ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 8, Issue, 08, pp.35528-35536, August, 2016*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

## RESEARCH ARTICLE

# INHIBITORY EFFECTS OF LEAF EXTRACT FROM *CYMBOPOGON FLEXUOSUS* AND ITS IMPACT IN CONJUNCTION WITH ANTIBIOTICS AGAINST PATHOGENIC WOUND BACTERIA

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**ARTICLE INFO**

***Article History:***
Received 13th May, 2016
Received in revised form
25th June, 2016
Accepted 10th July, 2016
Published online 20th August, 2016

***Key words:***

Lemon grass,
Wound associated bacteria,
Antibacterial effect,
Synergistic effect,
MIC.

**ABSTRACT**

Medicinal plants contain a plethora of bioactive metabolites that have been used in various traditional indigenous preparations as treatment modalities of multiple disorders. *Cymbopogon flexuosus,* also known as lemon grass, is an important medicinal plant that has been a part of Indian traditional medicines. Essential oil from lemon grass has been reported to possess antimicrobial, anti-inflammatory, anticancer, antioxidant, allelopathic and anthelmintic activity. However, potential of leaf extract from lemon grass to inhibit the pathogen isolates from the wound has not been reported. The present study was undertaken to explore the potent inhibitory activity of leaf extract of lemon grass and to investigate its synergistic effect with antibiotics against pathogenic bacteria isolated from wound. Pathogenic bacteria from wound were isolated, characterized and were used as pathogens. Interestingly, leaf extract of lemon grass exhibited much potent antibacterial activity with maximum inhibition in methanolic and ethanolic extract. Susceptibility of different available antibiotics against wound pathogenic bacteria was determined. Extracts from different solvent system were employed in conjunction with antibiotics and a synergistic effect was observed. Further, phytochemical analysis provides a detailed insight of the probable presence of phytochemical constituents in leaf extract. These findings for the first time highlights the prospective of leaf extract of lemon grass against wound associated pathogenic bacteria and also pave the path of exploring the synergistic effects with antibiotics as treatment modality.



**Citation: Keerti Singh, Lokesh Gambhir and Kunal Kishor, 2016.** "Inhibitory effects of leaf extract from *Cymbopogon flexuosus* and its impact in conjunction with antibiotics against pathogenic wound bacteria", *International Journal of Current Research*, 8, (08), 35528-35536.

## INTRODUCTION

Plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to gain from them a wondrous assemblage of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any of the part from a plant may yield a potent bioactive molecule (Castello *et al*., 2002). The medicinal action of plants is unique to particular plant species or groups as the combination of secondary products in a particular plant is taxonomically distinct. Since there is a global concern about an alarming increase in antibiotic-resistant microorganisms, dire need exist for new and effective therapeutic agents (Kamboj, 2000). The active constituents of plants possessing antimicrobial efficacy and medicinal properties, includes phenols, phenolic glycosides, salicylic acid, flavanoids, tannins, volatile oils etc which are used medically as an antiseptic, anti inflammatory and anti allergic agents (Fetrow Charles and Avila Juan, 2000). As per WHO report, 80% of the people living in developing countries almost exclusively use indigenous medicines. Medicinal plants form the principal component of these indigenous formulations. Medicinal plants used in traditional medicine should therefore be studied in depth to explore the potential to contain bioactive compounds and its toxic profile. *Cymbopogon flexuosus* is an important medicinal plant that has been part of Indian traditional medicines. It is also known as lemon grass which is widely cultivated on slopes, textured soils and even poor soil with hill slopes in southern India (Aniruddha Sarma *et al*., 2004). It is grown in Kerala, Assam, Maharashtra and Uttar Pradesh. Apart from India, it is also cultivated in large scale in Brazil, Mexico, Dominica, Haiti, Madagascar, Indonesia and China (Ganjewala *et al.,* 2008). Lemongrass leaf extract, essential oil called "lemon grass oil" or "citronella oil" and its constituents namely citral, geraniol, and geranyl acetate have been reported to possess a number of bioactivities viz., antimicrobial, anti-

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inflammatory, anticancer, antioxidant, allelopathic, anthelmintic, and insect and mosquito repellent (Aniruddha Sarma *et al*., 2004; Anjali Parashar *et al*., 2003; Asadipour *et al*., 2003). Some elite cultivars of lemon grass are krishna, cauveri, pragati, chirharit, neema and suvarna which produce essential oil of wide applications in flavors, pharmaceutical and food industries. This grass has potential to propagate from seeds and rooted slips. The stem is used in the manufacture of rayon and cellulose derivatives along with Leaves and essential oil are extensively used for medicinal purposes (Patra *et al*., 1990). The essential oil from *Cymbopogon flexuosus* contains Citral (43.80%), Z-citral (18.93%), geranly acetate (5.27%), trans-geraniol (3.66%) (Pandey,2002), Meeugenol (23.0%), Meisoeugenol (20.5%) and D-I limonene (16.5%) (Mazoor-I-khuda *et al*., 1986). Another different species of this plant (GRL-1) consist of very high geraniol (88.3%), other constituents includes limonene (1.2%), linalool (1%), $\beta$. Caryophyllene (1.8%) and $\gamma$-cadinene (2.2%). The anti-biofilm activity of lemon grass against *Staphylococcus aureus* has been attributed to its essential oil content. These oils along with Ag+ when used synergistically induce an inhibition of infectious pathogens. The present study was undertaken to investigate the antibacterial potential of lemon grass leaf extract against a spectrum of pathogenic bacteria. Further, a synergistic approach along with the commonly used antibiotic was adopted to evaluate the antibacterial activity that shall pave a way for synergistic formulations.

## MATERIALS AND METHODS

### Extraction of active principles from leaves

Soxhlet extraction is modification of simple percolation where a small volume of hot liquid is made to percolate through a column of material again and again. The extraction process in Soxhlet is repeated many times automatically until complete extraction is making the effects. The extracted compound may be isolated from extracted liquid after distillation. The menstrum of Soxhlet assembly is filled with about 500g of dried leaves. The solvents used for extraction purposes were petroleum ether, ethyl acetate, ethanol, methanol and distilled water.

### Extraction of essential oil from leaves

In Clevenger's apparatus a weighed amount of the material was taken in the RBF and was heated repeatedly. The vapors of the essential oil extract of the material condensed and were collected in the receiving tube. In Clevenger's apparatus, water column was maintained and as the oil starts accumulating, the level of the water increased to form a continual flow in the RBF from where it comes along in the form of steam. The oil was collected by oil eluting passage like that in burette. Oil received by Clevenger's apparatus was mixed and extracted with ether using separating funnel, ether layer was dried over using anhydrous sodium sulphate and collected the yield of essential oil.

### Preparation of extract solution

Concentrated extracts were dissolved in Dimethyl sulphoxide (DMSO) and were used for antimicrobial testing. With high polarity, DMSO is an ideal solvent at 1-2% and perhaps the least toxic for assay systems.

### Isolation, characterization and identification of Bacteria

The isolation of different pathogenic bacteria was done from various samples of wounds obtained from different hospitals of the Dehradun. Sterilized cotton swab was moistened in saline aseptically and swabbed from the skin lesions. The swab was spreaded over the respective media plates, which was then incubated for 24 - 48 hours at 37°C in an inverted position. After the incubation, the colonies were streaked for isolation (Larsen and Mahon, 2000). For collection of samples Cray and Blair Transport medium was used. Nutrient agar medium and Nutrient broth media were used for the primary isolation, followed by selective isolation by utilizing differential and selective media like MacConkeys agar, Mannitol salt agar, Blood agar, Eosin methylene blue agar, MRS agar media, Fluorescent pseudomonas agar, Marshal's agar and as per the requirements (all are from Hi-Media), followed by the morphological identification by the Gram's staining. The biochemical characterization includes IMViC test, Hydrogen sulphide production, Lactose, dextrose utilization, Nitrate reduction, Urease activity, Catalase test, Oxidase test as per the Bergey's manual of determinative bacteriology.

### *In vitro* antibacterial assay of the extract/oils against bacterial cultures

Bacterial cultures isolated from wounds from different clinical sources of Dehradun are maintained in the department of Microbiology, includes *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogens, Staphylococcus epidermidis, Staphylococcus aureus, Lactobacillus acidophilus, Clostridium perfringens.* The bacterial suspensions were standardized using McFarland turbidity standards. The standard was prepared by adding specific volumes of 99.5mL of 1% sulfuric acid and 0.5mL of 1.175% barium chloride to obtain a barium sulfate solution. This provides the bacterial suspension containing 1.5x108 CFU/ml (Hindler and Jorgensen, 2000).

### Preparation of 0.5 Mcfarland standard

0.5 ml of 0.048M $BaCl_2$ (1.17% w/v $BaCl_2$) was added to 99.5 ml of 0.18M $H_2SO_4$ (1% w/v) with constant stirring. The O.D. of the solution was recorded; it should be in the range of 0.08-0.1 at 625 nm ($1.5x10^8$ cells/ml for yeast). Standard was stored in amber colour bottle to prevent it from light at room temperature. Standard was briskly vortexed on a vortex mixer prior to use. (NCCLS, 1997)

### KB disc diffusion assay

5mm size discs were used with loading capacity of 20µl. Prepared samples were loaded in sterile discs and dried under aseptic conditions. Plates containing Muller Hinton Agar media were swabbed with 0.5 McFarland adjusted 16-18 hour old culture of the isolated test organisms. Sample loaded discs were then placed on the swabbed media plates and incubated at $37^0$C overnight for 24h. Disc loaded with solvent used for dilution served as control. Antibacterial and antifungal activity

was determined by measuring diameter of zone of inhibition (Kumar *et al*., 2007).

**Determination of Minimum Inhibitory Concentration (MIC) level of most active extracts**

Minimum inhibitory concentration (MIC) level of any antimicrobial substance is the lowest concentration of the drug inhibiting the microbial growth. The MIC value of the extracts was determined against a particular microorganism that exhibited a maximum activity in preliminary screening process. The methanol and ethanol extracts of *Cymbopogon flexuosus* were tested against *Escherichia coli, Streptococcus pyogens, Staphylococcus epidermidis, Staphylococcus aureus,* and *Clostridium perferingens*. Preparation of Assay medium Petri plate, Growth conditions and other parameters were similar to those of preliminary studies was followed. The MIC value of those microorganisms against a particular fraction is considered, which exhibit maximum activities in preliminary screening processes by disk diffusion method. MIC was determined by using different concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%) of extract solution and were screened for antimicrobial potential. The antibacterial activity was measured in terms of the inhibition zone diameter (Prescott *et al*., 2006).

**Preliminary evaluation of antibiotic susceptibility**

Preliminary evaluation of antibiotic susceptibility was done by employing multiple potent antibiotics like $Va^{10}$ – Vancomycin (10 $\mu$ g), $R^{30}$ – Rifampicin (30 $\mu$ g), $M^{30}$ – Methicillin (30 $\mu$ g), $Cf^{30}$ – Ciprofloxacin (30 $\mu$ g), $K^{30}$ –Kanamycin (30 $\mu$ g) , $Am^{30}$ – Amoxycillin (30 $\mu$ g), $G^{10}$ –Gentamycin (10 $\mu$ g), $S^{25}$ –Streptomycin (25 $\mu$ g), $Co^{25}$ – Co-trimazine (25 $\mu$ g), $A^{25}$ – Ampicllin (25 $\mu$ g) and $Tr^{25}$ – Trimethoprim (5 $\mu$ g) (Baur *et al*., 1966). The antimicrobial susceptibility test disks were used in accordance to National Committee for Clinical Laboratory Standards (NCCLS, 2000).

**Evaluation of synergistic and antagonistic effects with antibiotics**

The effect of extracts and antibiotics was evaluated in terms of antagonistic and synergistic effects, if the activity of the added composition increases in terms of the inhibition zone diameter it may be regarded as synergistic effect, on contrary if the composition reduces or give rise to negligible inhibition zone diameter it is regarded as antagonistic effect.

To evaluate combined antimicrobial activity of disc antibiotics, $Va^{10}$ – Vancomycin (10 $\mu$ g), $R^{30}$ – Rifampicin (30 $\mu$ g), $M^{30}$ – Methicillin (30 $\mu$ g), $Cf^{30}$ – Ciprofloxacin (30 $\mu$ g), $K^{30}$ – Kanamycin (30 $\mu$ g) , $Am^{30}$– Amoxycillin (30 $\mu$ g), $G^{10}$ – Gentamycin (10 $\mu$ g), $S^{25}$ –Streptomycin (25 $\mu$ g), $Co^{25}$ – Co-trimazine (25 $\mu$ g), $A^{25}$ – Ampicllin (25 $\mu$ g), $Tr^{25}$ – Trimethoprim (5 $\mu$ g), along with plant extracts were placed aseptically on each plates and mean inhibition zone diameter was measured (Smith *et al*., 1985).

**Preliminary determination of phytochemicals present in the extracts**

Preliminary determination of phytochemicals which may be present in the extracts was determined for the presence of different constituents (Baser *et al*., 2004).

**Test for alkaloids**

Small fractions of solvent free extract was stirred with a few drops of diluted HCl and filtered, the filtrate was tested for following colour tests: Mayer's Test: - Mayer's reagent (potassium mercuric iodide) was added to the test solution. It gives green colour precipitate. Hager's Test: - The test solution mixed with Hager's solution (saturated picric acid) gives yellow precipitate. Wagner's Test: - Wagner's reagent (Potassium iodide) was added to test solution, it gives brown coloured precipitate.

**Test for triterpenoids**

Salkowaski's Test: - Few drops of concentrated $H_2SO_4$ were added to test solution, shaken and allowed to stand for 10min, yellow lower layer confirms the presence of triterpenoids. Liebermann Burchardt Test: - The test solution was treated with acetic anhydride, mixed well and conc. $H_2SO_4$ was added from sides of test tube a deep red colour confirms the presence of triterpenoids.

**Test for flavanoids**

Ferric-Chloride Test: - Few drop of ferric chloride solution was added to test solution; intense green colour shows the presence of flavanoids. Zinc Hydrochloride Test: - Zn dust and few drops of HCl were added to test solution, a magenta red colour confirms the presence of flavanoids.

**Test for proteins**

Million's Test: - When a million's reagent was added to test solution and heated on water bath, a protein is stained red on warming. Biuret Test: - Test solution was mixed with 40% sodium hydroxide and diluted copper sulphate, gives blue colour. Ninhydrin Test: - On adding reagent of test solution, blue colour was observed.

## RESULTS

**Yield of extract from *Cymbopogon flexuosus* leaves**

Leaves of *Cymbopogon flexuosus* were dried and compounds were extracted using multiple solvents. After the extraction of the dried leaves, the percentage yield in each solvent system was determined. Total soluble fraction of *Cymbopogon flexuosus* was found to be 30% (Fig. 1).

**Isolation, characterization and identification of the wound associated bacteria**

The isolation of the wound associated bacteria from different clinical sources by utilizing multiple differential and selective media. The characterization and isolation of pure strains were done according to the Bergey's manual of determinative bacteriology. Further, it was observed that the isolated strains

of *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogens, Staphylococcus epidermidis, Staphylococcus aureus, Lactobacillus acidophilus, Clostridium perfringens* showed different activities in terms of IMViC test, Hydrogen sulphide production, Lactose, dextrose utilization, Nitrate reduction, Urease activity, Catalase test and Oxidase test as per the Bergey's manual of determinative bacteriology (Table 1).

**Antimicrobial potential of various extracts from Cymbopogon flexuosus leaves against the isolated wound bacteria**

All the extracts in multiple solvent systems were analyzed for their antimicrobial efficacy against wound associated bacteria. The methanol extract shows range of antibacterial activity in terms of inhibition zone diameter against *Escherichia coli* (28mm), *Pseudomonas aeruginosa* (4mm), *Streptococcus pyogens* (23mm), *Staphylococcus epidermidis* (20mm), *Staphylococcus aureus* (20mm), *Lactobacillus acidophilus* (14mm) and *Clostridium perfringens* (12mm). The ethanol extract showed range of antibacterial activity in terms of inhibition zone diameter which was comparable with the methanolic extract following the same pattern. Ethanol extract against *Escherichia coli* (22mm), *Pseudomonas aeruginosa* (4mm), *Streptococcus pyogens* (26mm), *Staphylococcus epidermidis* (20mm), *Staphylococcus aureus* (20mm), *Lactobacillus acidophilus* (17mm) and *Clostridium perfringens* (27mm). The ethyl acetate extract shows range of antibacterial activity in terms of inhibition zone diameter against *Escherichia coli* (10mm), *Pseudomonas aeruginosa* (2mm), *Streptococcus pyogens* (12mm), *Staphylococcus epidermidis* (10mm), *Staphylococcus aureus* (10mm), *Lactobacillus acidophilus* (25mm) and *Clostridium perfringens* (2mm). However, petroleum ether and distilled water extracts showed no antibacterial activity (Fig. 2).

**Determination of Minimum Inhibitory concentration (MIC) level active extracts**

Since methanol and ethanol extracts of *Cymbopogon flexuosus* showed antimicrobial activity, these extracts were analyzed for MIC levels.

The minimum inhibitory concentration of methanol at different concentrations of the extract, at different dilutions for the respective bacteria is *Escherichia coli* (3.12mm), *Streptococcus pyogens* (12.5mm), *Staphylococcus epidermidis* (50mm) and *Staphylococcus aureus* (12.5mm) (Fig. 3.). The MIC of ethanol at different concentrations of the extract, at different dilutions for the respective bacteria is *Escherichia coli* (3.12mm) and *Staphylococcus aureus* (25mm) (Fig. 4A and B).

**Preliminary evaluation of antibiotic susceptibility**

The susceptibility pattern of different bacteria against various antibiotics was analyzed (Table 2). It is evident that the test indicated that *Lactobacillus acidophilus* was highly sensitive organisms against most of the antibiotics, followed by *S. aureus*, while *P. aeruginosa* was resistant to most of the antibiotics. Trimethoprim ($Tr^{25}$) was not effective against microbes except *Lactobacillus acidophilus*. All the bacterial species were susceptible with Ciprofloxacin ($Cf^{30}$) while Amoxycillin ($Am^{30}$) showed moderate to good antibacterial activity against all the test organisms except *P. aeruginosa*. Rifampicin (30 $\mu$ g) and Streptomycin (25 $\mu$ g) showed good susceptibility pattern against all species except *P. aeruginosa.*

**Determination of Synergistic and Antagonistic effects of extracts in conjunction with antibiotics**

A synergistic approach was adopted to use available antibiotics in conjunction with the extracts to gain potent bioactivity. Interestingly, the result from this combination study using the disk diffusion method showed that the combination of the four different leaf extracts of *Cymbopogon flexuosus* (Petroleum ether, ethyl acetate, methanol and ethanol) produced synergistic effects with most of the disk antibiotics against *Pseudomonas aeruginosa* and *S. aureus* (Table 3 and Fig. 5). Antagonistic effects were observed against *E. coli*.

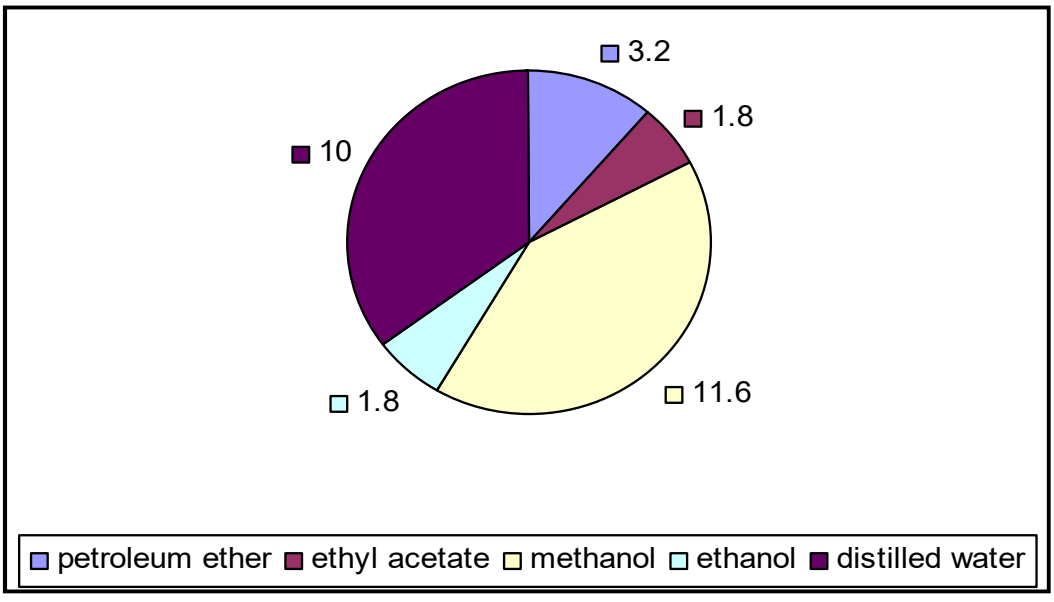


**Fig. 1. Percentage yield of the *Cymbopogon flexuosus***

**Table 1. Biochemical characterization of the isolated pathogenic bacteria from wound**

| Bacteria | Lactose | Dextrose | $H_2S$ | $NO_3$ | Indole | MR | VP | Citrate | Urease | Catalase | Oxidase |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* | + | + | - | - | + | + | + | - | - | - | - |
| *Pseudomonas aeruginosa* | - | - | - | + | - | - | - | + | - | + | + |
| *Streptococcus pyogens* | + | + | - | - | - | - | - | - | - | - | - |
| *Staphylococcus epidermidis* | - | + | - | - | - | - | + | - | - | + | + |
| *Staphylococcus aureus* | + | + | _ | + | - | + | + | - | - | + | - |
| *Lactobacillus acidophilus* | - | + | _ | + | _ | _ | - | - | _ | _ | + |
| *Pseudomonas aeruginosa* | - | - | - | + | - | - | - | + | - | + | + |
| *Clostridium perfringens* | + | - | - | - | - | - | - | - | - | + | + |

**Table 2. Preliminary evaluation of antibiotic susceptibility against pathogenic bacteria. $Va^{10}$ – Vancomycin (10 $\mu$ g), $R^{30}$ – Rifampicin (30 $\mu$ g) , $M^{30}$ – Methicillin (30 $\mu$ g), $Cf^{30}$ – Ciprofloxacin (30 $\mu$ g), $K^{30}$ –Kanamycin (30 $\mu$ g) , $Am^{30}$ – Amoxycillin (30 $\mu$ g), $G^{10}$ –Gentamycin (10 $\mu$ g), $S^{25}$ –Streptomycin (25 $\mu$ g), $Co^{25}$ – Co-trimazine (25 $\mu$ g), $A^{25}$ – Ampicllin (25 $\mu$ g), $Tr^{25}$ – Trimethoprim (5 $\mu$ g)**

| Bacteria | Antibiotic sensitivity (mean inhibition zone diameter in mm) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $Va^{10}$ | $R^{30}$ | $M^{30}$ | $Cf^{30}$ | $K^{30}$ | $Am^{30}$ | $G^{10}$ | $S^{25}$ | $Co^{25}$ | $A^{25}$ | $Tr^{25}$ |
| *Escherichia coli* | 0 | 21 | 0 | 30 | 0 | 18 | 23 | 20 | 0 | 0 | 0 |
| *Pseudomonas aeruginosa* | 0 | 14 | 0 | 28 | 0 | 0 | 17 | 15 | 0 | 0 | 0 |
| *Streptococcus pyogens* | 0 | 14 | 0 | 27 | 0 | 7 | 19 | 16 | 0 | 16 | 0 |
| *Staphylococcus epidermidis* | 14 | 14 | 0 | 35 | 18 | 11 | 22 | 29 | 0 | 11 | 0 |
| *Staphylococcus aureus* | 20 | 15 | 17 | 29 | 0 | 36 | 12 | 19 | 19 | 39 | 0 |
| *Lactobacillus acidophilus* | 15 | 24 | 18 | 14 | 0 | 39 | 12 | 11 | 14 | 32 | 38 |
| *Clostridium perfringens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 3. Effect of Extracts of C*ymbopogon flexuosus* with Antibiotics. * -ve sign indicates reduction in IZD from both the previous IZD of extract and antibiotic. * -ve sign indicates reduction in IZD from both the previous IZD of extract and antibiotic. *Nd indicates least or negligible effect**

| | | | IZD of Antibiotics/Extract (mm) | | | | % Variation | | | | Effects | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteria | Disc Antibiotic | IZD (mm) | P.E. | E.A | METH | ETH | P.E | E.A | METH | ETH | P.E | E.A | METH | ETH |
| *E. coli* | $R^{30}$ | 21 | 14 | 14 | 17 | 15 | -33.33 | -33.33 | -19.04 | -28.57 | Ant | Ant | Ant | Ant |
| | $S^{25}$ | 20 | 0 | 0 | 19 | 14 | 0 | 0 | -5 | -30 | Nd | Nd | Ant | Ant |
| | $Tr^{5}$ | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | Nd | Nd | Nd | Nd |
| *P. aeruginosa* | $R^{30}$ | 14 | 26 | 25 | 28 | 30 | 85.71 | 78.57 | 100 | 114.28 | Syn | Syn | Syn | Syn |
| | $S^{25}$ | 15 | 20 | 19 | 23 | 30 | 33.33 | 26.66 | 53.33 | 100 | Syn | Syn | Syn | Syn |
| | $Tr^{5}$ | 0 | 24 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | Syn | Nd | Nd | Nd |
| *S.aureus* | $R^{30}$ | 15 | 22 | 26 | 26 | 21 | 46.66 | 73.33 | 73.33 | 40 | Syn | Syn | Syn | Syn |
| | $S^{25}$ | 19 | 26 | 28 | 27 | 26 | 36.84 | 47.36 | 42.10 | 36.84 | Syn | Syn | Syn | Syn |
| | $Tr^{5}$ | 0 | 0 | 0 | 10 | 19 | 0 | 0 | 100 | 100 | Nd | Nd | Syn | Syn |

**Table 4. Preliminary phytochemical determination of *C. flexuosus* leaf extract in different used solvent systems**

| Phytochemical Tests | *Cymbopogon flexuosus* | | | | |
|---|---|---|---|---|---|
| | Petroleum ether extract | Ethyl acetate extract | Methanol extract | Ethanol extract | Aqueous extract |
| **1.Test for alkaloids** | | | | | |
| a) Mayer's Test | +++ | ++ | ++++ | +++ | - |
| b) Hager's Test | ++ | + | +++ | ++ | - |
| c) Wagner's Test | + | + | ++ | + | - |
| **2. Test for Proteins** | | | | | |
| a) Million's Test | - | - | - | - | - |
| b) Biuret Test | - | - | - | - | - |
| c) Ninhudrin Test | - | - | - | - | - |
| **3. Test for Triterpenoids** | | | | | |
| a) Salwkowski's Test | - | - | - | + | - |
| b) Libbermann-Burchard's Test | - | - | - | + | - |
| **4. Test for flavonoids** | | | | | |
| a) Ferric chloride Test | - | - | ++ | - | + |
| b) Alkaline reagent Test | - | - | + | - | + |
| c) Zinc-hydrochloride acid Test | - | - | + | - | + |

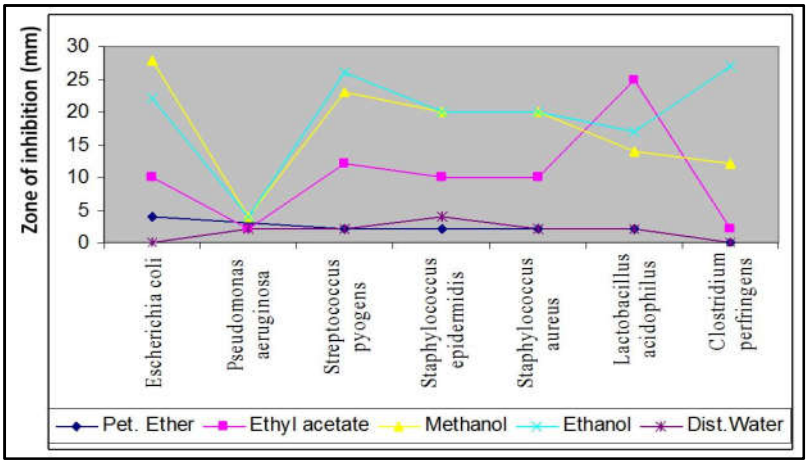


**Fig. 2. Antibacterial efficacy of different solvent based extracts of *Cymbopogon flexuosus***

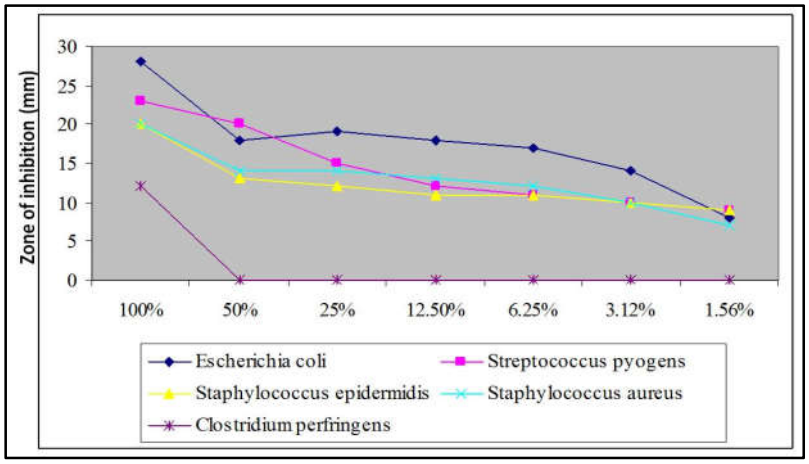


**Fig. 3. MIC of Methanol extract from leaves of *Cymbopogon flexuosus* against wound isolates**

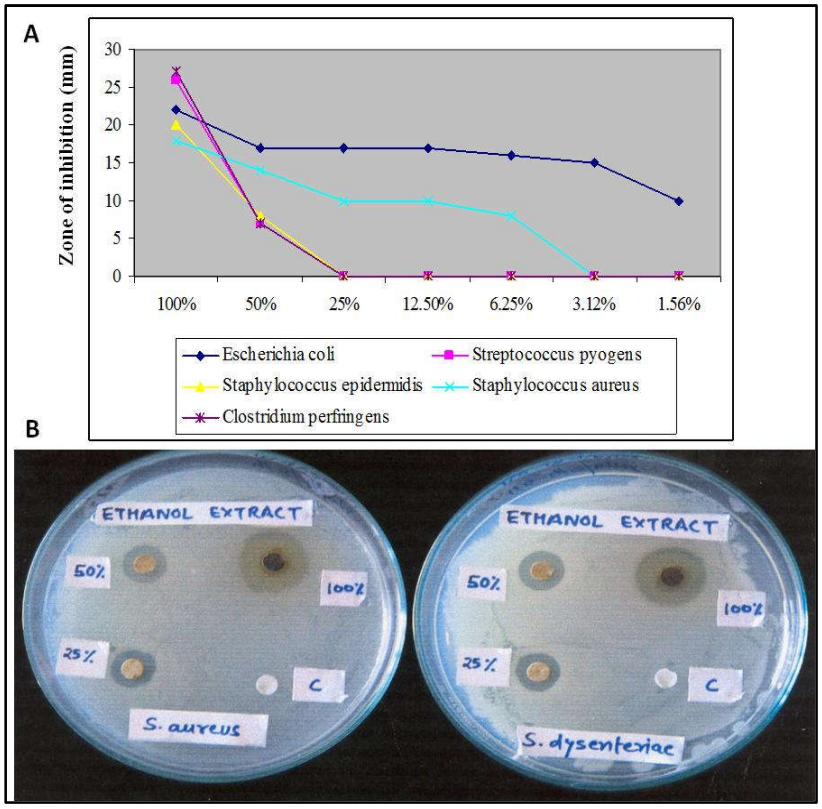


**Fig. 4. (A) MIC of ethanol extract from leaves of *Cymbopogon flexuosus* against wound isolates. (B) Image showing the zone of inhibition of ethanolic extract in different percentage against *S. aureus* and *S. dysenteriae***

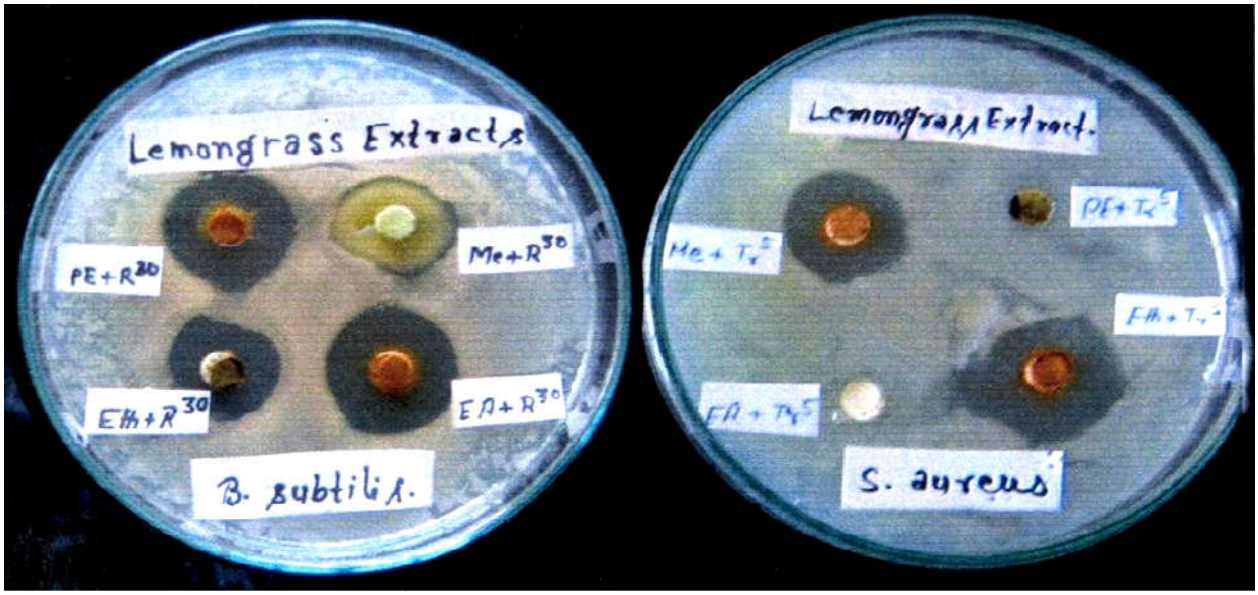


**Fig. 5. Synergistic Effect: Activity of leaf extracts from *C. flexuosus* in conjunction with $R^{30}$ and $Tr^{5}$against *B. subtilis* and *S.aureus* respectively**

### Preliminary phytochemical determination

Preliminary phytochemical determination of the different extracts of the *Cymbopogon flexuosus* was done which showed the presence of different compounds (Table. 4). The analysis of the *Cymbopogon flexuosus* conferred the presence of alkaloids which was exhibited in petroleum ether extract and ethyl acetate extract, alkaloids and flavanoids were present in methanol extract, alkaloids and triterpenhoids were abundant in ethanol extract whereas only flavanoids were present in the aqueous extract.

## DISCUSSION

Plants have been used right through human history for their medicinal properties and herbal medicines are gaining popularity worldwide. Since all herbal medicines are mixtures of more than one ingredient, such combination of many substances obviously increases the potential of interactions taking place due to pleiotropic effect (Olayinka A Aiyegoro and Anthony I Okoh, 2010; Chaudary, 1994). Essential oil extracted from *Cymbopogon winterianus* has shown good antibacterial activity against both Gram negative and Gram positive bacteria, but very less in comparison to the essential oil of *Cymbopogon flexuosus* (Evans *et al*., 1986; Chandrashekar and Joshi, 2004; Kumaran *et al*., 2003; Oyedele *et al*., 2002; National Committee for Clinical Laboratory Standards: Approved standard, 2000; Novick and Bounchand. 1971). In the present study it has been observed that *Cymbopogon flexuosus* leaf extracts encompass moderate to better antimicrobial efficacy against the isolated wound associated bacteria. The essential oil extracted from *Cymbopogon flexuosus*, in the present study, has shown potent antibacterial efficacy against both Grams positive as well as Gram negative isolated wound associated bacteria. The activity was more pronounced in case of Gram negative bacteria except *Pseudomonas aeruginosa*. The minimum inhibitory concentration was determined for the most active extracts, which showed good to moderate results against the tested bacteria. Further, employing a spectrum of antibiotics having different mechanism of action was tested against isolated bacterial strains. Their sensitivity pattern was determined by disc diffusion method. Resistant to some antibiotics may be attributed to poor penetration of the antibiotics in the cell or rapid efflux of antibiotics due to production of inactive enzymes by the organism, which rendered antibiotic less effective. In the present study, we hypothesized that a conjunction modality (combination of leaf extract and antibiotics) may serve as a potent antibacterial strategy. Therefore, synergistic and antagonistic activity of the extract in conjunction with antibiotics was determined. Interestingly, it was observed that the *Cymbopogon flexuosus* extract exhibited synergistic effect which resulted in enhancing the antibacterial activity evident from increased zone of inhibition. The phytochemical evaluation of the extracts showed the presence of the alkaloids, triterpenoids and flavanoids in the methanol and ethanol extract of the *Cymbopogon flexuosus* could be correlated well with their potent antimicrobial efficacy. In conclusion, the present study was undertaken to explore the antimicrobial efficacy of leaf extract/oil of *Cymbopogon flexuosus*.

The study provides a detailed insight of the antibacterial action of lemon grass extract against pathogenic bacteria isolated from wounds. Further, a synergistic effect was observed between extracts and available antibiotic modalities. Our findings may help in identifying *Cymbopogon flexuosus* as a storehouse of potent bioactive compounds of medicinal value and also proposes synergistic use of extracts with existing antibiotics to achieve potent antibacterial activity as an amenable approach.

### Conflict of interest

There is no actual or potential conflict of interest

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