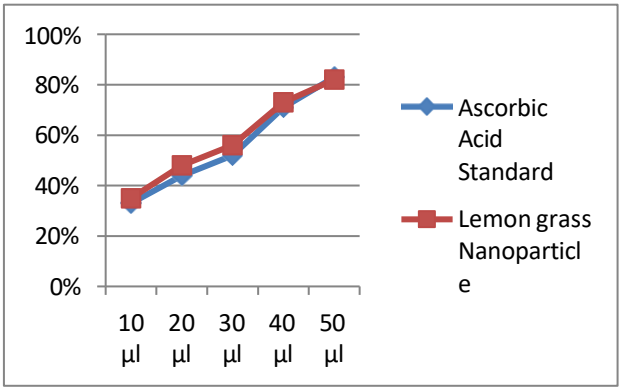
Graph:3 DPPH Assay

5.3.5 FRAP Assay:

The concentration of antioxidant has a ferric-TPTZ reducing ability equivalent to that of 1 mmol/l FeSO4.7H2O. EC1 was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution determined using the corresponding regression equation.

FRAP Assay:		
concentration	**Ascorbic Acid Standard**	**Lemon grass Nanoparticle**
10 µl	33 %	35%
20 µl	44 %	48%
30 µl	52 %	56%
40 µl	71 %	73%
50 µl	83 %	82%

Table: 4 FRAP Assay



Graph:4 FRAP Assay

5.3.6 Nitric oxide (NO) scavenging activity:

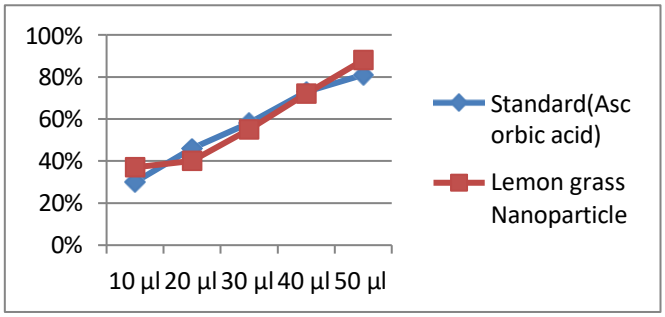
The antioxidant activity of plant extract was evaluated by nitric oxide scavenging activity using different concentrations 0.2µl, 0.4µl, 0.6µl, 0.8µl, 1.0µl.

Finally absorbance is observed at 540 nm. All the reactions were performed in triplicates, and their percentage inhibition was calculated by the following formula

Concentration	**Standard (Ascorbic acid)**	**Lemon grass Nanoparticle**
10 µl	15 %	31%
20 µl	38 %	31%
30 µl	56 %	59%
40 µl	73 %	66%
50 µl	86 %	76%

Nitric oxide (NO) scavenging activity		
Concentration	**Standard(Ascorbic acid)**	**Lemon grass Nanoparticle**
10 µl	30 %	37%
20 µl	46 %	40%
30 µl	58 %	55%
40 µl	73 %	72%
50 µl	81 %	88%

Table: 4 Nitric oxide (NO) scavenging activity



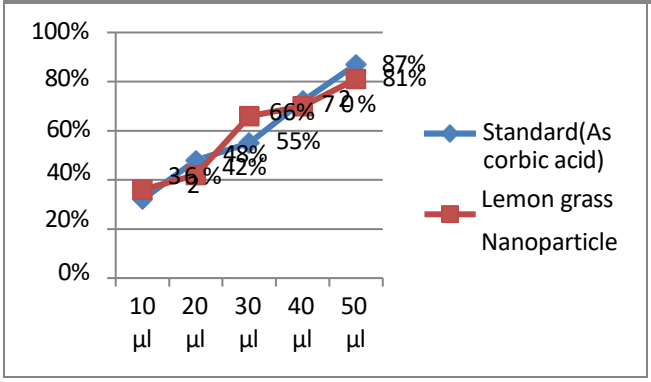
Graph: 5 Nitrite oxide (NO) scavenging activity

5.3.7 Hydrogen peroxide scavenging activity (H_2O_2)

The hydrogen peroxide scavenging activity was measured absorbance was read at 230 nm. BHT was taken as a positive control and the reaction was carried out in triplicates. Percent inhibition of the assay was calculated.

Table: 5 Hydrogen peroxide scavenging activity (H_2O_2)

Concentration	*E. Coli*	*Bacillus subtilis*	*Pseudomonas aeroginosa*	*Streptococcus pneumoniae*
25µl	0.1cm	0.7 cm	0.5cm	0.6cm
50µl	1.0cm	0.9 cm	0.8cm	0.8 cm
75µl	1.2 cm	1.1cm	1.2cm	1.0 cm
100µ	1.5 cm	1.3cm	1.5cm	1.5 cm
Standard	1.2cm	1.5cm	1.3cm	1.2cm



Graph: 6 Hydrogen peroxide scavenging activity (H_2O_2)

5.4 ANTIBACTERIAL ACTIVITY:

Antibacterial activity was performed by agar diffusion method. Van der Watt *et al.*, 2001. The stock culture of bacteria (*Pseudomonas, Staphylococcus, Bacillus, klebseilla, proteus E.coli*) were received by inoculating in nutrient broth media and grown at 37% for 18 hours. The agar plates of the above media were prepared. Each plates was inoculated with 18 hours old cultures the bacteria were swab in the sterile plates. Cut the 5 wells Pour the plant extract in ratio 25µl, 50µl, 75µl, 100µl. All the plates were incubated at 37°C for 24 hours and the diameter of inhibition zone was noted in Cm.

Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations or plant extracts against Gram positive, Gram negative bacteria. The extracts exhibited antibacterial activities against tested microorganisms.

5.4.1 Antibacterial activity was performed by agar diffusion method

Hydrogen peroxide scavenging activity (H_2O_2)		
Concentration	**Standard(Ascorbic acid)**	**Lemon grass Nanoparticle**
10 µl	32 %	36%
20 µl	48 %	42%
30 µl	55 %	66%
40 µl	72 %	70%
50 µl	87 %	81%

Table: 7- Antibacterial activity was performed by agar diffusion method

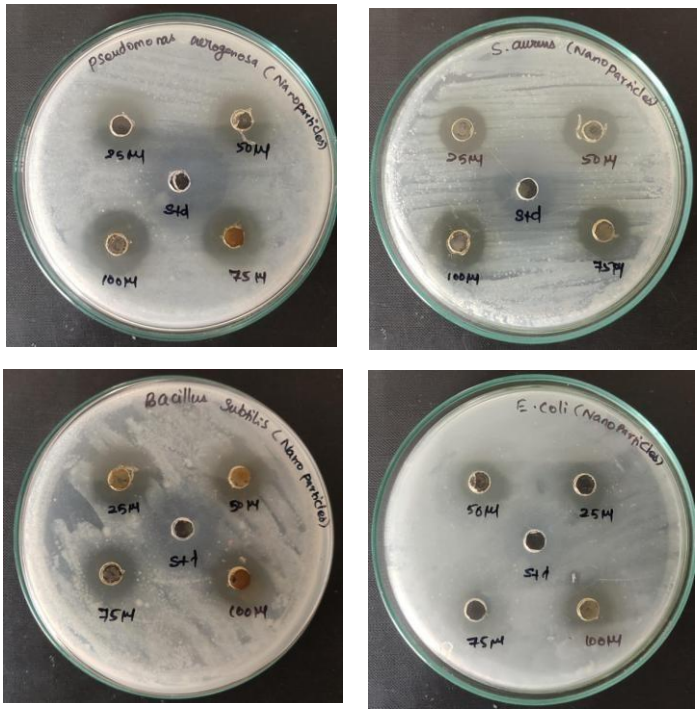
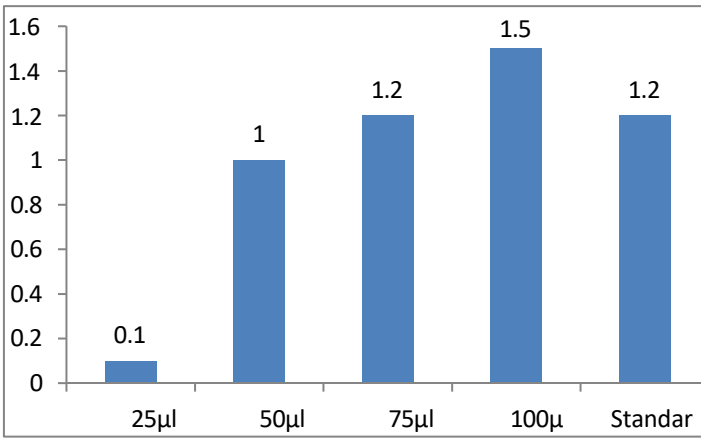


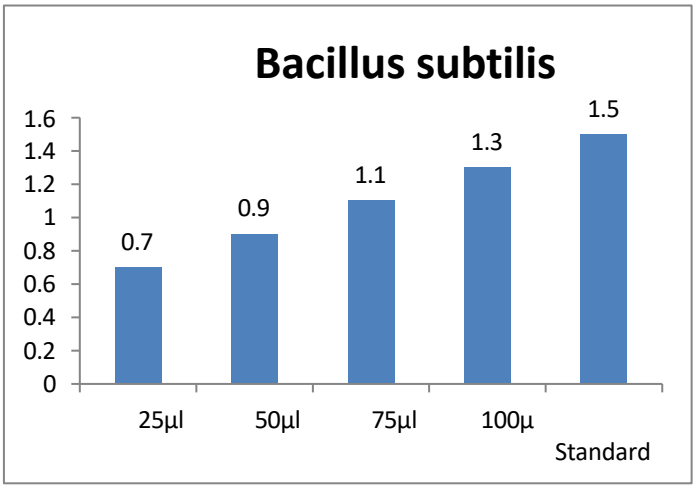
Fig 7: Antibacterial activity was performed by agar diffusion method



Graph: 7-Antibacterial activity was performed by agar diffusion method

Lemon grass Nanoparticle showed inhibition zone of 0.1 cm, 1.0 cm, 1.2 cm and 1.5 cm for 25 µl 50µl, 75 µl and 100µl respectively against *E. Coli* respectively and Standard chloramphenicol shows against 1.5 cm.

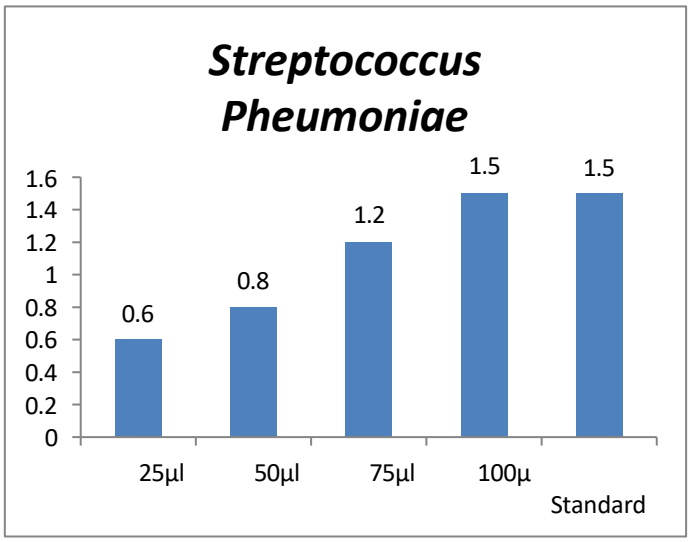
5.4.2 Antibacterial activity against the *Bacilllus subtilis*



Graph:8 Antibacterial activity against the *Bacilllus subtilis*

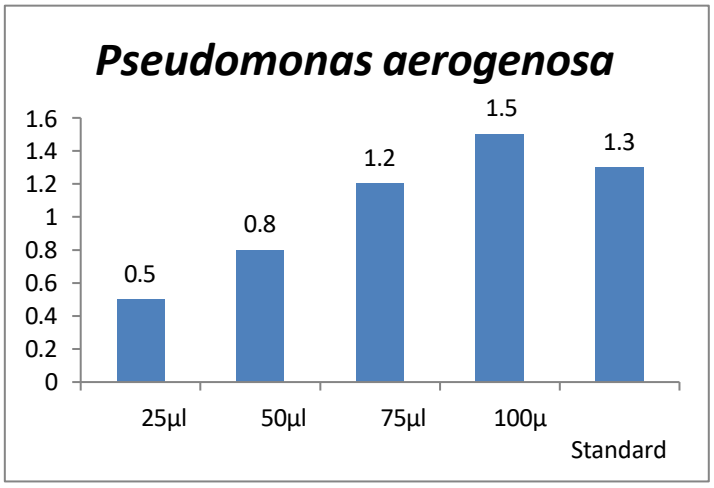
Lemon grass Nanoparticle showed inhibition zone of 0.7 cm, 0.9 cm, 1.1 cm and 1.3cm for 25 µl 50µl, 75µl and 100µl respectively against *Bacillus subtilis* respectively and Standard chloramphenicol shows against 1.5 cm.

5.4.3 Antibacterial activity against the *Streptococcus pheumoniae*



Graph: 9- Antibacterial activity against the *Streptococcus pheumoniae*

5.4.4 Antibacterial activity against the *Streptococcus pheumoniae*



Graph:10- Antibacterial activity against the *Streptococcus pheumoniae*

Lemon grass nanoparticle showed inhibition zone of 0.6 cm, 0.8 cm, 1.2 cm and 1.5 cm for 25 µl 50µl, 75µl and 100µl respectively against to *Pseudomonas aerogenosa* respectively and Standard chloramphenicol shows against 1.5 cm.

5.5 Anticancer Activity:

The result of MTT assays revealed that the Nanoparticle extract was decreased the percent viability of all the cells but to different extent. Nanoparticle was found to induce more cytotoxicity towards cancer cell lines. These results revealed morphological changes and shrinkage of cells leading to cell death induced by the

extracts in the prostate cancer cell lines. The IC_{50} values of plant extracts of against the Liver cancer cell line

5.5.1 Results

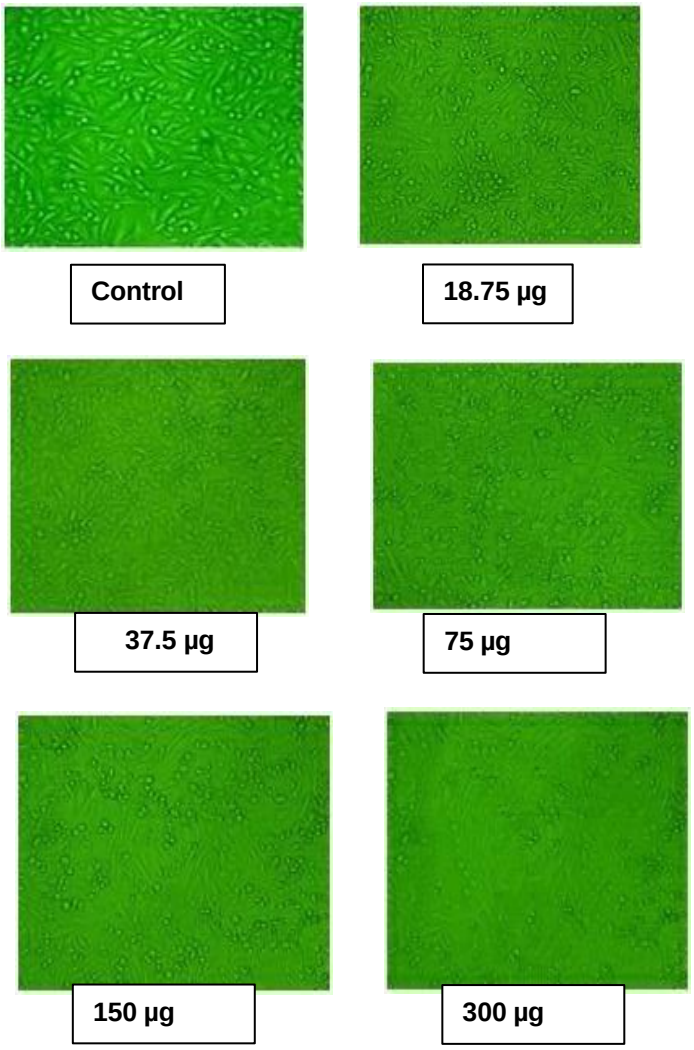
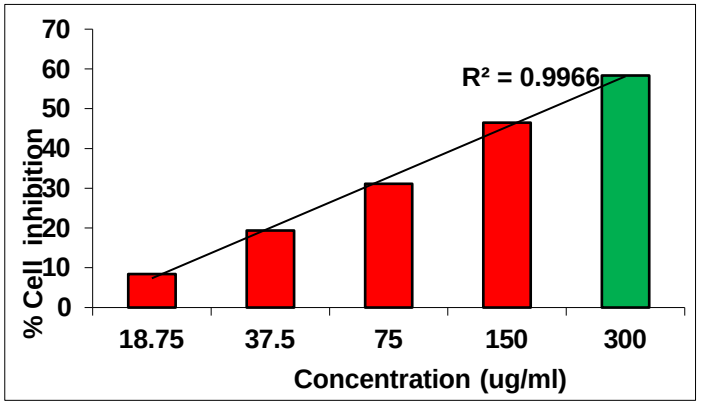


Figure:8 Anticancer Activity Result

5.5.2 Calculation:

Conc	18.75 µg	37.5 µg	75 µg	150 µg	300 µg	Cont
ABS	0.065	0.151	0.244	0.367	0.456	0.781
	0.066	0.151	0.245	0.362	0.457	0.785
	0.067	0.152	0.242	0.363	0.457	0.782
Avg	**0.066**	**0.151333**	**0.243667**	**0.364**	**0.4566666667**	**0.7826667**
Conc (µg/ml)	**% cell inhibition**				**IC 50 174.29**	**µg/ml**
18.75	8.432709					
37.5	19.3356				**R^2**	**0.996**
75	31.13288					
150	46.50767					
300	58.34753					

Table: 8 Anticancer Activity Calculation



Graph:11-Concentration of Cell Inhibition

6. CONCLUSIONS

This study demonstrated that lemongrass essential oil has the potential to be used as an antimicrobial treatment against pathogenic bacteria's. The activity of lemongrass oil increased over storage time, with the greatest reductions in bacterial populations seen on 24 hrs of sampling. The antibacterial activity also increased with initial exposure time However, there was minimal difference in the activity among the samples stored under refrigeration and abuse temperatures. The results of the present study suggest that lemongrass oil could potentially be used in the organic fresh produce industry as an alternative decontaminant for fresh leafy greens. Sensory properties of treated leafy greens merit further study.

7. REFERENCES

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