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Phytochemical Analysis and Antibacterial Activities of *Cymbopogon citratus* from Banke, Nepal

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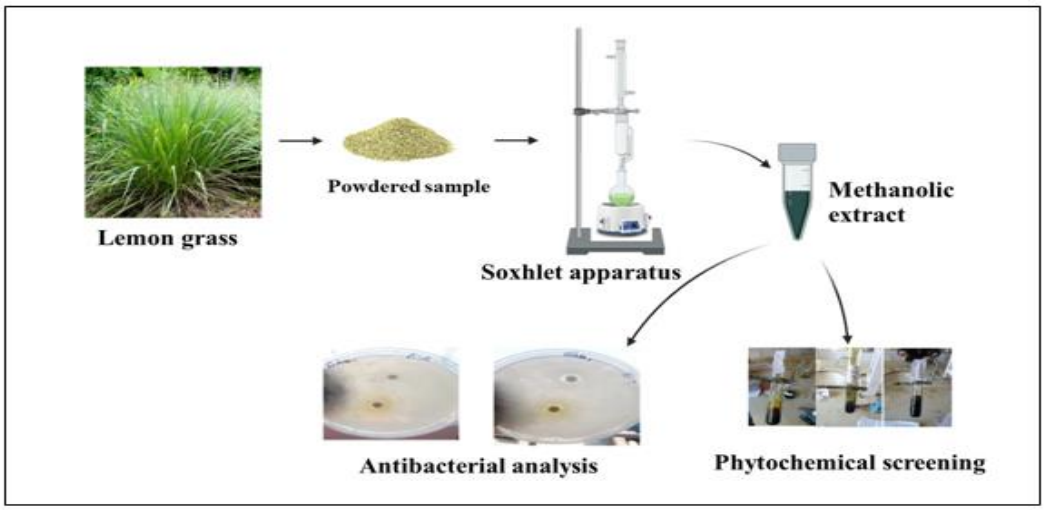
Abstract

Plant extracts are being used in the development of antibacterial agents because they offer promising sources of new and effective antibacterial compounds. This study aimed to investigate the phytochemical composition and antibacterial properties of the methanolic extract of Cymbopogon citratus, commonly known as "Kagate Ghas" (कागते घाँस). Freshly harvested and shade-dried leaves of Cymbopogon citratus were subjected to methanolic extraction using a Soxhlet apparatus. The extract was tested for its natural chemical compounds and was found to contain alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, proteins, and carbohydrates. Interestingly, steroids were not detected in the analysis. The disc diffusion method was used for the assessment of antibacterial activity against gram-positive Bacillus subtilis (ATCC 6051) and the gram-negative Enterobacter aerogenes (ATCC 29007), with a zone of inhibition (ZOI) measuring 12 mm against B. subtilis and 7 mm against E. aerogenes. This indicates methanolic extract of C. citratus is more effective against gram-positive than gram-negative bacteria.

Keywords: antibacterial activity, *Cymbopogon citratus,* phytochemical analysis, zone of inhibition

Figure 1 *Scheme 1: Graphical Representation of Methanolic Extraction of C. citratus and its Antibacterial Study*



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Introduction

People have been relying on medicinal plants for centuries as a natural source of healing and treatment, making them an important part of traditional medicine throughout history. According to research, around 3.3 billion people in less developed nations regularly use medicinal herbs, making these plants the foundation of traditional medicine (Awuchi, 2019). Local customs and beliefs continue to serve as the foundation for healthcare in several emerging nations. The use of plant-based medicine in human healthcare is essential. Natural products and their derivatives account for more than 50% of contributions in the clinical field (Gurib-Fakim, 2006). The traditional medicine system is widely popular for treating various diseases due to its easy accessibility, low preparation cost, and minimal undesirable side effects, making it the only affordable healthcare option for the poorest patients (Swargiary, 2017). Plants can synthesize the different organic compounds, which may be primary or secondary metabolites. These primary or secondary metabolites produced by living organisms in nature are called natural products (Herbert, 2012). Plant-derived drug discovery has global importance as the necessity for the development of new effective therapeutic agents is increasing because of the rise in drug-resistant bacteria. The secondary metabolites produced by plants have a role in defense against pathogens. Herbs have medicinal properties due to the presence of different active principles like alkaloids, volatile essential oils, glycoside, resins, oleoresins, steroids, tannins, terpenoids, flavonoids, and phenolics that have toxicological, pharmacological, and ecological importance (Hussein et al., 2018). A large number of phytochemicals, such as taxol, vinblastine, aspirin, quinine, and hypericin, which are used in modern medicine, are plant-derived (Daniel, 2006). Among the bioactive secondary metabolites, phenolics are a large group of natural compounds that include simple phenol, phenolic acid, flavonoids, tannins, coumarins, ligans, xanthones, and stilbenes (Balasundram et al., 2006). *C. citratus* (lemongrass), locally known as "Kagate Ghas" (कागते घाँस) is widely used for its flavor in tea in Nepal.

This *C. citratus* is also known as West Indian lemongrass or simply lemongrass (*USDA Plants Database*) and belongs to the Poaceae family (Vyshali et al., 2016). It is considered a medicinal plant valued for its rich array of bioactive compounds, which has led to its increasing interest in natural product research (Acimovic et al., 2020). The plant's characteristic lemon-like odor is primarily due to the presence of citral, a cyclic monoterpene (Ahire et al., 2022); therefore, the name is Lemongrass. This plant has been extensively used in Ayurvedic medicine, with freshly cut and partially dried leaves serving as the source of its essential oil (Manvitha & Bidya, 2014). Several studies have demonstrated the plant's diverse pharmacological activities, anti-bacterial, anti-diarrheal, anti-filarial, anti-fungal, and anti-inflammatory properties (Telangi et al., 2022). Some research has also explored its potential anti-malarial (Chukwuocha et al., 2016), antioxidant

(Manvitha & Bidya, 2014), hypoglycemic, and neurobehavioral effects (Ahire et al., 2022). In addition, *C. citratus* has shown promise in treating various conditions, including diarrhea, inflammation, and diabetes. The essential oil from *C. citratus* is widely used in the food, cosmetic, and pharmaceutical industries due to its pleasant aroma and biological activities (Oladeji et al., 2019). The phytochemicals, flavonoids, tannins, terpenoids, and essential oils like citral are linked to a range of biological activities, notably antibacterial effects (Kigigha et al., 2018).

In this work, the phytochemical screening of methanolic extract and its antibacterial activity of lemongrass from Banke, Nepal, are conducted. The antibacterial activity was conducted against American Type Culture Collection (ATCC) in both gram-positive bacteria *(B. subtilis* ATCC 6051) and gram-negative bacteria (*E. aerogenes* ATCC 29007). The results support the promising potential of *C. citratus* as a natural source for developing alternative antibacterial agents and advancing plant-based therapeutic applications.

Geographical Distribution and Taxonomy of *C. citratus*

C. citratus is a plant of the tropics and subtropics, where it is found at elevations up to 1400 meters. The plant is native to Southeast Asia and widely distributed across tropical regions (Magotra et al., 2021; Oladeji et al., 2019). Lemongrass is cultivated in several regions of Nepal, especially in tropical and subtropical climates. In Nepal, *C. citratus* is cultivated for its essential oil, which is extracted from freshly cut and slightly dried leaves (Pokhrel & Yadav, 2018). The terai plains and parts of the mid-hills offer favorable conditions for its growth, characterized by warm temperatures and moderate rainfall. Key areas where lēmongrass is commonly grown include Banke, Kailali, Dang, Chitwan, Makwanpur, and Sunsari, Nepal.

Table 1 *Taxonomical Arrangement of C. citratus*

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliposida
Order	Poales
Family	Poaceae
Subfamily	Panicoideae
Genus	*Cymbopogon*
Species	*Citratus*

(Karunamoorthi et al., 2010)

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Materials and Method

Chemicals

All the necessary organic solvents (Mayer's reagent, Wagner reagent, Dragendorff's reagent, Molisch reagent, Fehling's reagent, Benedict's reagent) and chemicals such as methanol, sodium hydroxide, chloroform, glacial acetic acid, hydrochloric acid, concentrated sulphuric acid, distilled water (AR grade) manufactured by Himedia Laboratories Pvt. Ltd., India, supplied from local suppliers in Kathmandu and were used without further purification.

Collection and Preliminary Treatment of Samples

The collection of leaves of *C. citratus* were done from Raptishonari-02, Banke, Nepal, during the month of September. The collected sample was well washed with distilled water and shade-dried for two weeks until it became completely dry. The dried sample was finally crushed into fine powder using a grinder and processed in further steps.

Preparation of Plant Extract

40g of powdered sample was weighed and placed in the thimble of a Soxhlet apparatus, and 200 mL of methanol was used for extraction. The process continued for 3-4 hours until a clear solvent was observed. The crude extract was concentrated using a water bath maintained at 40-50°C. Initially, the heating mantle was set at 50°C until the methanol boiled, then reduced to 40°C. The concentrated extract was stored in a conical flask, sealed with aluminum foil, and subjected to phytochemical screening.

Phytochemical Screening

Detailed phytochemical examinations were carried out for the methanolic extracts as per the standard methods of (Tiwari et al., 2011).

Alkaloid Test

Wagner's Test: Approximately 10 mg of the plant extract was placed in a test tube, followed by the addition of a few drops of Wagner's reagent. The appearance of a reddish-brown precipitate was taken as a positive indication of alkaloids.

Flavonoid Test

i) Shinoda test: A 10 mg sample of the extract was combined with magnesium turnings and 1-2 drops of concentrated hydrochloric acid. The development of a pink color suggests the presence of flavonoids.

ii) Lead Acetate test: A 10 mg sample of the extract was treated with a few

drops of 10% lead acetate solution. The appearance of a yellow precipitate signifies the presence of flavonoids.

Phenols Test

Lead acetate test: 10 mg of extract was mixed with 0.5 mL of 1% lead acetate solution. The appearance of a white precipitate suggests the presence of phenolic compounds.

Test for Tannins

Ferric chloride test: 5 mg of extract was mixed with 0.5 mL of 5% ferric chloride. The development of a dark bluish-black precipitate indicates the presence of tannins.

Test for Steroids and Sterols (Salkowski's Test)

5 mg of extract, 2 mL of chloroform, and conc. H_2SO_4 were added in the test tube. A red-colored upper layer and yellow with green fluorescence in the lower layer indicate the presence of steroids and sterols.

Carbohydrates Tests

i) Fehling's test: To 0.5 mg of extract, 5 ml of Fehling's solution was added and the mixture was heated in a water bath. The development of yellow or red precipitate indicates the presence of reducing sugar.

ii) Benedict's test: A 5 mL of Benedict's reagent was combined with 0.5 mg of extract, then heated in water bath. The formation of precipitate with colors ranging from red to yellow or green suggests the presence of reducing sugar.

Saponins Test

Foam test: 0.5 mg of extract was mixed with 20 mL of distilled water, then vigorously shaken in a graduated cylinder for 15 minutes. The presence of saponins was indicated by the formation of foam layer reaching up to 1 cm in height.

Glycosides Test

A 0.5 mg sample of the extract was dissolved in 1 mL of water, followed by the aqueous NaOH solution was. The appearance of a yellow color suggests the presence of glycosides.

Test for Protein & Amino Acids

i) Biuret test: 0.5 mg of the extract was combined with an equal volume of 40% sodium hydroxide solution and two drops of 1% copper sulphate solution in a test tube. The development of a violet color indicates the presence of proteins.

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ii) Ninhydrin test: 0.5 mg of the extract was mixed with 2 drops of freshly prepared 0.2% ninhydrin solution in a test tube and heated. The formation of a pink or purple color indicates the presence of proteins, peptides, or amino acids.

Antibacterial Assay of *C. citratus Extract*

The antibacterial activity of the methanolic extract of *C. citratus* was evaluated against two bacterial strains, the gram-positive *B. subtilis* and the gram-negative *E. cymaerogenes*. All the strains of bacteria were cultured in Nutrient Broth (NB) and incubated for 24 hours. Sterile Mueller-Hinton agar (MHA) plates were prepared with a uniform thickness of 4 mm. Respective inoculums of organisms of selected bacterial strains were carpet cultured using a sterile cotton swab uniformly. The plates were first left undisturbed for 15 minutes at room temperature inside a laminar flow hood to allow initial diffusion. After that, they were further kept at room temperature for approximately 60 minutes before being incubated at 37°C for 24 hours. This antibacterial test was conducted by the disc diffusion method (Balouiri et al., 2016). 10 µL extract sample was pipetted and dispensed on a 6 mm filter paper disc kept on the MHA plate. It was incubated overnight at 37°C, and the next day, the antibacterial activities were recorded by measuring the ZOI.

Results and Discussion

Phytochemical Screening

Preliminary Phytochemical analysis of methanolic extract of *C. citratus* revealed the presence of various primary and secondary metabolites, including alkaloids, flavonoids, glycosides, tannins, saponins, carbohydrates, and proteins. However, steroids were found to be absent in the extract. A summary of the screening results is presented in Table 2.

Each of the phytochemicals is characterized by various protective and therapeutic effects; i.e., secondary metabolites played a vital role in the antibacterial, anti-diabetic, and antioxidant process (Tang et al., 2024). Likewise, primary metabolites such as carbohydrates, lipids, proteins, amino acids, etc., were important for various life processes such as growth, reproduction, and development. The selective presence of phenolic groups such as tannins and flavonoids indicated having antioxidant properties (Banjarnahor & Artanti, 2014). Lemongrass extract can also be used as an insecticide against Aedes aegypti (Aditama & Sitepu, 2019).

Table 2 *Phytochemical Screening of Methanol Leaf Extract of C. citratus*

S.N.	Phytochemicals	Test assay	Observation	Result of test
1	Alkaloids	Wagner's test	Reddish brown colored precipitate	+
2	Flavonoids	a. Shinoda test	Pink colored	+
			Yellow colored precipitate	+
		b. Lead acetate test		
3	Glycosides		Yellow color	+
4	Tannins	Ferric chloride test	Dark bluish-black colored precipitate	+
5	Saponins	Foam test	Foam	+
6	Carbohydrates	Fehling's test	Reddish colored precipitate	+
		Benedict's test	Green colored precipitate	+
7	Phenols	Lead acetate test	White colored precipitate	+
8	Steroids	Salkowski's test	Reddish yellow colored precipitate	-
9	Proteins	Biuret test	Violet color	+
		Ninhydrin test	Pink color	+

Evaluation of Antibacterial Activity of *C. citratus Extract*

The antibacterial activity of the methanolic extract of *C. citratus* was assessed using the disc diffusion method against two bacterial strains: the gram-positive *B. subtilis* and the gram-negative *E. aerogenes.*

Table 3 *ZOI of C. citratus Methanolic Extract*

S.N.	Bacteria	ZOI (mm)
1.	*B. subtilis* (gram-positive)	12
2.	*E. aerogenes* (gram-negative)	7

Table 3 presents the observed antibacterial activity of the methanolic extract of *C. citratus* against both gram-positive (*B. subtilis*) and gram-negative (*E. aerogenes*) bacterial strains. A ZOI of 12 mm was measured for *B. subtilis,* and a comparatively smaller ZOI of 7 mm for *E. aerogenes* was recorded. Methanol was used as the control, and it showed no antibacterial activity against the bacteria,

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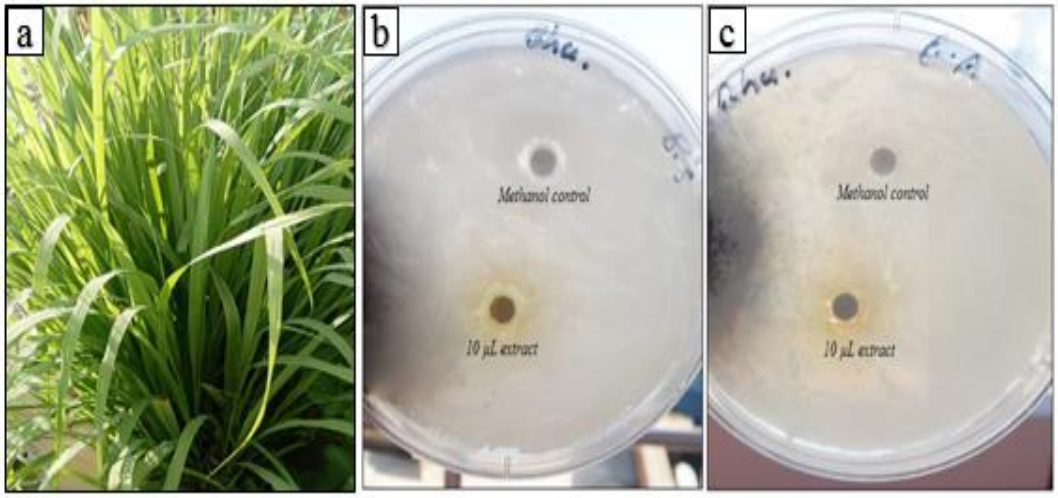
which is shown in Figure 2. The result obtained from the antibacterial analysis in other similar works shows similar results of higher susceptibility against gram-positive bacteria than gram-negative bacteria (Balakrishnan et al., 2014). The variation in antibacterial activity can be attributed to the structural and compositional differences between the cell membranes of gram-positive and gram-negative bacteria. The Gram-negative bacteria have a thinner peptidoglycan layer but possess an additional outer membrane composed of lipopolysaccharides (Huang et al., 2008). This outer membrane functions as a barrier, restricting the penetration of antibacterial compounds, phenolic compounds, and flavonoids from the methanolic extract of lemongrass (Balakrishnan et al., 2014). In contrast, the Gram-positive bacteria have a thick peptidoglycan layer that is relatively porous and lacks an outer membrane of lipopolysaccharides (Pasquina-Lemonche et al., 2020), allowing antimicrobial compounds to enter inside the cell, causing the inhibition of bacteria (Balakrishnan et al., 2014). In several studies, methanolic extracts have demonstrated higher antibacterial activities compared to aqueous extracts against various pathogens (Aina et al., 2014). This may be due to the higher polarity, which helps in the extraction of both lipophilic and hydrophilic substances. Besides, the methanol can work easily at room temperature because of its highly volatile nature (Borges et al., 2020).

Additionally, the secondary metabolites in the samples vary based on different climatic factors such as temperature, humidity, rainfall, altitude, and others (Pant et al., 2021; Qaderi et al., 2023). Moreover, qualities of secondary metabolites are also affected by the maturity period of the harvested lemongrass plant (Tajidin, N. E, 2012).

Figure 2 *Antibacterial Susceptibility Test of Methanolic C. citratus Extract: (a) Lemongrass Plant (b) ZOI Observed against B. subtilis (gram-positive) (c) ZOI Observed against E. aerogenes (gram-negative)*



Conclusion

The qualitative phytochemical analysis of methanolic leaf extracts of *C. citratus*, collected from Banke, Nepal, indicated the presence of several bioactive compounds such as alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, carbohydrates, phenols, and proteins. However, steroids were not detected. The antibacterial susceptibility testing revealed substantial activity against both gram-positive and gram-negative bacteria, with a notably greater susceptibility observed in the gram-positive *B. subtilis* (ATCC 6051) compared to the gram-negative *E. aerogenes* (ATCC 29007). This differential susceptibility suggests that the active phytochemicals may disrupt bacterial cell wall integrity or metabolic pathways more effectively in gram-positive strains. This brings out the huge potential for developing natural antibacterial agents from *C. citratus*.

Availability of Data and Materials

The data will be made accessible upon request at any time.

Competing Interests

All the authors have read and approved the manuscript and declare that they do not have any kinds of conflicts of interest.

Authors' Contribution

BBM: Sample collection, wet lab work, data analysis, interpretation, and drafting first manuscript

NR: Sample collection, wet lab work, data analysis, interpretation, and drafting first manuscript

MMS: Sample collection, data analysis, design of illustrations, and manuscript revision

IS: Data interpretation, manuscript editing

BDB: Conceptualization, supervision, manuscript editing

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