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STUDIES ON ANTIMICROBIAL ACTIVITY OF CYMBOPOGON SPECIES OF KARNATAKA AGAINST CLINICAL ISOLATES

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ABSTRACT: Antimicrobial activity of the various species of *Cymbopogon* plants like *Cymbopogon citrates*, *Cymbopogon martini*, *Cymbopogon nardus*, *Cymbopogon flexuosus*, *Cymbopogon schoenanthus* against various pathogens like *E. coli*, *Bacillus subtilis*, *Streptococcus species*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Serratia marcescens* were studied with the help of agar well diffusion method. The samples were extracted with solvents like Methanol, Ethanol, Chloroform, Acetone, Diethyl ether to measure the antimicrobial activity by using Muller-Hinton agar plates. The Chloroform and Diethyl ether extract of *Cymbopogon flexuosus*, *Cymbopogon martini* showed the highest zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In *Cymbopogon citrates*, all the pathogens were resistant to all the extracts except for *Streptococcus species* and *Pseudomonas aeruginosa*, which showed the least susceptibility to all the extracts except for Methanol and Diethyl ether extract. In *Cymbopogon schoenanthus*, Methanol extract showed the highest zone of inhibition against *Staphylococcus aureus* and *Bacillus subtilis*, but *Klebsiella pneumonia* showed resistance to all the extracts except for Diethyl ether which showed the least zone of inhibition. In *Cymbopogon flexuosus*, Methanol, Chloroform, Diethyl ether showed the highest zone of inhibition against *Staphylococcus aureus* and *Serratia marcescens*, but *Klebsiella pneumonia* and *Bacillus subtilis* showed resistance in all the extracts. In *Cymbopogon nardus*, all the pathogens showed resistance in all the extracts except for the Chloroform and Methanol, which showed the highest zone of inhibition against *Bacillus subtilis*.

INTRODUCTION: Medicinal plants have been known to cure many diseases due to the presence of active bioconstituents. The bacteremia, fungemia, and death have been caused by asymptomatic colonization by the various spectrum ranges of infection¹. The highest incidence of fungal skin diseases was witnessed in 2016, by accounting for (2.10 billion, 1.88 billion to 2.34 billion) which were among the top four of the ten².

The infections that are unresponsive to all known antibiotics were reported³. Therefore, the oldest form of medicine is obtained from one of the candidates available is used for the treatment of skin disorders which is the natural product⁴.

In the present study, the antimicrobial activity of various species of *Cymbopogon* has been tested against various pathogens like *E. coli*, *Bacillus subtilis*, *Streptococcus species*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Klebsiella pneumonia* by using various extracts of Methanol, Ethanol, Chloroform, Acetone, and Diethyl ether. The gastrointestinal tract of humans and other vertebrates is the habitat of various pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, *Kleb-*

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siella pneumonia, *Salmonella typhimurium*. Some of the serious infections are caused by some harmful strains, while some of them help in food digestion are beneficial to humans. Gram-positive bacteria like *Staphylococcus aureus*, *Streptococcus species*, *Bacillus subtilis*, *Serratia marcescens* is an anaerobe that grows in the absence of oxygen. The toxins produced on the surface of the cell wall proteins of some of the strains make them pathogenic which leads to many infectious diseases by inactivating the antibodies after binding to it.

The antimicrobial activity of *Cymbopogon* species has been studied by the agar well diffusion method by measuring the zone of inhibition against the pathogens treated with the various extracts. In our study, it has found that Gram-positive bacteria like *Staphylococcus aureus* is more susceptible to the leaf extracts of *Cymbopogon* due to the presence of thick-walled peptidoglycan, which undergoes degradation when treated with all the extracts, whereas Gram-negative bacteria like *Klebsiella pneumonia* is more resistant to all the extracts of *Cymbopogon* species due to the presence of three layers of Lipopolysaccharide (LPS) around their plasma membrane.

MATERIALS AND METHODS: The plant samples of different species maintained in the greenhouse of Visveshwarapura College of Science, Bangalore, India, were obtained from Sanjeevani Vatika, Department of Horticulture, GKVK, UAS (B), Bangalore, India, having authentication no. 81. The obtained leaf samples were cleaned with distilled water, dried under shade, powdered, and stored in airtight bottles.

Solvent Extract Preparation: 5 g of each powdered sample was extracted with 50 mL of methanol for 48 h at room temperature with continuous stirring. The filtration method was employed after 48 h to collect the supernatant. The crude extract of the solvent was obtained by the process of evaporation. The Antimicrobial analysis method was performed to reveal the antimicrobial activity of the plant samples with the help of the agar well diffusion method. The Solvent extraction was done by the Soxhlet apparatus. Muller-Hinton agar plates were prepared to evaluate the antimicrobial activity of the samples with the following solvent extracts Methanol, Ethanol,

Acetone, Chloroform, and Diethyl ether against selected human pathogens viz., *Streptococcus sp.*, *Salmonella sp.*, *E. coli*, *Bacillus sp.*, *Pseudomonas sp.*, *Serratia marcescens*, *Staphylococcus aureus*, and *Klebsiella pneumonia*. The Muller-Hinton agar plates were spread with 100 µl inoculum of each selected pathogen uniformly with the help of a swab. A sterile cork borer is used to punch a well of 6mm in diameter after 5 min of incubation. The well was loaded with 80 µl of the concentrated sample. The plates were incubated at 37 °C overnight, and after incubation, plates were observed for the zone of inhibition^{9,10}.

Minimum Inhibitory Concentration: The Minimum inhibitory concentration was carried out for the samples showing higher antimicrobial activity against the pathogens. Different concentrations of the samples were checked for antimicrobial activity to determine the concentration at which the samples showed the least activity. Five different concentrations of the samples were used for the determination of the MIC.

RESULTS AND DISCUSSION: Medicinal plants play a significant role in treating diseases by acting against pathogens. The agar well diffusion method is used to study the antimicrobial properties of the *Cymbopogon* species. The new drug compounds present in the plants have been contributed to human well-being. The plant extracts have antimicrobial properties that can be used for therapeutic purposes. The essential oil of *Cymbopogon martini* was determined for its MIC by using agar dilution assay⁵.

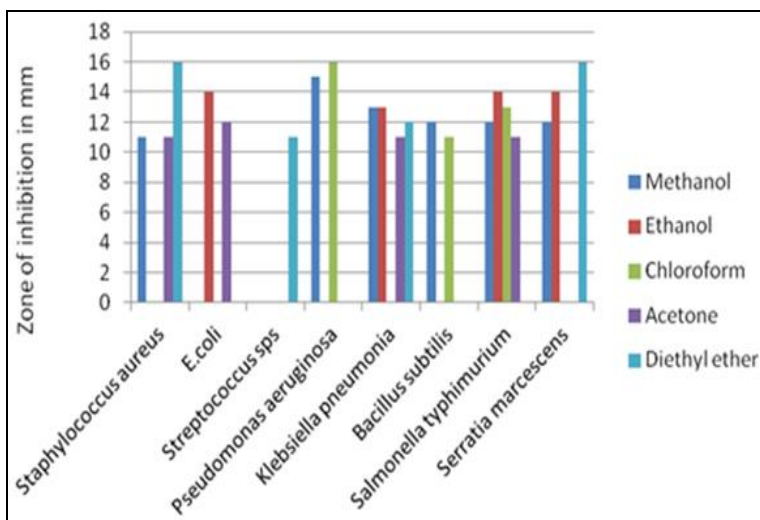
The chemical compounds are the complex mixtures of monoterpene and sesquiterpene hydrocarbons and alcohols, aldehydes (their oxygenated derivatives), ketones, and other chemical families like oxides, sulphur derivatives, and fatty acids present in the *Cymbopogon martini* essential oil were known to have the broad spectrum of antimicrobial activity by its different mode of action⁶. The antimicrobial activity of the essential oil of *Cymbopogon martini* has been reported among the ethnobotanically available aromatic plants. The pathogens causing skin diseases have not been evaluated by the topical formulation of antimicrobial activity.

The acceptability and availability of the practices in the Ethiopian Society were taken into account ⁷. The antimicrobial activity of *Cymbopogon* species has been tested by using various solvents of Methanol, Ethanol, Chloroform, Acetone, Diethyl ether against various pathogens like *E. coli*, *Bacillus subtilis*, *Streptococcus species*, *Salmonella*

typhimurium, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Serratia marcescens*. *Cymbopogon citratus* is not effective in inhibiting *Klebsiella pneumonia* whereas effective in inhibiting *Streptococcus species* and *Pseudomonas aeruginosa* in the Chloroform and Acetone extract **Table 1, Graph 1** and **Fig. 1**.

TABLE 1: EVALUATION OF ANTIMICROBIAL ACTIVITY OF CYMBOPOGON CITRATUS USING VARIOUS SOLVENTS AGAINST SELECTED HUMAN PATHOGENS (THE VALUES REPRESENTS THE ZONE OF INHIBITION IN mm)

Solvents	Methanol	Ethanol	Chloroform	Acetone	Diethyl ether
<i>Staphylococcus aureus</i>	0	0	0	0	9
<i>E. coli</i>	0	0	0	15	14
<i>Streptococcus species</i>	0	9	14	13	0
<i>Pseudomonas aeruginosa</i>	12	0	13	12	0
<i>Klebsiella pneumonia</i>	0	0	0	0	0
<i>Bacillus subtilis</i>	0	10	10	0	13
<i>Salmonella typhimurium</i>	11	0	10	0	0
<i>Serratia marcescens</i>	14	13	0	0	13



GRAPH 1: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF CYMBOPOGON CITRATUS

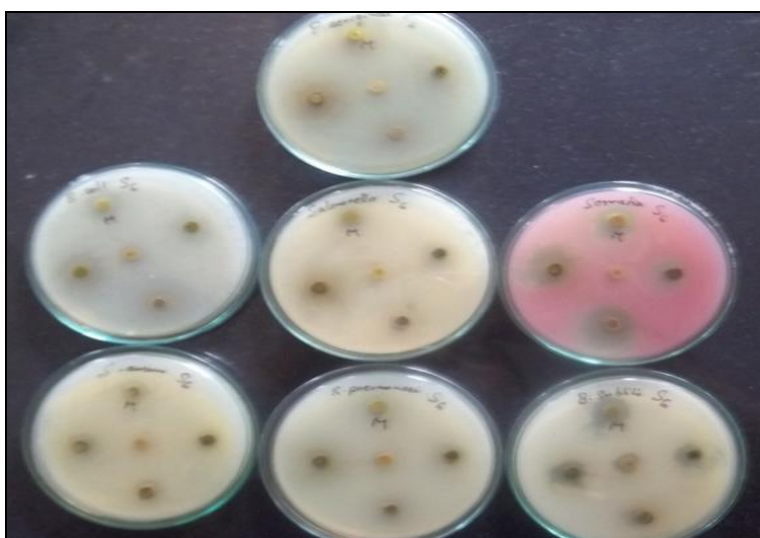


FIG. 1: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF CYMBOPOGON CITRATUS AGAINST VARIOUS PATHOGENS

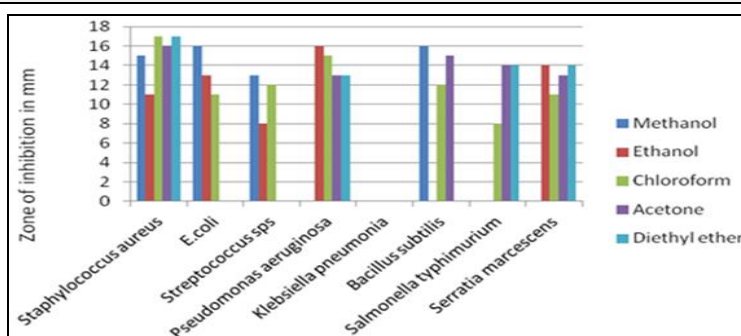
Sushant *et al.*, (2013) ⁸ have studied the antimicrobial activity of *Cymbopogon citratus* by using methanol extract, which was more effective against *Staphylococcus* species. The zone of inhibition observed for *Cymbopogon citratus* was 25 mm. *Staphylococcus aureus* was very sensitive to the methanol extract of *Cymbopogon citratus*. Balachandar Balakrishnan *et al.*, (2014) ⁹ showed that all the solvents like Chloroform, Methanol, and water were used to extract *Cymbopogon citratus*, which showed maximum zone of inhibition against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas vulgaris*. The minimum inhibition activity exhibited against *Nocardia species*, *Escherichia aerogenes*, *Serratia species*, and *Staphylococcus aureus* in all the solvent extracts of lemongrass. The chloroform extract of *Cymbopogon citratus* showed a maximum zone of inhibition against *Pseudomonas aeruginosa* (20

mm) and *Bacillus subtilis* (21 mm). The methanolic extract of *Cymbopogon citratus* showed the highest zone of inhibition against *Pseudomonas aeruginosa* (20 mm) and *Bacillus subtilis* (18 mm). The results of antibacterial activity were good against Gram-positive and Gram-negative organisms exhibited by the leaf extracts of the plant *Cymbopogon citratus*. In *Cymbopogon martini*, all the extracts were very effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The Chloroform extract and Diethyl ether extract were very effective in inhibiting *Staphylococcus aureus* by exhibiting a zone of inhibition (17 mm). *Klebsiella pneumonia* showed resistance to all the extracts. *Salmonella typhimurium* showed the least susceptibility to the chloroform extract of *Cymbopogon martini* by exhibiting a zone of inhibition of 8 mm **Table 2, Graph 2, and Fig. 2.**

TABLE 2: EVALUATION OF ANTIMICROBIAL ACTIVITY OF CYMBOPOGON MARTINI USING VARIOUS SOLVENTS AGAINST SELECTED HUMAN PATHOGENS (THE VALUES REPRESENTS THE ZONE OF INHIBITION IN MM)

Solvents	Methanol	Ethanol	Chloroform	Acetone	Diethyl ether
<i>Staphylococcus aureus</i>	15	11	17	16	17
<i>E. coli</i>	16	13	11	0	0
<i>Streptococcus species</i>	13	8	12	0	0
<i>Pseudomonas aeruginosa</i>	0	16	15	13	13
<i>Klebsiella pneumonia</i>	0	0	0	0	0
<i>Bacillus subtilis</i>	16	0	12	15	0
<i>Salmonella typhimurium</i>	0	0	8	14	14
<i>Serratia marcescens</i>	0	14	11	13	14



GRAPH 2: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF CYMBOPOGON MARTINI

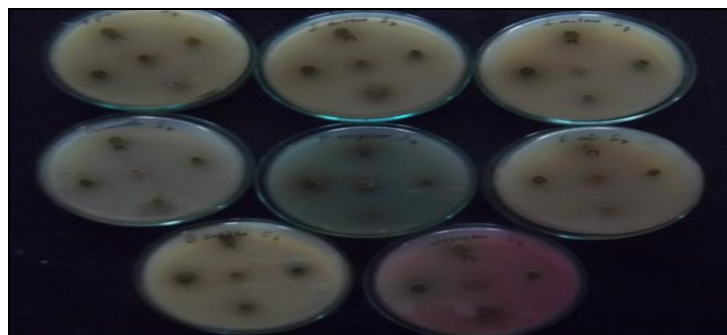


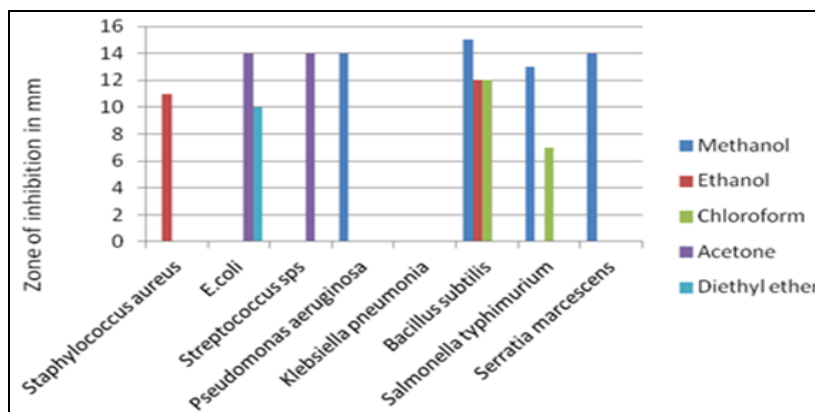
FIG. 2: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF CYMBOPOGON MARTINI AGAINST VARIOUS PATHOGENS

Huma Jafri *et al.*, (2014)¹⁰ showed that the disc diffusion method was used to determine the antibacterial activity of essential oils against *Staphylococcus aureus* strains. The antibacterial activity was screened for plants like *Cymbopogon nardus*, *Cymbopogon martini*, *Cymbopogon citratus*, *Thymus vulgaris*, *Syzygium aromaticum*, Cinnamaldehyde, geraniol, eugenol, and citral. *Cymbopogon nardus*, *Cymbopogon citratus*, *Cymbopogon martini*, geraniol, Citral were not effective in inhibiting strains like JSA01, JSA03, JSA05, JSA06, JSA09, JSA10, JSA11. The eugenol and thyme oil were effective against *Staphylococcus aureus* strains which showed a

zone of inhibition ranging from 10 to 19 mm. In *Cymbopogon nardus*, *Klebsiella pneumonia* was resistant to all the extracts, whereas *Bacillus subtilis* and *Serratia marcescens* showed maximum zone of inhibition to the methanol extract, which showed a zone of inhibition of 15 mm, 14 mm respectively. All the other pathogens like *E.coli*, *Staphylococcus aureus*, *Streptococcus species*, *Pseudomonas asaeruginosa*, *Salmonella typhimurium* were resistant to all the extracts except for Acetone were *E. coli* and *Streptococcus species* which showed the least zone of inhibition of 14 mm **Table 3, Graph 3 and Fig. 3.**

TABLE 3: EVALUATION OF ANTIMICROBIAL ACTIVITY OF CYMBOPOGON NARDUS USING VARIOUS SOLVENTS AGAINST SELECTED HUMAN PATHOGENS (THE VALUES REPRESENTS THE ZONE OF INHIBITION IN MM)

Solvents	Methanol	Ethanol	Chloroform	Acetone	Diethyl ether
<i>Staphylococcus aureus</i>	0	11	0	0	0
<i>E. coli</i>	0	0	0	14	10
<i>Streptococcus species</i>	0	0	0	14	0
<i>Pseudomonas aeruginosa</i>	14	0	0	0	0
<i>Klebsiella pneumonia</i>	0	0	0	0	0
<i>Bacillus subtilis</i>	15	12	12	0	0
<i>Salmonella typhimurium</i>	13	0	7	0	0
<i>Serratia marcescens</i>	14	0	0	0	0



GRAPH 3: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF CYMBOPOGON NARDUS

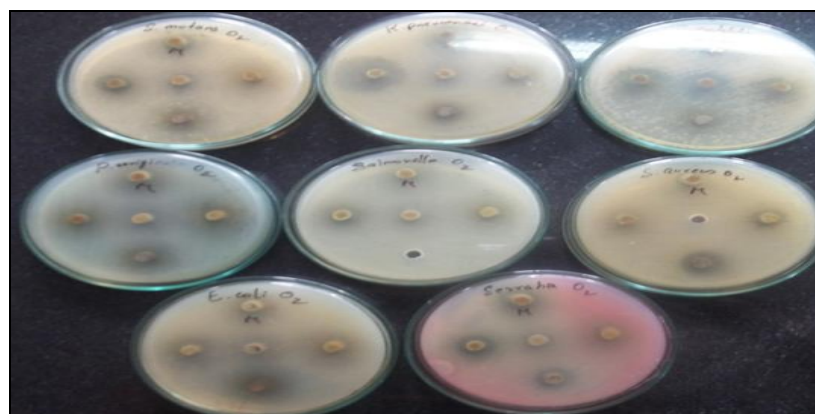


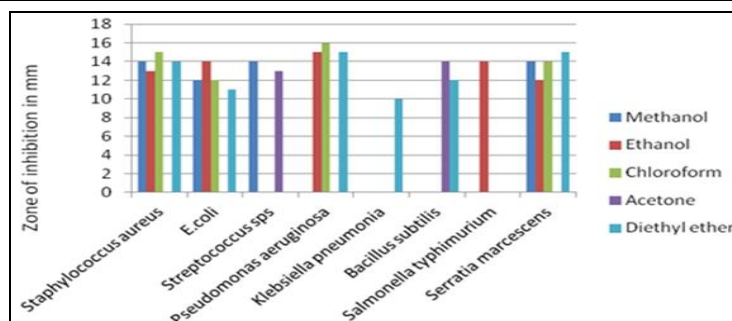
FIG. 3: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF CYMBOPOGON NARDUS AGAINST VARIOUS PATHOGENS

The essential oil of *Cymbopogon nardus* inhibited 36 bacterial strains growth isolated from cultural aquatic animals and also 7 ATCC bacterial strains type. The *Cymbopogon nardus* essential oil exhibited MIC values ranging from 0.244 mg/ml to 0.977 mg/ml against the bacterial isolates tested. The MIC values of the essential oil of *Cymbopogon nardus* were lesser than the MIC values of Hammer et al., which indicated that human pathogens were less susceptible to the *Cymbopogon nardus* essential oil compared to the bacterial isolates from aquatic animals. The *Cymbopogon martini* essential oil was known for its remarkable activity due to the chemical composition, amount, structural configuration, and their functional groups, and possible synergistic interactional best components¹¹. The essential oil of *Cymbopogon martini* showed strong efficacy against tested fungi compared to bacteria and against gram-positive bacteria than to gram-negative bacteria with higher resistance toward *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The biological activity of the essential oils was involved in many mechanisms of actions (inhibition of efflux pump, inhibition of cell wall formation, dysfunction of the fungal mitochondria, and cell membrane disruption); the inhibition of ergosterol synthesis is responsible for the superior potency of the *Cymbopogon martini* essential oil in this study

against fungal pathogen compared to bacterial pathogen¹². *Staphylococcus pyrogenes*, gram-positive bacteria, were recorded for the higher inhibitions at all tested concentrations compared to *Pseudomonas aeruginosa*, gram-negative bacteria. This is attributed to the overexpression of efflux pumps responsible for innate antimicrobial resistance¹³. The presence of sodium lauryl sulphate can be attributed to the increased activity of the hydrophilic ointment formulation, which is used as a surfactant, has biological activity against fungal and bacterial strains, while the presence of polyethylene glycols have excellent solubility in water attributes for the better antimicrobial activities of the macrozol blend ointment formulation and can enhance the diffusion bioactive constituents into the media which are more hydrophilic¹⁴. In *Cymbopogon flexuosus*, all the extracts showed the maximum zone of inhibition against the *Staphylococcus aureus*, *E.coli*, *Serratia marcescens*, and *Pseudomonas aeruginosa*, except for Acetone extract, which showed the least susceptible to it. *Klebsiella pneumonia* and *Salmonella typhimurium* showed resistance to all the extracts except for Diethyl ether and Ethanol, which showed the least susceptibility by exhibiting the zone of inhibition of 10 mm and 14 mm respectively **Table 4, Graph 4 and Fig. 4.**

TABLE 4: EVALUATION OF ANTIMICROBIAL ACTIVITY OF CYMBOPOGON FLEXUOSUS USING VARIOUS SOLVENTS AGAINST SELECTED HUMAN PATHOGENS (THE VALUES REPRESENTS THE ZONE OF INHIBITION IN MM)

Solvents	Methanol	Ethanol	Chloroform	Acetone	Diethyl ether
<i>Staphylococcus aureus</i>	14	13	15	0	14
<i>E. coli</i>	12	14	12	0	11
<i>Streptococcus species</i>	14	0	0	13	0
<i>Pseudomonas aeruginosa</i>	0	15	16	0	15
<i>Klebsiella pneumonia</i>	0	0	0	0	10
<i>Bacillus subtilis</i>	0	0	0	14	12
<i>Salmonella typhimurium</i>	0	14	0	0	0
<i>Serratia marcescens</i>	14	12	14	0	15



GRAPH 4: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF CYMBOPOGON FLEXUOSUS

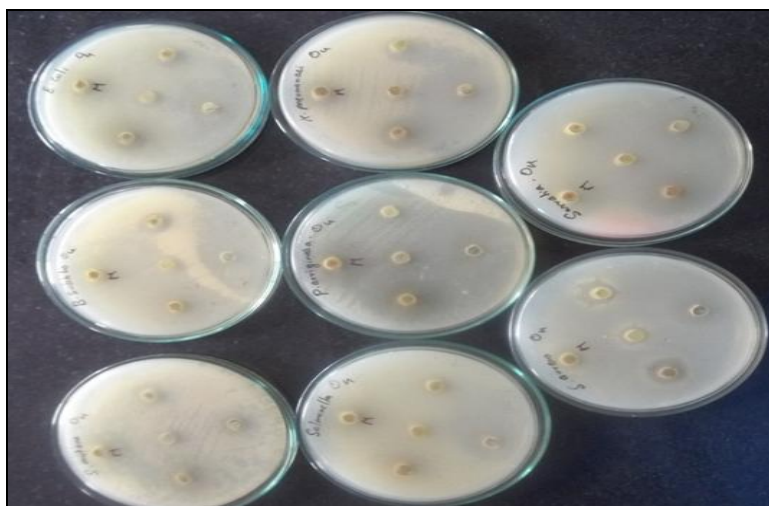


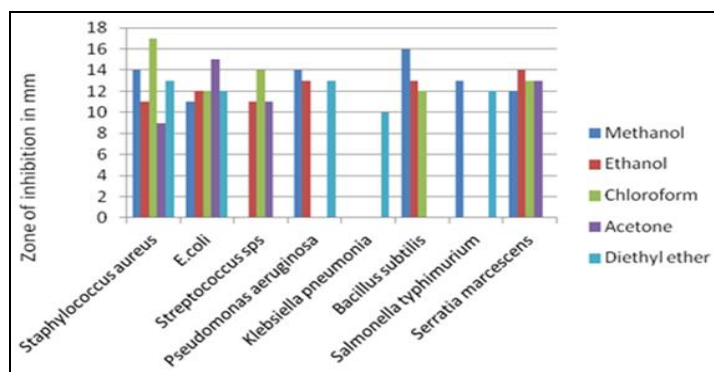
FIG. 4: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF CYMBOPOGON FLEXUOSUS AGAINST VARIOUS PATHOGENS

Shalini kakarla *et al.*, (2009) ¹⁵ has illustrated that the Essential oils of Lemongrass possessed higher antibacterial than antifungal activity. The ‘Krishna’ and ‘Cauveri’ essential oils exhibited strong activity against *Bacillus subtilis* by showing 45-47 mm of a zone of inhibition. They also exhibited strong inhibitory effects against *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli*. The EO constituents were screened for its antimicrobial activity revealed Citral has the highest activity against all bacteria except *Staphylococcus aureus* whereas the geraniol and geranyl acetate, the 2

other constituents, has a small effect against *Staphylococcus aureus* except for geranyl acetate showing some inhibitory activity. In *Cymbopogon schoenanthus*, all the extracts effectively inhibited the growth of *Staphylococcus aureus* and *E. coli* by exhibiting the highest zone of inhibition of 17 mm to the chloroform extract and 15 mm to the Acetone extract, respectively. All the extracts were not effective in inhibiting *Klebsiella pneumonia* which showed the least susceptibility to the Diethyl ether extract by exhibiting the zone of inhibition of 10 mm **Table 5, Graph 5 and Fig. 5.**

TABLE 5: EVALUATION OF ANTIMICROBIAL ACTIVITY OF CYMBOPOGON SCHOENANTHUS USING VARIOUS SOLVENTS AGAINST SELECTED HUMAN PATHOGENS (THE VALUES REPRESENTS THE ZONE OF INHIBITION IN MM)

Solvents	Methanol	Ethanol	Chloroform	Acetone	Diethyl ether
<i>Staphylococcus aureus</i>	14	11	17	9	13
<i>E. coli</i>	11	12	12	15	12
<i>Streptococcus species</i>	0	11	14	11	0
<i>Pseudomonas aeruginosa</i>	14	13	0	0	13
<i>Klebsiella pneumonia</i>	0	0	0	0	10
<i>Bacillus subtilis</i>	16	13	12	0	0
<i>Salmonella typhimurium</i>	13	0	0	0	12
<i>Serratia marcescens</i>	12	14	13	13	0



GRAPH 5: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF CYMBOPOGON SCHOENANTHUS

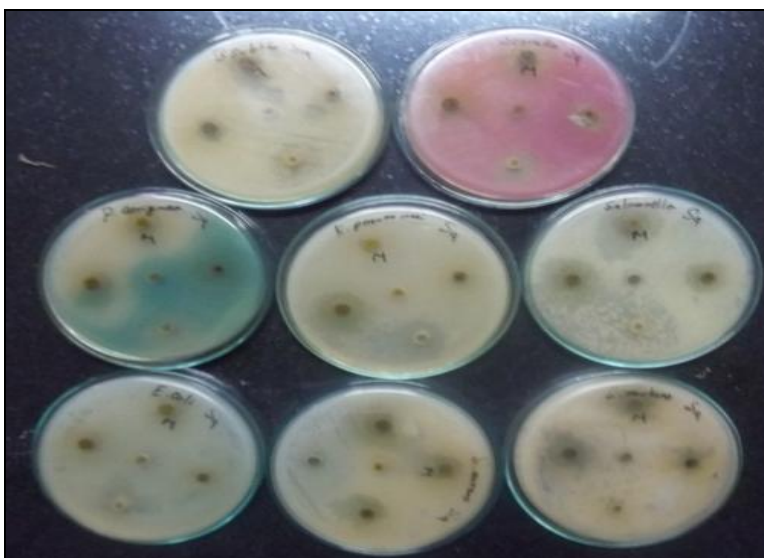


FIG. 5: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *CYBOPOGON SCHOENANTHUS* AGAINST VARIOUS PATHOGENS

In the current study, *Cymbopogon* species have been tested for antimicrobial activity by agar well diffusion method. *Klebsiella pneumonia* has shown resistance to all the extracts, whereas chloroform extracts of *Cymbopogon martini* **Table 2**, *Cymbopogon flexuosus* **Table 4**, and *Cymbopogon schoenanthus* **Table 5** showed the highest zone of inhibition against *Staphylococcus aureus* by exhibiting the zone of inhibition of 17 mm, 15 mm, and 17 mm respectively.

From the above study, it is clear that all the extracts in *Cymbopogon martini* **Table 2**, *Cymbopogon flexuosus* **Table 4** and *Cymbopogon schoenanthus* **Table 5** were effective in inhibiting *Staphylococcus aureus* and *Pseudomonas aeruginosa* which exhibited the highest zone of inhibition evaluated its antibacterial properties by disc diffusion method except for Acetone extract which was resistant to it.

All the extracts of *Cymbopogon* species **Table 1-5** were not effective in inhibiting the growth of *Klebsiella pneumonia*, indicating it is highly resistant to all the extracts of *Cymbopogon* species. Therefore, organisms like *Staphylococcus aureus* and *Pseudomonas aeruginosa* were sensitive and got inhibited against the leaf extracts of *Cymbopogon martini* **Table 2**, *Cymbopogon flexuosus* **Table 4** and *Cymbopogon schoenanthus* **Table 5**. The sensitivity of the organisms was studied by Agar well diffusion method, and the zone of inhibition around discs helps in differentiating the plant extracts effectively against

certain pathogens, which is very helpful in using these medicinal plants in the field of pharmacology.

CONCLUSION: The organic compounds which are present naturally in the plants can be extracted by using solvents. The antimicrobial activity exhibited by the plant crude extracts plays a significant role in fighting against pathogens and thrown the limelight in phytomedicine in combating against the pathogens resistant to multiple drugs. The extraction of the organic compounds depends upon the solubility property of the compound in the solvent. The extraction method differs between various products and the compound. The organic compounds present in medicinal plants have paved the pathway to treat various diseases in the field of pharmacology.

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