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Kewat RR
M.V.Sc. (Scholar) Department of
Veterinary Pharmacology and
Toxicology, College of Veterinary
Sciences & A.H., NDVSU,
Jabalpur, Madhya Pradesh,
India

Sharma RK
Professor & Head, I/C Dean
Department of Veterinary
Pharmacology and Toxicology
College of Veterinary Sciences &
A.H., NDVSU, Jabalpur,
Madhya Pradesh, India

Gautam Vidhi
Assistant Professor, Department
of Veterinary Pharmacology and
Toxicology, College of Veterinary
Sciences & A.H., NDVSU,
Jabalpur, Madhya Pradesh,
India

Shraman K
Assistant Professor, Department
of Veterinary Pharmacology and
Toxicology College of Veterinary
Sciences & A.H., NDVSU,
Jabalpur, Madhya Pradesh,
India

Jogi J
Assistant Professor, Department
of Veterinary Pharmacology and
Toxicology College of Veterinary
Sciences & A.H., NDVSU,
Jabalpur, Madhya Pradesh,
India

Dinodiya N
PhD Scholar, Department of
Veterinary Pharmacology and
Toxicology College of Veterinary
Sciences & A.H., NDVSU,
Jabalpur, Madhya Pradesh,
India

Correspondence
Kewat RR
M.V.Sc. (Scholar) Department of
Veterinary Pharmacology and
Toxicology College of Veterinary
Sciences & A.H., NDVSU,
Jabalpur, Madhya Pradesh,
India

Isolation, morphological identification and *in vitro* antibacterial activity of endophytic bacteria isolated from *Cymbopogon citratus* leaves

Kewat RR, Sharma RK, Gautam Vidhi, Shraman K, Jogi J and Dinodiya N

Abstract

The present study was conducted to isolate and identify endophytic bacteria from leaves of *Cymbopogon citratus* (Lemongrass) and *in vitro* antibacterial activity was observed on Gram positive and Gram negative bacteria. Twenty leaves samples from *Cymbopogon citratus* were taken. The leaves were sterilized and incubated into King's B agar medium and then again sub-cultured into blood agar and then transferred into BHI broth. The morphological and biochemical characteristics of endophytic bacteria isolated from *Cymbopogon citratus* were studied. Antibacterial activity was studied by the disc diffusion method with known antibiotic ciprofloxacin (CIP) as standard. Twenty bacterial isolates from leaves of *Cymbopogon citratus* were isolated. The microscopic examination of endophytic bacteria showed that isolates from leaves of *Cymbopogon citratus* were gram positive rods. The biochemical characterization of endophytic bacterial isolates from *Cymbopogon citratus* showed positive reaction to catalase and oxidase and negative reaction to coagulase test. All the isolates had shown negative reaction to various enzymic activity tests. Endophytic bacteria from leaves of *Cymbopogon citratus* had shown antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus*.

Keywords: Antibacterial, ciprofloxacin, *Cymbopogon citratus*, endophytes

Introduction

Antibiotics encompass a chemically heterogeneous group of organic, low-molecular weight compounds produced by microorganisms that are deleterious to the growth or metabolic activities of other microorganisms (Thomashow *et al.*, 1997) [17]. Antibiotics have very specific mode of action, affecting the vital processes like DNA, RNA, protein and cell wall synthesis. However, the recent upsurge of antibiotic resistant microbes has created havoc. Antibiotic resistance has turned out to be a global problem wherein more and more bacteria are developing resistance to antibiotics mainly conferred by randomly mutated genes (Westh *et al.*, 2004) [18]. The problem of resistance has resulted in increased morbidity, mortality and costs of health care. This has prompted for more research for potent antimicrobial compounds to tackle drug resistant organisms like *Staphylococcus* sp., *Mycobacterium tuberculosis* and *Streptococcus* sp. (Zajicek, 1996) [20].

Endophytic bacteria found ubiquitous in all plant species in the world, reside in the inner tissues of living plants without causing apparent symptoms of infection. An enormous, relatively untapped source of microbial diversity is represented by the endophytes (Tan and Zau, 2001) [16]. They have attracted increasing attention as they are efficient producers of antimicrobial agents and seem to have unique genetic and biological systems that may have applications outside the host plant in which they normally reside. Antibiotics are potent antimicrobial agents with high specificity. However the relentless emergence of antibiotic resistant strains of pathogens, together with the retarded discovery of novel antibiotics has led to the urgent need to find alternative treatments. Thus screening for antimicrobial compounds from bacterial endophytes is a promising way to overcome the increasing threat of drug resistant strains of human and plant pathogen (Whipps *et al.*, 2012) [19].

The objective of the present study was to isolate endophytic bacteria from *Cymbopogon citratus* (lemongrass) leaves, their identification and investigation of their antibacterial activity against three gram positive bacteria viz. *Staphylococcus aureus*, *Streptococcus pyogenes* and *Bacillus cereus* and gram negative bacteria *Escherichia coli*, *Salmonella Typhimurium* and *Klebsiella pneumoniae*.

Materials and methods

Collection of Samples

Fresh leaves of *Cymbopogon citratus* (lemongrass) were procured from Department of Botany, J.N.K.V.V. and N.D.V.S.U campus, Jabalpur. Mature healthy plant leaves were collected from two different places of Jabalpur. Ten samples from each area were taken and processed for further isolation of endophytic bacteria. Samples were immediately brought to laboratory and were used within 24 hrs and finally processed for isolation of endophytic bacteria.

Sterilization of leaves

The sterilization of leaves and isolation of endophytic bacteria from the leaves was done according to Mahajan *et al.* (2014)^[12], with some modifications (Fig. 1, 2 & 3).

Sterility check

To confirm that the surface of leaves were effectively sterilized, 1 ml of the sterile distilled water that was used in final rinse of surface sterilization procedures were plated on to nutrient agar media and incubated at 37 °C for 24 hrs. Bacterial growth was observed after 24 hrs. Also surface sterilized leaves were rolled on nutrient agar plates and incubated at 37 °C for 24 hrs and checked for possible microbial growth.

Preparation and sterilization of media

King's B (KB) media, mueller hinton media, blood agar media and BHI broth were prepared by adding agar into the distilled water. Hot plate was used for the proper mixing of media and autoclaved at 121 °C for 15-20 minutes at 15 lbs.

Inoculation of leaves and isolation of endophytic bacteria

The media was poured into different autoclaved Petri plates and leaves of the plants were embedded in Petri plates. These plates were then incubated at 37 °C for 24 hrs. Characterization of the bacteria was done according to its morphology and by Gram's staining. After that a single colony was transferred into BHI broth and incubated at 37 °C for 24 hours.

Purification of endophytic bacteria

For purification of endophytic bacteria, subculturing was mainly done by streaking a loop full of BHI broth on the fresh pre solidified blood agar plates and then incubated at 37 °C for 24 hrs. After incubation the colony was transferred into BHI broth and then incubated at 37 °C for 24 hrs and purity was checked by Gram's staining and stored for further work.

Antibacterial activity of endophytic bacteria *In vitro* study

Table 1: List of procured culture from Himedia

S. No.	Bacteria	ATCC Catalogue No.
1.	<i>Escherichia coli</i>	25922
2.	<i>Klebsiella pneumonia</i>	700603
3.	<i>Salmonella Typhimurium</i>	13311
4.	<i>Bacillus cereus</i>	11778
5.	<i>Staphylococcus aureus</i>	6538
6.	<i>Streptococcus pyogenes</i>	12386

Preparation of inoculums of known culture

Mc-Farlands standard was used for the determination of concentration of known culture as described by Henric *et al.* (1956)^[6]. 1 ml of known culture containing 3.0×10^9 cfu/ml was used for antibacterial activity of endophytic bacteria.

Preparation of Antibacterial disc

For determination of antibacterial activity of endophytic bacteria preparation of antibacterial disc was done according to Kirubaharan *et al.* (1999)^[10] with slight modifications.

Antibacterial test

The prepared bacterial inoculums were evenly spread on a sterile Mueller Hinton agar plate as per method described by Bauer *et al.* (1969)^[2]. The known antibiotic Ciprofloxacin (CIP) disc was simultaneously placed as a control for antibiotic sensitivity. The dried disc was incubated at 37 °C for 24 hrs. Result was recorded as positive (growth) or negative (no growth) and zone of inhibition of growth exerted by these impregnated discs.

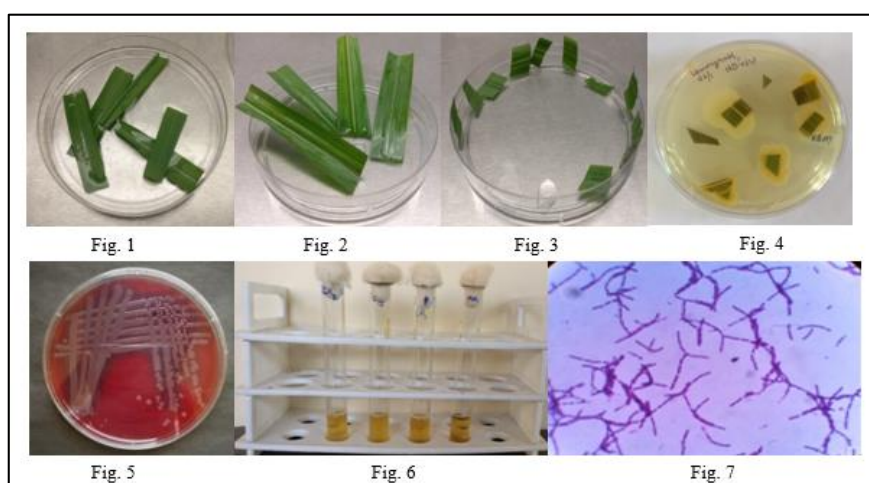


Fig 1: Washing of *Cymbopogon citratus* leaves,

Fig 2: Drying of *Cymbopogon citratus* leaves,

Fig 3: Cut pieces of *Cymbopogon citratus* leaves,

Fig 4: Growth of endophytic bacteria from *Cymbopogon citratus* leaves on King's B media,

Fig 5: Growth of endophytic bacteria from *Cymbopogon citratus* leaves on sheep blood agar,

Fig 6: Growth of endophytic bacteria from *Cymbopogon citratus* leaves in BHI broth,

Fig 7: Gram's staining of endophytic bacteria isolated from *Cymbopogon citratus* leaves

Results and Discussion

Total twenty strains of endophytic bacteria were isolated from leaves of *Cymbopogon citratus*, ten strains from each place viz. Department of Botany, J.N.K.V.V. and N.D.V.S.U. campus were obtained and identified by morphological, biochemical and molecular methods. *In vitro* antibacterial activity was evaluated against six known pathogenic bacteria. Knowledge on the diversity of endophytic bacteria is important for both ecological and biotechnological studies. Endophytic bacteria are found virtually in every plant on earth (Ryan *et al.*, 2008) [13]. The population density of endophytic bacteria vary from 10^2 to 10^9 and depends on many factors, including the plant being studied, the part under analysis, developmental stage of the plant cultivar (genotype) and the interaction with other organisms, as well as other environment related factors (Costa *et al.*, 2012) [4]. Different plant parts such as root, stem and nodule (Hung and Annapurna, 2004), leaves, stems and root (Sobral *et al.*, 2005) [14] can also be used for isolation of endophytic bacteria. Costa *et al.* (2012) [4] had isolated culturable endophytic bacteria from common bean (*Phaseolus vulgaris*) leaves.

Growth characteristics of endophytic bacteria isolated from *Cymbopogon citratus* from Department of Botany, J.N.K.V.V. and N.D.V.S.U. campus on King's B media showed that 75 per cent colonies were circular in shape while 25 per cent were irregular, 60 per cent colonies had flat elevation on petri plate while 40 per cent had raised elevation, margin of 80 per cent colonies were entire while 20 per cent were filamentous, the surface of the growth was smooth for 70 per cent of the colonies while 30 per cent were rough and 65 per cent growth were opaque and white in color while 35 per cent were translucent (Table 02, Fig. 4).

The endophytic bacterial colonies grown on King's B agar were transferred to blood agar plates and incubated at 37 °C for 24 hrs. The growth of endophytic bacteria from leaves of *Cymbopogon citratus* were then observed. All the isolates from leaves of *Cymbopogon citratus* were non-haemolytic in nature (Fig. 5).

Colonies of endophytic bacteria which were grown on blood agar were transferred to the sterile BHI broth tubes and incubated at 37 °C for 24 hrs. The growth of endophytic bacteria from leaves of *Cymbopogon citratus* were observed. Endophytic bacteria from *Cymbopogon citratus* leaves collected from Department of Botany, J.N.K.V.V. and N.D.V.S.U. campus shown characteristics as 40 per cent isolates with surface growth and 100 per cent isolates with turbidity. Sediment formation was seen in 70 per cent isolates and 45 per cent isolates showed odour formation. None of the isolates showed the presence of pigmentation (Table 03, Fig. 6).

The preliminary identification of the endophytic bacterial isolates was done based on various morphological features of isolated endophytic bacteria. The colony characteristics of endophytic bacteria isolated from leaves of *Cymbopogon citratus* were circular in shape, raised elevation on petri plate, entire colony margins, the surface of the growth was smooth, opaque and white in colour. Beiranvand *et al.*, (2017) [3] had identified bacterial isolates from medicinal plants of Iran by their morphology and characteristics of their colonies such as size, shape, colour and surface growth. Colonies with similar morphological features were grouped into the same species.

The microscopic examination of endophytic bacterial isolates had shown that all endophytic bacterial isolates from leaves of *Cymbopogon citratus* were Gram positive rods. The isolation of endophytic bacteria was in agreement with the findings of Baghat *et al.* (2014) [1] who found 90 per cent of Gram positive bacteria. However (Hung and Annapurna, 2004) [7] had found equal percentages of Gram positive 49 per cent and Gram negative 51 per cent bacteria (Table 04, Fig. 7).

The endophytic bacteria isolated from *Cymbopogon citratus* of Department of Botany, J.N.K.V.V. and N.D.V.S.U. campus shown positive reaction to catalase and sugar fermentation test (maltose) and negative reaction to coagulase, VP, ONPG, urease and arginine utilization tests (Table 05).

In the enzymic activity test cellulolytic, amylolytic and esterolytic activity was not observed with endophytic bacteria isolated from *Cymbopogon citratus* (Table 06). This was in agreement with the findings of Soman (2018) [15] who found that endophytic bacteria isolated from different varieties of babool leaves did not show any enzymic activity reaction viz. cellulase, amylase and protease activity test reaction. Khanam and Chandra (2015) [9] conducted a study in which the isolates from the dye yielding plant *Beta vulgaris* did not show any enzymic activity reaction. In contrast, El-Deeb *et al.* (2013) [5] observed that endophytic bacteria isolated from *Plectranthus tenuiflorus* had exhibited extracellular enzymatic activity.

The antibacterial activity of endophytic bacteria was evaluated against various Gram positive and Gram negative pathogenic bacteria namely *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella Typhimurium*. Results were recorded for the formation of zone of inhibition around the disc. The inhibitory zone around the disc indicated absence of bacterial growth reported as sensitive and absence of zone reported as resistant. Isolated endophytic bacteria from *Cymbopogon citratus* shown antibacterial activity as 80 per cent isolates inhibited growth of *Staphylococcus aureus* and 80 per cent isolates inhibited growth of *Bacillus cereus* and none of the isolates inhibited the growth of *Streptococcus pyogenes* (Table 07). Isolated endophytic bacteria from *Cymbopogon citratus* did not shown any antibacterial activity against *Salmonella Typhimurium*, *Klebsiella pneumoniae* and *Escherichia coli* (Table 08).

Most of the isolates from *Cymbopogon citratus* had shown antibacterial activity against Gram positive (*Staphylococcus aureus*) and Gram negative bacteria (*Bacillus cereus*). This suggests that metabolites of endophytic bacteria might have diffused in the culture medium and suppressed the growth of pathogenic bacteria. The bacterial strains secrete different types of natural products to inhibit or kill a wide variety of harmful disease causing agents including, bacteria, fungi, viruses and protozoans that affect humans and animals (Kumar *et al.*, 2016) [11]. The bioactive compound could easily move into the bacterial cell membrane via the general bacterial porins, which might be responsible for several metabolic functions of the cell or they may enter from various pores in the outer cell membrane of bacteria, resulting in the leakage of internal substances to the outside, causing lysis of cell and death (Islam *et al.*, 2018) [8]. The bacterial strain secretes 2,4-diacetylphloroglucinol (DAPG), phycocyanin, siderophores, lytic enzymes, chitinase etc. which degrade the cell wall of pathogens and act as natural biological control.

Table 2: Growth of endophytic bacteria isolated from *Cymbopogon citratus* leaves on King's B media

S. No.	Isolate No.	Form	Elevation	Margin	Surface	Opacity	Chromogenesis
1.	JL-1a	Irregular	Raised	Entire	Smooth	Opaque	Absent
2.	JL-1b	Circular	Raised	Entire	Smooth	Opaque	Absent
3.	JL-1c	Circular	Raised	Entire	Rough	Translucent	Absent
4.	JL-1d	Circular	Flat	Entire	Smooth	Translucent	Absent
5.	JL-1e	Circular	Raised	Entire	Smooth	Opaque	Absent
6.	JL-2a	Circular	Flat	Entire	Smooth	Opaque	Absent
7.	JL-2b	Irregular	Raised	Filamentous	Smooth	Opaque	Absent
8.	JL-2c	Circular	Flat	Entire	Smooth	Translucent	Absent
9.	JL-2d	Irregular	Flat	Filamentous	Rough	Opaque	Absent
10.	JL-2e	Circular	Raised	Entire	Smooth	Opaque	Absent
11.	NL-1a	Circular	Raised	Entire	Rough	Translucent	Absent
12.	NL-1b	Circular	Raised	Entire	Smooth	Opaque	Absent
13.	NL-1c	Circular	Raised	Entire	Rough	Translucent	Absent
14.	NL-1d	Irregular	Raised	Entire	Smooth	Opaque	Absent
15.	NL-1e	Circular	Flat	Entire	Smooth	Opaque	Absent
16.	NL-2a	Irregular	Raised	Entire	Smooth	Opaque	Absent
17.	NL-2b	Circular	Flat	Entire	Smooth	Translucent	Absent
18.	NL-2c	Circular	Raised	Filamentous	Rough	Opaque	Absent
19.	NL-2d	Circular	Flat	Entire	Smooth	Opaque	Absent
20.	NL-2e	Circular	Flat	Filamentous	Rough	Translucent	Absent

Table 3: Growth of endophytic bacteria isolated from *Cymbopogon citratus* leaves in BHI broth

S. No.	Isolate number	Surface growth	Turbidity	Sediment	Odour	Pigmentation
1.	JL-1a	Absent	Present	Present	Absent	Absent
2.	JL-1b	Present	Present	Absent	Present	Absent
3.	JL-1c	Absent	Present	Present	Absent	Absent
4.	JL-1d	Present	Present	Absent	Present	Absent
5.	JL-1e	Present	Present	Present	Absent	Absent
6.	JL-2a	Absent	Present	Present	Absent	Absent
7.	JL-2b	Absent	Present	Absent	Absent	Absent
8.	JL-2c	Absent	Present	Present	Present	Absent
9.	JL-2d	Absent	Present	Present	Present	Absent
10.	JL-2e	Present	Present	Present	Absent	Absent
11.	NL-1a	Present	Present	Absent	Present	Absent
12.	NL-1b	Absent	Present	Present	Absent	Absent
13.	NL-1c	Present	Present	Present	Present	Absent
14.	NL-1d	Present	Present	Absent	Absent	Absent
15.	NL-1e	Absent	Present	Present	Absent	Absent
16.	NL-2a	Absent	Present	Present	Absent	Absent
17.	NL-2b	Absent	Present	Present	Present	Absent
18.	NL-2c	Absent	Present	Present	Present	Absent
19.	NL-2d	Present	Present	Absent	Absent	Absent
20.	NL-2e	Absent	Present	Present	Present	Absent

Table 4: Gram's staining of endophytic bacteria isolated from *Cymbopogon citratus* leaves

S. No.	Isolate number	Gram's staining	Shape	Types of bacteria
1.	JL-1a	Positive	Rod	1
2.	JL-1b	Positive	Rod	1
3.	JL-1c	Positive	Rod	1
4.	JL-1d	Positive	Rod	1
5.	JL-1e	Positive	Rod	1
6.	JL-2a	Positive	Rod	1
7.	JL-2b	Positive	Rod	1
8.	JL-2c	Positive	Rod	1
9.	JL-2d	Positive	Rod	1
10.	JL-2e	Positive	Rod	1
11.	NL-1a	Positive	Rod	1
12.	NL-1b	Positive	Rod	1
13.	NL-1c	Positive	Rod	1
14.	NL-1d	Positive	Rod	1
15.	NL-1e	Positive	Rod	1
16.	NL-2a	Positive	Rod	1
17.	NL-2b	Positive	Rod	1
18.	NL-2c	Positive	Rod	1
19.	NL-2d	Positive	Rod	1
20.	NL-2e	Positive	Rod	1

Table 5: Biochemical tests of endophytic bacteria isolated from *Cymbopogon citratus* leaves

S. No.	Isolate No.	Catalase test	Coagulase test	Oxidase test	V P Test	ONPG Test	Urease test	Arginine utilization test	Sugar fermentation test		
									Sucrose	Maltose	Lactose
1.	JL-1a	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
2.	JL-1b	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
3.	JL-1c	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
4.	JL-1d	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
5.	JL-1e	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
6.	JL-2a	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
7.	JL-2b	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
8.	JL-2c	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
9.	JL-2d	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
10.	JL-2e	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
11.	NL-1a	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
12.	NL-1b	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
13.	NL-1c	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
14.	NL-1d	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
15.	NL-1e	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
16.	NL-2a	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
17.	NL-2b	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
18.	NL-2c	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
19.	NL-2d	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative
20.	NL-2e	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative

Table 6: Enzymic activity test reaction of endophytic bacteria isolated from *Cymbopogon citratus* leaves

S. No.	Isolate No.	Cellulase activity	Amylase activity	Esterase activity
1.	JL-1a	Negative	Negative	Negative
2.	JL-1b	Negative	Negative	Negative
3.	JL-1c	Negative	Negative	Negative
4.	JL-1d	Negative	Negative	Negative
5.	JL-1e	Negative	Negative	Negative
6.	JL-2a	Negative	Negative	Negative
7.	JL-2b	Negative	Negative	Negative
8.	JL-2c	Negative	Negative	Negative
9.	JL-2d	Negative	Negative	Negative
10.	JL-2e	Negative	Negative	Negative
11.	NL-1a	Negative	Negative	Negative
12.	NL-1b	Negative	Negative	Negative
13.	NL-1c	Negative	Negative	Negative
14.	NL-1d	Negative	Negative	Negative
15.	NL-1e	Negative	Negative	Negative
16.	NL-2a	Negative	Negative	Negative
17.	NL-2b	Negative	Negative	Negative
18.	NL-2c	Negative	Negative	Negative
19.	NL-2d	Negative	Negative	Negative
20.	NL-2e	Negative	Negative	Negative

Table 7: *In vitro* antibacterial activity of endophytic bacteria isolated from *Cymbopogon citratus* leaves against Gram positive bacteria

S. No.	Isolate No.	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Bacillus cereus</i>
1.	JL-1a	S	R	S
2.	JL-1b	S	R	S
3.	JL-1c	S	R	S
4.	JL-1d	R	R	R
5.	JL-1e	S	R	S
6.	JL-2a	S	R	S
7.	JL-2b	S	R	S
8.	JL-2c	R	R	R
9.	JL-2d	S	R	S
10.	JL-2e	S	R	S
11.	NL-1a	S	R	S
12.	NL-1b	S	R	S
13.	NL-1c	S	R	S
14.	NL-1d	R	R	R
15.	NL-1e	R	R	R
16.	NL-2a	S	R	S
17.	NL-2b	S	R	S

18.	NL-2c	S	R	S
19.	NL-2d	S	R	S
20.	NL-2e	S	R	S

Table 8: *In vitro* antibacterial activity of endophytic bacteria isolated from *Cymbopogon citratus* leaves against Gram negative bacteria

S. No.	Isolate No.	<i>Salmonella Typhimurium</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>
1.	JL-1a	R	R	R
2.	JL-1b	R	R	R
3.	JL-1c	R	R	R
4.	JL-1d	R	R	R
5.	JL-1e	R	R	R
6.	JL-2a	R	R	R
7.	JL-2b	R	R	R
8.	JL-2c	R	R	R
9.	JL-2d	R	R	R
10.	JL-2e	R	R	R
11.	NL-1a	R	R	R
12.	NL-1b	R	R	R
13.	NL-1c	R	R	R
14.	NL-1d	R	R	R
15.	NL-1e	R	R	R
16.	NL-2a	R	R	R
17.	NL-2b	R	R	R
18.	NL-2c	R	R	R
19.	NL-2d	R	R	R
20.	NL-2e	R	R	R

Table 9: Over all *in vitro* antibacterial activity of endophytic bacteria isolated from *Cymbopogon citratus* leaves

S. No.	Bacteria	No. of isolate which showed sensitivity	Total no. of isolate showed sensitivity
1.	<i>Staphylococcus aureus</i>	16	16
2.	<i>Streptococcus pyogenes</i>	0	0
3.	<i>Bacillus cereus</i>	16	16
4.	<i>Salmonella Typhimurium</i>	0	0
5.	<i>Klebsiella pneumoniae</i>	0	0
6.	<i>Escherichia coli</i>	0	0

Conclusion

It is concluded from this investigation that phenetic characterization and biochemical tests indicated the presence of endophytic bacteria in the leaves of *Cymbopogon citratus*. Gram negative rods were present in the leaves of *Cymbopogon citratus*. The endophytic bacteria obtained from *Cymbopogon citratus* had shown antibacterial activity against *Staphylococcus aureus* and *Salmonella Typhimurium*.

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