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Antimicrobial activity of *Cynodon dactylon* against MDR bacteria

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Abstract
The antimicrobial activity of *Cynodon dactylon* (Grass) were determined and compared against 2 Gram+ve bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and 2 Gram-ve bacteria (*Salmonella typhi* and *Escherichia coli*), which are Multi Drug Resistant (MDR). The *Cynodon dactylon* leaves were crushed and their extract was taken in Propanol. The efficiency of the extract were tested against MDR bacteria through well diffusion assay. In this endeavor the antimicrobial assay extract show inhibitory or antimicrobial activity against MDR bacteria. The propanol extract of *Cynodon dactylon* showed maximum antimicrobial activity against *Staphylococcus aureus* (Gram+ve) followed by *Bacillus cereus* (Gram+ve) while there is no antimicrobial activity obtained against *Salmonella typhi* (Gram-ve) and *Escherichia coli* (Gram-ve). On the basis of present finding it was concluded that the extract possess antimicrobial and pharmacological properties, hence can be used parallel to synthetic drugs which have undesirable side effects.

Keywords: *Cynodon dactylon*, MDR, antimicrobial activity, *S. aureus, B. cereus, S. typhi, E. coli*

Introduction
Infectious disease accounts for high proportion of health problems. Mortality due to these infections continues to be a major problems. Infections due to variety of microbial agents, such as pathogenic *Staphylococcus aureus, Bacillus cereus, Salmonella typhi* and *Escherichia coli* which are Multi Drug Resistant (MDR) are most common (Mukherjee *et al*.,1998) [7]. In present time multiple drug resistance in pathogenic microbes become a serious problem of humankind world wide (Peng *et al*.,2006) [8]. Synthetic drugs are not only expensive but also often associated with side effects. Therefore we have to control microbial infection via new infectious fighting strategies. However, the previous studies of rapid, widespread emergence of resistance for new antimicrobial agents indicates that even new families of these agents will have a short life expectancy while there are some advantages of using medicinal plants, such as often better patience tolerance, fewer side effects, relatively less expensive, acceptance due to long history of use and being renewable in nature. For all these reasons, researcher are increasingly turning their attention to herbal products, for development of new better drugs against multiple drug resistant microbial strains (Benkeblia, 2004) [3]. With the increasing incidence of antibiotic resistance by several pathogenic microbial agents, antimicrobial evaluation of medicinal plants has become the need of the hour. Biomolecules derived from plants have an advantage of being less toxic in comparison to synthetic agents (Gideon *et al*.,2016) **[6]**. According to WHO more than 80% worlds' population depends upon traditional medicine for their primary healthcare needs. Herbal medicine support about 75-80% of whole population and major part of routine therapy involves use of plant extract and active constituents (Akerele,1993) [1]. Medicinal plants are rich in various secondary metabolites of antimicrobial activities such as terpenoids, flavenoids, saponins, alkaloids alkenyl phenols, tannins, phorbol esters and glycoalkaloids. The screening of products of plants for antimicrobial properties have shown that the higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). The increased incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to unselective use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from medicinal plants. However, very few information is available on such activity of plants and out of 4 lakhs plant species on earth, only some has been studied for antimicrobial activities. Plant based medicinal substances are basis of many of the modern pharmaceuticals we use today for our various ailments. The plant kingdom harbors an inexhaustible source of active ingredients invaluable in intractable disease.

Bioactive compounds are normally gathered as secondary metabolites in cells of all plant but their concentration varies as per the plant parts, season climate and particular growth phase. Leaf is one of the highest accumulated part of the plant of such compounds and people are generally preferred it for therapeutic purposes some of the active compounds inhibit the growth of disease causing microbes either singly or in combination (Selvamohan *et al*., 2012) [9]. The present study was undertaken to evaluate the antimicrobial activity of propenolic extract of *Cynodon dactylon* leaves on 4 MDR (Multiple Drug Resistant) bacteria namely *Staphylococcus aureus, Bacillus cereus, Salmonella typhi* and *Escherichia coli*. *Cynodon dactylon* belonging to family Poaceae is considered as a sacred herb (Balasubramanian, *et al*., 2008) [2]. It posses various medicinal properties as an antidiabetic agent in traditional system of medicine. The aqueous plant extract is used as anti-inflammatory, anti-emetic, antioxidative, cardioprotative diuretic and purifying agents (Singh *et al*., 2007) [10].

Family Poaceae is one of the most commonly occurring weeds which is a hardy, perennial, creepy grass finding a wide distribution around the globe particularly in tropical areas and high temperature. The weed is fast growing, drought resistant, light green and very tough with a course texture and are found in short cylindrical pieces of 2-4mm in diameter and 3-20mm long (Gideon *et al*.,2016) **[6]**. Resistant Gram-positive pathogens, *Staphylococcus aureus* have become a serious problem in the medical community. *Staphylococcus aureus* is an organism with several virulent factors and resistance mechanisms at its disposal. It is a significant cause of a wide range of infectious disease in humans. *Staphylococcus aureus* often causes life-threatening deep seated infections like pneumonia bacteremia and endocarditis (Jajani *et al*., 2011) [6]. In the light of above mentioned fact, the present study was carried out to screen the antimicrobial activity Of *Cynodon dactylon* against MDR bacteria in Allahabad city.

Materials and Methods

1. **Place of Work:** The present study was carried out in Centre For Microbiology, Department of Botany, Ewing Christian College, Prayagraj.
2. **Study Sample:** The medicinal plant used for evaluating their antimicrobial activity was *Cyanodon dactylon.* For the study young fresh leaves of the plant was used.
3. **Collection of Plant Sample:** Fresh young leaves of Cyanodon dactylon were collected from the garden of Department of Botany, Ewing Christian College, Allahabad.
4. **Preparation of Extract:** leaf samples were properly washed and dried in Hot Air Oven. These were then grounded into fine powder using morter and Pestle. This powder were then used for extract preparation with solvent i.e. Propanol.
5. **Propanolic extract of *Cyanodon dactylon*:** for this 10 gm dried and finely ground leaves of Cyanodon dactylon were taken in air tight bottle and 50 ml of propanol were added and kept under dark. After two days, the content were stirred well and filtered using Whatman no. 1 filter paper. The filtrate was collected and stored in sterile glass beaker for further study (Hema *et al*., 2013) [5].
6. **Bacterial strains / Test organisms:** The antimicrobial activity of propanolic extract of Cyanodon dactylon was evaluated against two gram positive viz. *Staphylococcus aureus* and *Bacillus cereus* and two gram negative bacteria viz. *Escherichia coli* and *Salmonella typhi*.
7. **Inoculum preparation:** Nutrient broth were prepared and was transferred into test tubes (5ml each) and autoclaved for 15 – 20 mins at 121 ^{0}C. It was used for preparing bacterial suspensions which was incubated for approximately 24 hours and used for experiment.

Screening of antimicrobial activity: The plant extracts were tested on Nutrient Agar Medium to detect their antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhi* through well diffusion method. This whole work was carried out in Laminar Air Flow chamber. The Muller Hinton Agar (MHA) medium was poured in all the petriplates. One MHA plate was marked as media control plate. When the media solidified in all the plates, the plates were swabbed with test bacteria. Now wells of 5mm diameter were made on the swabbed media surface with the help of sterile media borer and the well were filled with prepared extract of *Cyanodon dactylon*. After this plates were kept at 37^{0}C for 24 hours. After proper incubation plates were examined for zone of inhibition around the wells and the zone was measured and recorded in mm (Hema *et al*., 2013) [5].

Result and Discussions

The result indicated that the Propanolic extract of *Cynodon dactylon* showed inhibitory or antimicrobial activity against MDR bacteria *S. aureus* and *B. cereus.* There is no inhibitory or antimicrobial activity obtained against *S. typhi* and *E.coli*.

Table 1: Antimicrobial activity of *Cynodon dactylon* against pathogens (in mm)

S. No.	Zone of Inhibition (in mm) / Test Organism				
1.	Sample	*S. aureus*	*B. cereus*	*E. coli*	*S. typhi*
2.	Propanolic Extract	19	12	00	00
3.	Solvent or Control	00	00	00	00

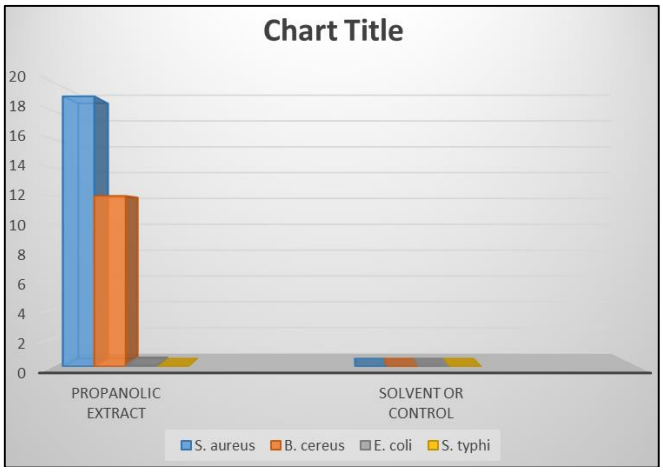

Fig 1: Antimicrobial Activity of *Cynodon dactylon* against Pathogens (in mm)

The diameter of zone of inhibition for each sample was recorded against each MDR bacteria (Table showing the zones of inhibition obtained against MDR of extract of *Cynodon dactylon*). The propanolic extract of *Cynodon dactylon* plant (leaves) showed the highest antimicrobial activity against *Staphylococcus aureus* (Gram +ve), the mean zone of inhibition (ZOI) being 19mm. The lowest antimicrobial activity of *Cynodon dactylon* in propanol

extract was observed against *Bacillus cereus* (Gram+ve) with mean ZOI being 12mm. While there is no zone of inhibition (ZOI) was obtained against *Escherichia coli* (Gram-ve) and *Salmonella typhi* (Gram-ve). The resistance of Gram-ve bacteria could be due to its cell wall structure which is biochemically more complex than Gram+ve and appears usually trilayered, Besides peptidoglycan, there are phospholipids, proteins and lipopolysaccharides in the cell wall. Hema *et al*., (2013) [5] also conducted study which support this study, showing the inhibitory activity of *Cynodon dactylon* leaves against Gram+ve but Gram-ve bacteria were resistant to the extract. They observed 11mm zone of inhibition against *S.aureus*, while in this study, mean zone of inhibition of *S. aureus* was observed to be 19mm and 11mm zone of inhibition against *B. cereus*, while in this study, mean zone of inhibition of *B. cereus* was observed to be 12mm. Gideon *et al*., (2016) [6] through their study 12mm zone of inhibition was observed against *Bacillus* spp. Which support this study, showing 12mm zone of inhibition of *B. cereus.* Selvamohan *et al*., (2012) [9] in their study recorded the maximum zone of inhibition produced by the extract of *Cynodon dactylon* against *Staphylococcus aureus* which support this study but they observed 13mm zone of inhibition against *S. aureus* while in this study, mean zone of inhibition of *S. aureus* was observed to be 12mm. Srinivasan *et al*., (2001) prepared an extract from the leaves of *Cynodon dactylon* and examined it's antimicrobial activity against some Gram+ve and Gram-ve bacteria, however they did not find any antimicrobial effects for this extract while in this study Gram+ve bacteria showing inhibitory or antibacterial activity and Gram-ve bacteria showing resistant perperties.

Conclusion

The research in medicinal plants has gained a renewed focus recently. The prime reason is that other system of medicine although effective comes with many side effects leading to serious complications. Plant based system of medicine being natural does not pose this serious problem. Hence, this alternative way of treatment can serve as an important platform for the development of inexpensive, safe and effective medicines. *C. dactylon* along with other medicinal plants has been playing a significant role from ancient time in traditional medicine, Ayurveda and in tribal medicine. *Cynodon dactylon* leaves offer an array of health benefits, being packed with antiseptic, analgesic, anti-inflammatory, wound healing, astringent, antioxidant, immunomodulatory, antidiabetic and anticancer activities. In conclusion, the demonstrated antimicrobial activities of leaf extract of *Cynodon dactylon* in this study has given a preface of their potentials as antimicrobial agents and replacement of synthetic drugs. The observed inhibitory effects of the extracts in the present study on the tested MDR bacteria is a justification for the need to explore the various traditional modes of disease treatments. This is very important as it will assist in standardizing traditional herbal medicine treatments. Lack of standardization has been described by WHO, 2000 as one of the problems mitigating against recognition of traditional medical practitioners. The antimicrobial activities observed in this study puts forth the importance of preserving and encouraging these medicinal plants according to the standards of scientific achievement and open evidence based views towards a more rational policy on medicinal plant research.

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