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Medicinal Benefits of Aqueous Extract of Lemongrass (*Cymbopogon citratus*): Effects of Some Biochemical Evaluations, Anti-microbial Properties and Characterization of Three Metallic Nanoparticles

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ABSTRACT

Introduction: *Cymbopogon citratus* (lemongrass) is known for its medicinal properties, offering therapeutic potential against various diseases, including cancer. This study investigates the nutritional profile, antinutrient content, minerals, phytochemicals, antioxidants, and antimicrobial properties of *C. citratus*, alongside the green synthesis of metallic nanoparticles from its aqueous extract, to assess its medicinal potential.

Method: The leaves of *C. citratus* were analyzed for proximate composition, antinutrient content, mineral profile, and both qualitative and quantitative phytochemical constituents.

Results: The analysis showed high levels of ash, crude fiber, and protein, with low concentrations of antinutrients and moderate amounts of essential minerals. Phytochemical screening identified the presence of bioactive compounds such as saponins, alkaloids, flavonoids, and phenolic compounds, which are known for their potent antioxidant properties that could potentially suppress malignancy and other degenerative diseases. The antimicrobial activity indicated that both the aqueous extract and the synthesized metallic nanoparticles exhibited significant activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Spectroscopic characterization through UV-Vis and FTIR techniques revealed chemical structures and molecular components relevant to the medicinal properties.

Conclusion: The aqueous extract of *C. citratus* contains notable medicinal constituents, highlighting its potential for the development of new drugs for the prevention and treatment of cancer and other degenerative diseases. This study underscores the therapeutic value of lemongrass and supports its further exploration in pharmaceutical applications.

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Introduction

Cancer progression and the development of clinical therapies for different cancer types have evolved dramatically in recent years, making this a critical area of scientific research (Loi et al., 2023). The chemical structures of isolated compounds are essential for cancer treatments. Among the various natural and synthetic substances explored for their potential anti-cancer properties, medicinal plants and metallic nanoparticles have garnered significant attention (Mbele et al., 2017). Lemongrass (Cymbopogon citratus), commonly found in tropical regions, especially in Southeast Asia, is a widely used herb known for its aromatic and therapeutic properties. The primary compounds in C. citratus are terpenes, alcohols, ketones, aldehydes, and esters. Notably essential oils in lemongrass include geranial (citral α), neral (citral β), citronellal, terpinolene, geranyl acetate, myrecene, and terpinol methylheptenone. Additionally, the plant contains flavonoids and phenolic compounds such as luteolin, isoorientin 21-O-rhamnoside, quercetin, kaempferol, and apiginin. Research indicates that C. citratus exhibit a range of pharmacological activities, including anti-amoebic, antibacterial, anti-cancer, antifilarial, antifungal, and anti-inflammatory activities (Shah et al., 2011). Traditionally, it has been employed in various cultures for its medicinal benefits, which include anti-inflammatory, analgesic, and anti-microbial effects. Recent scientific studies have further expanded on these traditional uses, highlighting the potential anti-cancer properties of lemongrass.

Phytochemical analysis of lemongrass has revealed a rich composition of bioactive compounds, including flavonoids, phenolic acids, and essential oils such as citral, which are known for their antioxidant properties (Muala et al., 2021). These compounds play a crucial role in neutralizing free radicals, preventing cellular damage, and reducing cancer risk. Additionally, lemongrass extracts can induce apoptosis (programmed cell death) in cancer cells and inhibit their proliferation (Ruvinov et al., 2019). Recent studies have explored lemongrass as a valuable natural source for developing new anti-cancer therapies, highlighting its medicinal properties and traditional uses across many cultures (Philion et al., 2017). In parallel with natural remedies, the use of metallic nanoparticles in medicine, particularly in cancer treatment, has shown tremendous promise. These nanoparticles can enhance cancer treatments efficacy by serving as carriers for drugs, ensuring targeted delivery to cancer cells while minimizing side effects on healthy tissues. They can also be designed to release drugs in response to specific stimuli in the tumor microenvironment, allowing for controlled and sustained release of the medication (Ghazal et al., 2024). Beyond drug delivery, metallic nanoparticles themselves can exhibit cytotoxic effects on cancer cells through the generation of reactive oxygen species (ROS) and disruption of cellular functions (Sofowora, 1996). Their ability to disrupt cellular signaling pathways or alternating permeability of membranes establishes their potential as chemotherapeutic agents (Augustine et al., 2020). The use of metallic nanoparticles as alternative chemotherapeutic agents is on the rise in cancer treatment, representing a significant advancement in precision medicine. Preliminary studies have looked into the interaction between metallic nanoparticles and bioactive compounds from lemongrass. Wang et al. (2013) explained that nanoparticles may enhance the solubility and bioavailability of specific phytochemicals, thereby enhancing their anticancer effects (Vimala & Kannan, 2021). By combining the inherent medicinal properties of these nanoparticles with conventional treatments, it is possible to enhance treatment outcomes and address some of the limitations of traditional chemotherapy and radiation therapy (Ghazal et al., 2024).

This study aimed to determine the medicinal importance of lemongrass. (Cymbopogon citratus) leaves, focusing on their nutritional content, anti-nutritional factors, mineral composition, phytochemical constituents, antioxidants compounds, and scavenging abilities. Furthermore, this study will explore the characterization and potential medicinal applications of metallic nanoparticles in cancer therapy, highlighting their synergistic effects with natural plant extracts.

Materials and Methods

Preparation of Plant Leaf Extract

The leaves of lemongrass (C. citratus) were harvested, washed under running tap water to remove dust, and air-dried for several days. Once dried, the leaves were crushed into a powder. Twenty grams of the powdered leaves were then mixed with 100 mL of distilled water and shaken overnight to ensure effective extraction. The resulting mixture was filtered through muslin cloth, and the filtrate was collected for further phytochemical analyses, including in-vitro antioxidant assessment, and green synthesis analyses.

Phytochemical Analysis

previous studies reported that chemical screening was performed on the aqueous extract of Cymbopogon citratus to identify its phytochemical constituents, including flavonoids, alkaloids, saponin, phenolic compounds, and steroidal nucleus (Sofowora, 1996; Harborne, 1984).

In-Vitro Antioxidant Activity of the Aqueous Extract of Cymbopogon Citratus Leaf Sample

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was done on Cymbopogon citratus extract, as described by Talaiekhozani and Amani (2019).

The nitric oxide (NO) radical scavenging assay was conducted on the aqueous extract, as described by Gyamfi et al. (1999).

The ferric-reducing antioxidant power was determined using a modified method of Jagetia and Baliga (2004), whereby the ferric reducing power of the extract was ascertained by comparing the antioxidant power to the reduction of $Fe3+$ ferricyanide in stoichiometric excess.

The chelation ability for $Fe2+$ was determined using a modified technique reported by Pulido et al. (2000) and Minotti & Aust (1987) .

The total phenolic content of the aqueous extract was determined based on the method of Puntel et al. (2005).

The total flavonoid content was assessed using a colorimetric assay developed by Singleton et al., 1999.

Ascorbic acid content in the extract was analyzed using the 2,4- Dinitrophenylydrazine (DNPH) reagent and a modified spectrophotometric method described by Roe and Kuether (1943).

Green Synthesis Analyses of the Aqueous Extract of Cymbopogon Citratus Leaf Sample

Separate 0.1 M solutions of silver, zinc, and copper were prepared in deionized water. 60 g of powdered C. citratus leaves were boiled with 500 mL of de-ionized water in a 1000 mL round-bottom flask for 1 hour to ensure proper extraction. The mixture was then filtered using a Buckner funnel lined with filter paper. A second time filtration was done to obtain a clean filtrate, which was stored in the fridge at 40C for nanoparticle synthesis. Three separate conical flasks, each containing 20 mL of the 0.1 M metal ion solutions of AgNO3, ZnCl, and CuSO4, were prepared. 20 mL of the plant extract was added separately to each flask. The mixture was stirred for 15 minutes at 70 °C on a hot plate, and the color shift indicating the reduction of metallic ions to nanoparticles was observed. The concept of nanomedicines promotes the development of personalized treatments with the potential to revolutionize medical practice compared to conventional and molecular medicine. Regulatory policies are being implemented to reduce the toxicity of nanomaterials.

Characterizations of the Synthesized Silver, Zinc, and Copper Nanoparticles

A double-beam spectrophotometer (Shimazdu, model UV-1800, Kyoto, Japan) was used to record UV-visible spectra with a resolution of 1 nm between 200 and 800 nm against a distilled water blank. The biosynthesis of silver (AgNPs), zinc (ZnNPs), and copper nanoparticles (CuNPs) was tracked by measuring the UV-visible spectra of the reaction mixtures.

The organic functional groups present in the leaf extract and metallic nanoparticles (AgNPs, ZNNPs, and CuNPs) were analyzed using Fourier transform infrared (FTIR) spectroscopy. FTIR measurements were performed in diffuse reflectance mode using KBr pellets at a resolution of 4 $cm-1$ with an instrument from Shimazdu, Kyoto, Japan.

Anti-Microbial Analyses of Aqueous Extract and Metallic Nanoparticles of the Leaf of C. Citratus

Antibacterial and antifungal analyses of the aqueous extract and metallic nanoparticles of C. citratus were conducted using the disk diffusion plate method. Nutrient agar and potato dextrose agar were prepared, poured into sterile plates, and allowed to gel. Cultures of bacteria and fungi were inoculated into the respective agars. Then, 8 mm wells were made in each plate, and the pre-diffused disks of the synthesized samples were introduced into the wells.

The plate was incubated at 37 0C for bacteria and at 25 0C for fungi. The plates were examined a day after they were introduced. The zones of inhibition, measured in millimeters, depicts the anti-microbial power of the extract and nanoparticles against the organisms.

Results

The results of the proximate compositions, displayed in table 1, indicate high levels of ash, protein and fiber contents, along with significant amounts of nutritionally valuable minerals and vitamin C. The lemongrass leaf also contains relatively low levels of antinutrients but high tannin contents.

Table 2 showed the qualitative phytochemical screening results of the aqueous and ethanol extracts of lemon grass leaves, indicating the presence of saponin, flavonoids, alkaloids, and glycosides. However, steroids were not detected in both solutions. Additionally, phenolic compounds and tannins were also not present in the ethanol extract of the lemongrass leaves.

Table 3 presents the quantitative results of antioxidant and phytochemical compounds, as well as the radical scavenging abilities of the aqueous extract of lemongrass leaves.

Table 4 shows the antimicrobial potentials of the aqueous extract of lemongrass and its metallic nanoparticles against various bacteria species, showed that both plants extract and the nanoparticles possessed antibacterial activity.

Figure 1 displays the wavelength scanning graph of the aqueous extract of lemongrass (*C. citratus*) leaves and its metallic nanoparticle. Figure 2 showed the FTIR spectrum of the aqueous extract of lemongrass (*C. citratus*) leaves.

Table 1: Proximate, minerals, vitamin c, and antinutrient compositions of lemongrass (*Cymbopogon citratus*) leaf

Parameters	Results
Proximate composition (mg/g)	
Moisture content	6.66±0.02
Ash	11.73±0.05
Crude Fat	7.34±0.01
Crude Protein	12.20±0.05
Crude Fiber	13.84±0.15
Mineral Composition (mg/g)	
Potassium	6.18
Sodium	4.21
Calcium	8.00
Magnesium	1.45
Phosphorus	34.00
Vitamin C (mg/g)	
Lemon grass	20.05±1.01
Antinutrient (mg/g)	
Oxalate	1.08±0.10
Tannin	46.45±0.03
Phylate	0.12±0.001
Saponin	2.00±0.10
Cyanide	4.05±0.15

Table 2. Phytochemical screening of lemon grass (*Cymbopogon citratus*) leaf aqueous and ethanolic extracts

Samples	Saponin	Phenolics	Flavanoids	Alkanoids	Taninns	Steroids	Glycosides
Aqueous extract	++	+	+	+	+	-	+
Ethanol extract	+	-	+	++	-	-	+

Table 3. Antioxidants and some important phytochemical compounds of aqueous extract of lemongrass (*Cymbopogon citratus*) leaf

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Parameters	Results
Antioxidant compounds content (mg/g)	
Phenolic compounds	12.26±0.06
Flavonoids	0.56±0.01
Antioxidants properties	
FRAP (mg/g)	1.39±0.04
Fe2+ Chelating (%)	62.14±1.90
Antioxidants radical scavenging ability (%)	
TBARS	33.51±0.11
Nitric Oxide (NO)	49.19±2.15
Hydroxyl (OH)	87.50±1.73
DPPH radical	3.36±1.39
Phytochemical compounds (mg/g)	
Saponin	76.09±3.18
Tannin	0.35±0.01
Alkaloids	ND
Terpenoids	17.95±0.04
Steroids	7.58±0.02
Glycosides	23.04±0.05

Table 4. Anti-microbial potentials of aqueous extract of lemongrass (*Cymbopogon citratus*) leaf and its metallic nanoparticles

Samples	Concentration (mg/mL)	*Escherichia coli*	*Staphylococcus typhi*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Extracts	100	1.9	2.1	-	1.0
	50	1.2	1.6	-	1.0
AgNP	100	3.4	3.8	1.6	4.3
	50	2.9	3.1	1.2	3.8
CuNP	100	3.0	3.3	2.8	3.5
	50	2.4	3.0	2.6	3.0
ZnNP	100	3.1	5.2	3.7	2.9
	50	2.6	4.5	3.2	2.7

Zone diameter (mm); AgNP: Silver Nanoparticle; CuNP: Copper Nanoparticle; ZnNP: Zinc nanoparticle.

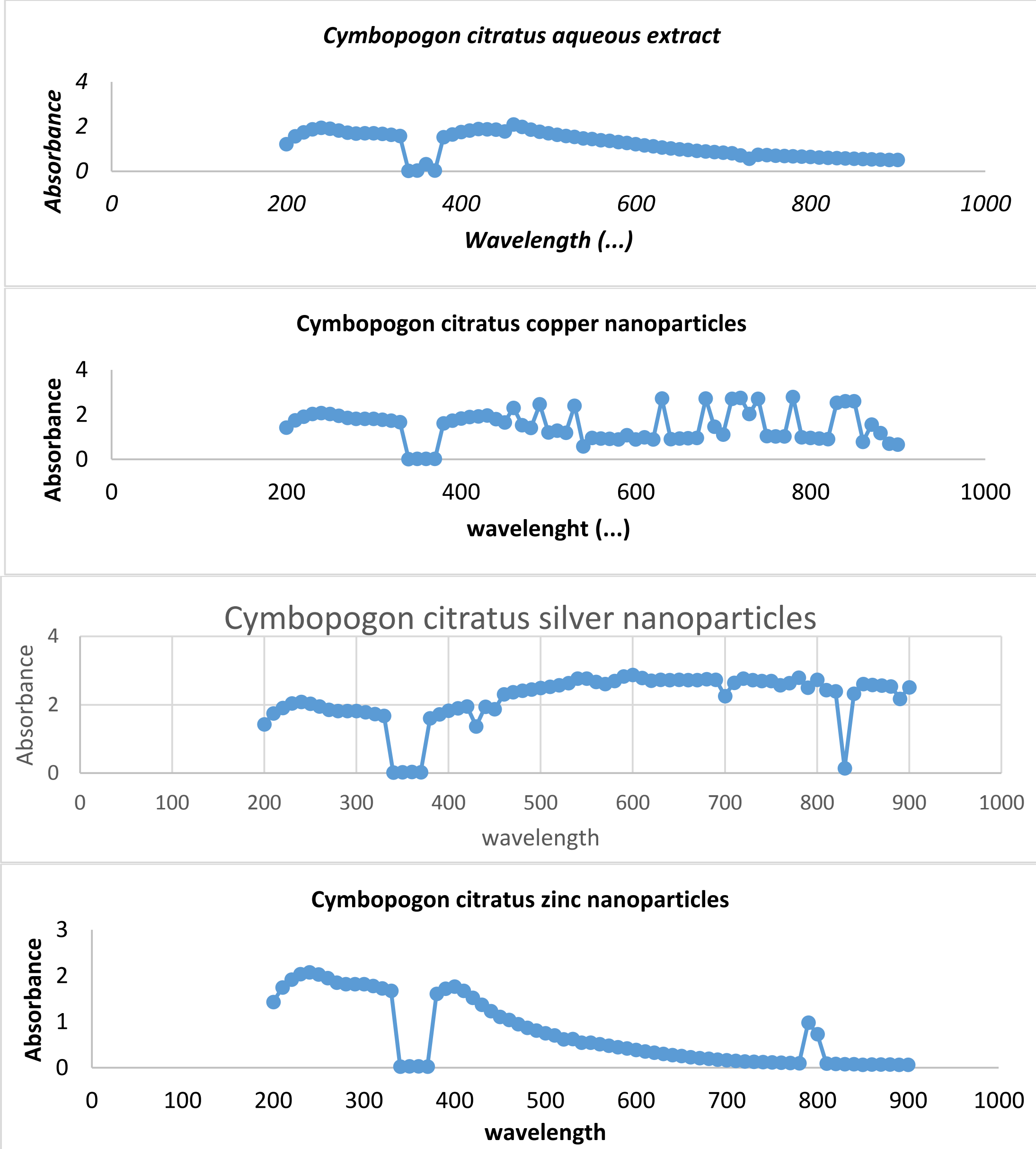

Figure 1. Wavelength scanning of lemongrass (*Cymbopogon citratus*) leaf aqueous extract and its metallic nanoparticles

Figure 3 shows the FTIR spectrum of synthesized silver nanoparticles of lemongrass (*C. citratus*) leaves.

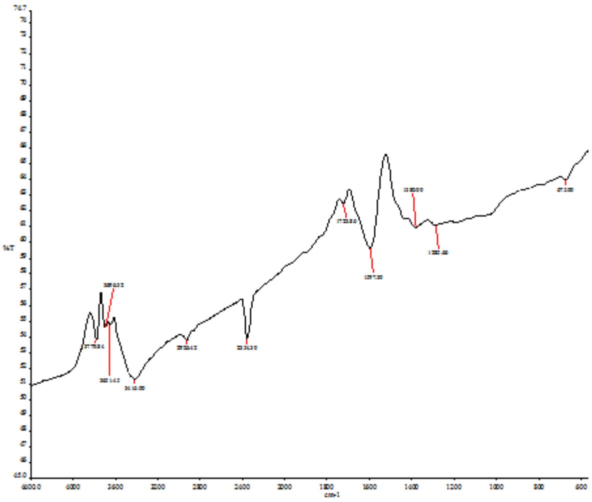

Figure 2. FTIR spectrum of aqueous extract of lemongrass (*Cymbopogon citratus*) leaf

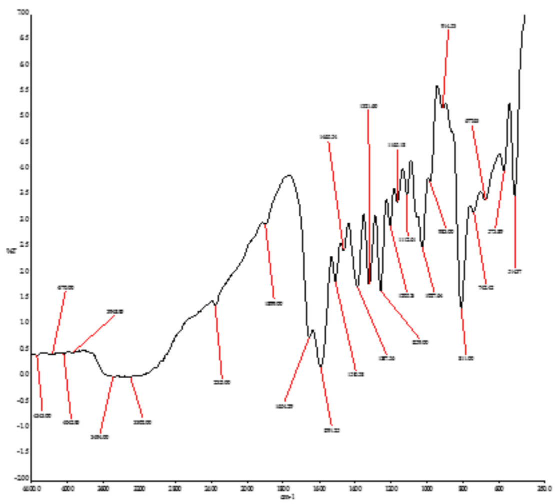

Figure 3. FTIR spectrum of silver nanoparticle of lemongrass (*Cymbopogon citratus*) leaf aqueous extract

Figure 4 presents the FTIR spectrum of synthesized zinc nanoparticles from lemon grass (*C. citratus*) leaves. Figure 5. displayed the FTIR spectrum of synthesized copper nanoparticles from lemon grass (*C. citratus*) leaves.

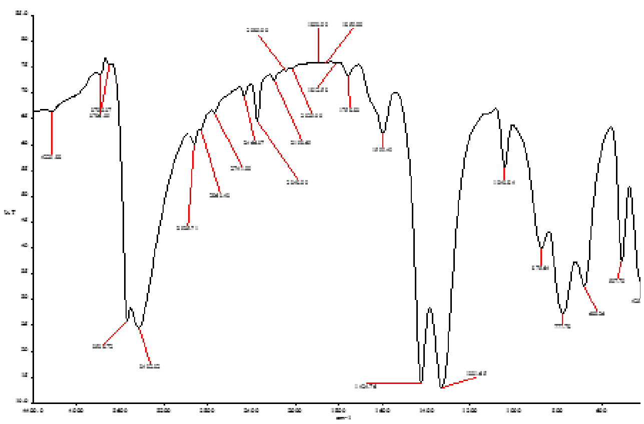

Figure 4. FTIR spectrum of Zinc nanoparticle of lemongrass (*Cymbopogon citratus*) leaf aqueous extract

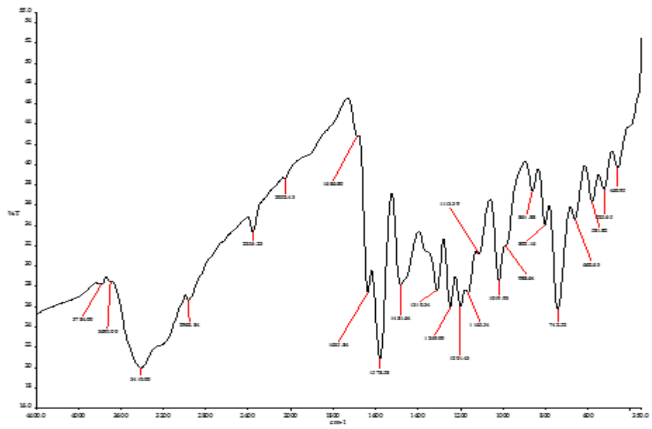

Figure 5. FTIR spectrum of copper nanoparticle of lemongrass (*Cymbopogon citratus*) leaf aqueous extract

Discussion

The continuous production of free radicals through enzymatic and non-enzymatic reactions is a normal physiological process. However, excessive free radical production can lead to oxidative and nitrative stress, which has been implicated in the development of numerous diseases, including cancer (Dreher & Junod, 1996). Highlighted, oxidative DNA damage is a frequent event in normal human cells and plays a critical role in mutagenesis and tumorigenesis. The lesions induced by oxygen free radicals (OFR) that do not result in immediate cell death can instead drive the development of cancer by stimulating the expansion of tumor clones and the acquisition of malignant properties (Klaunig et al., 2009). Furthermore, chronic inflammation, often associated with oxidative stress, fosters a micro environment conducive to cancer by producing additional ROS and reactive nitrogen species (RNS), perpetuating a cycle of damage and mutation (Hussain et al., 2003). Cancer remains a significant global health challenge, with 18.1 million new cases reported in 2018, expected to rise to 23.6 million annually by 2030 (Bray et al., 2018). Traditional treatment modalities, including surgery, radiation, and systemic chemotherapy, face limitations due to cancer recurrence, drug resistance, and severe side effects on non-target tissues (Nussbaumer et al., 2011). This necessitates the exploration of alternative therapies that offer better efficacy and reduced toxicity. Phytochemicals, bioactive compounds derived from plants, offer promising alternatives to cancer treatment. They have shown the potential to improve treatment efficacy and reduce adverse reactions associated with conventional chemotherapy. Numerous studies have demonstrated the antitumor potential of various phytochemicals, which have been evaluated at preclinical and clinical levels and are even available on the market.

Lemongrass (*C. citratus*) is a notable example, with studies showing its significant anticancer properties. In a work done by (Alwaili, 2022), it was found that lemongrass extract induces mitochondrial fission and apoptosis in SW1417 colon cancer cells. Similarly, Rahimi et al. (2023) demonstrated the efficacy of lemongrass essential oil (LEO) encapsulated in nanoliposomes against breast cancer cells, showing enhanced cytotoxicity and apoptosis compared to free LEO.

The redox-sensitive mechanisms of phytochemicals play a crucial role in inhibiting cancer cell proliferation. Loo (2003) discussed how cancer cells require a certain level of oxidative stress to maintain proliferation and survival, primarily through the generation of H_2O_2. Phytochemicals can scavenge excess H_2O_2, suppressing oxidative stress-responsive genes and inhibiting cancer cell growth. Conversely, some phytochemicals can induce oxidative stress to intolerable levels in cancer cells, leading to DNA damage and apoptosis. This dual mechanism underscores the versatility of phytochemicals in cancer therapy.

Lemongrass is rich in various nutrients and phytochemicals, contributing to its medicinal properties. Findings from this study on the proximate composition reveal significant amounts of crude protein (12.20 mg/g), crude fiber (13.84 mg/g), and crude fat (7.34 mg/g), along with essential minerals such as potassium (6.18 mg/g), sodium (4.21 mg/g), and calcium (8.00 mg/g). It also contains vitamin C (20 mg/g), which is known for its antioxidant properties. The phytochemical screening of lemongrass extracts shows the presence of saponins, phenolic compounds, flavonoids, alkaloids, tannins, steroids, and glycosides. These compounds are crucial for the plant's anti-cancer and anti-microbial activities. Notably, the aqueous extract of lemongrass is particularly rich in saponins and phenolics, while the ethanolic extract has a higher concentration of alkaloids and glycosides.

Lemongrass exhibits significant antioxidant activity, with high levels of phenol compounds (12.26 mg/g) and saponins (76.09 mg/g). It also shows substantial percentage radical scavenging abilities, particularly for hydroxyl (OH) radicals (87.50%) and nitric oxide (NO) radicals (49.19%). These nutritional components contribute to the overall health benefits of lemongrass.

The high phenolic and flavonoid content of lemongrass is primarily responsible for its antioxidant activity. Phenolic compounds act as hydrogen donors, neutralizing free radicals and thus mitigating oxidative stress (Rice-Evans et al., 1997). Flavonoids further enhance antioxidant capacity through metal chelation and free radical scavenging (Pietta, 2000). The $Fe2+$ chellating activity and TBARS scavenging ability of lemongrass underscore its potential in preventing lipid peroxidation, a process intimately linked to cancer progression. These antioxidant properties contribute to its anti-cancer effects by reducing oxidative stress in cancer cells.

In terms of anti-microbial potential, lemongrass aqueous extract and its nanoparticles (AgNP, CuNP, ZnNP) demonstrate effective inhibition against various bacterial strains, including E. coli, S. typhi, S. aureus, and P. aeruginosa. The nanoparticles, particularly ZnNP, exhibit higher anti-microbial activity compared to the extract alone, indicating the enhanced efficacy of nanoparticle formulations. The analysis of C. citratus extract and nanoparticles (AgNP, CuNP, ZnNP) further elucidates their chemical characteristics and biological activities.

Figure 1.0 illustrates the wavelength scanning of C. citratus leaf extract and its metal nanoparticles. The results show distinct absorption peaks corresponding to different components and nanoparticles, indicating the presence and interaction of bioactive compounds in these formulations. The FTIR spectrum of the extract of C. citratus leaf (Figure 2.0) shows characteristic peaks indicating the presence of functional groups such as hydroxyl, carbonyl, and aromatic compounds. These peaks correspond to bioactive compounds like flavonoids and phenolics, which contribute to the extract's antioxidant and anticancer properties. The FTIR spectrum of silver nanoparticles (Figure 3.0) synthesized from C. citratus leaf reveals distinct peaks indicating the successful capping and stabilization of AgNPs by the plant extract. The presence of AgNPs is confirmed by the characteristic Ag-O stretching vibrations. The FTIR spectrum of zinc nanoparticles (Figure 4.4) indicates similar stabilization by the plant extract, with peaks corresponding to Zn-O interactions. These nanoparticles exhibit enhanced anti-microbial activity, as evidenced by their significant inhibition zones against various bacterial strains. The FTIR spectrum of copper nanoparticles (Figure 4.0) shows characteristic peaks for Cu-O bonds, indicating successful nanoparticle formation. CuNPs also demonstrate notable anti-microbial properties, contributing to their potential therapeutic applications.

It is evident that lemongrass has a multifaceted role in cancer therapy due to its rich phytochemical composition, antioxidant properties, and anti-microbial potential. The dual mechanism of inducing and scavenging oxidative stress in cancer cells, as discussed by Loo (2003), provides a strong basis for its anticancer efficacy. This aligns with the ongoing search for more effective and less toxic cancer treatments, as conventional therapies continue to face significant challenges.

Despite numerous evidences of anticancer properties of lemongrass from laboratory studies, the clinical application remains underexplored. Further works on nanoparticle formulations focusing on extensive preclinical and clinical trials is essential to evaluate the efficacy and safety of lemongrass extract encapsulated in nanocarriers, such as copper (CuNP), silver (AgNP), and zinc (ZnNP) nanoparticles, for cancer therapy. Optimization of dosages is crucial to maximize therapeutic effects while minimizing toxicity. The enhanced anti-microbial activity exhibited by these nanoparticle formulations, particularly ZnNP, suggests their potential to address secondary infections that often complicate cancer treatment. Further study is needed to elucidate the specific mechanisms by which these nanoparticles exert their anti-microbial and anticancer effects, enabling the development of more targeted and effective therapeutic strategies. The enhanced anti-microbial activity exhibited by these nanoparticle formulations, particularly ZnNP, suggests their potential to address secondary infections that often complicate cancer treatment. The findings of this study demonstrate that lemongrass (C. citratus) possesses significant potential as an alternative and complementary therapy for cancer treatment due to its rich phytochemical composition, antioxidant properties, and ability to induce apoptosis in cancer cells. The proximate composition analysis revealed notable levels of crude protein (12.20 mg/g), crude fiber (13.84 mg/g), and vitamin C (20 mg/g), which contribute to its therapeutic potential. Nanoparticle formulations, including CuNP, AgNP, and ZnNP, were found to enhance the anti-microbial and anticancer activities of lemongrass extract. Notably, the antibacterial activity of these nanoparticles against pathogens such as E. coli and S. typhi was significantly higher compared to the extract alone. The FTIR spectra confirmed the successful incorporation of lemongrass extract into the nanoparticles, highlighting the potential of these formulations in targeted drug delivery systems. The extract of lemongrass, as well as its nanoparticle formulations, showed promising antioxidant properties, including high hydroxyl radical scavenging activity (87.50%) and nitric oxide radical inhibition (49.19%).

Conclusion

The findings of this study and numerous laboratory studies underscore the versatility and potential of plant-derived compounds in cancer therapy, meanwhile it is needed that clinical trials to evaluate the dosage, safety, and effectiveness of lemongrass extracts in cancer patients be evaluated, this would in turn give way for lemongrass to be incorporated into standard treatment protocols. By integrating lemongrass-based treatments into conventional cancer therapy, some current limitations, such as drug resistance and toxicity, can be addressed. The exploration of synergistic effects and enhanced bioavailability through advanced delivery systems could significantly improve the efficacy and safety of cancer treatments, ultimately leading to better patient outcomes and quality of life.

Declarations

Conflict of interest

There is no conflict of interest among the authors.

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Consent for publications

All authors have read and approved the manuscript for publication.

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None.

Authors' contributions

OOA Came up with the research idea and designed the work. OA carried out the experiment, OOA and OA wrote the first draft of the manuscript, (OA) carried out the literature search, (OMO) carried out the statistical analysis, and (OOA) supervised the study. All authors read and approved the final manuscript for publication.

Ethical Considerations

Ethical issues (including plagiarism, data fabrication, double publication and submission, redundancy) have been completely looked into by the author.

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