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SCREENING OF TRADITIONALLY USED PLANTS FOR ANTIBACTERIAL ACTIVITY AND ITS PHYTOCHEMICAL ANALYSIS

Singh M[1], Mishra P[2]

[1] Dr. M C Saxena College of Engineering and Technology, Lucknow, UP, India
[2]Integral University, Lucknow, U.P.

***Corresponding Author: Manish Singh**

Email ID: pallsharma91@gmail.com

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ABSTRACT

Cynodon dactylon is a perennial grass present in approximately everywhere (mostly in warm region), which is used as treatment of many diseases like diarrhea, tumor and cancer, wound etc. In this study the methanolic extract of *Cynodon dactylon* was investigated for their antibacterial activities against 6 bacteria that *is E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonela typhi, Mycobacterium tuberculosis, Staphylococcus aureus* with the help of NAM media and measured zone of inhibition and NB media for minimum inhibitory concentration on 620nm wavelength. In this, the ampicillin and rifampicin (Tb drug) was taken as positive control. In *Cynodon dactylon* the Carbohydrate, phenols and tanins, saponins, steroids, alkaloid, terpenoid, catecholic tanin was demonstrated by phytochemical analysis.

Key words: *Cynodon dactylon, Mycobacterium tuberculosis,* Phytochemicals, Alkaloid, Antibiogram.

INTRODUCTION

The large number of drug are isolated from plants, such like that one is *Cynodon dactylon* which is used as medicines from ancient time **[1]**. The doob grass is commonly known as dhruva grass, Bermuda grass etc. **[2]**. According to classification it belongs to Angiosperms-Phylum, Class- Monocots, Family- Poaceae **[3]**.

The doob grass is easly available around us and mostly found in warm climatic region. It is a perennial creepling herb **[2]**. It reproduce through seeds, runners, and rhizomes **[1]**. Doob grass is used as medicine from ancient times, it is used in treatment of cough, chronic diarrhea, cancer etc, that means the antibiotic resistance is found in doob grass **[4]**. Further, it has antimicrobial, antihistaminic, antidiabetic, and antioxidant activity **[5]**. Plants play a very important role in pharmaceutical industries in developing alternative drug **[6]**. The word antibiotics means against life. Many bacteria which live in our body and some are harmful to us and also from outside bacteria inter in our body, therefore some are also harmful **[7]**. They cause many types of diseases **[8]**. An antibiotic is a type antimicrobial substance active against bacteria. They may either kill or inhibit the growth of bacteria. The most important type of antibiotic medications is widely used in the treatment and prevention of such infections. Antibiotics have been used since ancient times (Antibiotics revolutionized medicine in the 20th century **[9]**. Many pharmacological industries can form many types of new antibiotic, with some modification of old antibiotic for better work. That's antibiotic are good to kill the micro-organism or suppress the division of micro-organism **[10]**. But we also know that in those bacteria, few bacteria and fungus are resistant from antibiotic, And then that antibiotic can't work properly **[11]**. Some antibiotics attack on aerobic bacteria, while others works against anaerobic bacteria **[12]**.

Amita SR. et al., (2011) studied on potential antibacterial and antifungal activity of Cynodon dactylon, they were used aqueous extract of whole part of plant to treat painful and inflammatory condition. From that study they were find them antimicrobial activity on *Pseudomonas aeruginosa, Klebsiella pneumonia, E.coli, Sa, Proteus mirabilis* and *Candida albicans*

They were find the antimicrobial activity through anti sensitivity test, and find the clear zone of inhibition and also find the phytochemical test on *Cynodon dactylon,* the result saponins, tanins, steroids, and flavonoids was present . All results was positive on bacteria except one that was Candida albicans **[13]**. Abdullah S. et al., *(*2012) suggested that preliminary phytochemical study and antimicrobial activity from various extract of *Cynodon dectylon.*

They can say that grass have possesses medicinal values. They were take 7 solvent that is acetone, chloroform, diethyl ether, ethanol, ethyl acetate, methanol, and n-pentane to study phytochemical test. And they were also test on some microbes like *Bacillus cereus, Bacillus subtilis E. coli, Klebsiella spp., Pa, staphylococcus pyogenes,* and *Straptococcus pneumonia* used diffusion method. In this, some solvents with sample give better result and some was less, they were also obtain zone of inhibition. The ethyl acetate extracts shows broad spectrum activity to all the tested pathogen where no activity observed for n-pentane extraction **[14]**. Solanki R and Nagori BP., (2012) studied on screening of antibacterial activity of hydroalcoholic extract of *Cynodon dactylon,*_from that's the grass plant is use to medical treatment like cramps, measles, tumors, wounds, and warts. They were take hydroalcoholic extract of *Cynodon dectylon* to investigate on two gram positive and two gram-negative bacteria that are *SA, staphylococcus albicus,* and *E.coli, PA* with the help of agar well diffusion methids and micro-dilution method. Their control was ciprofloxacin. They take ratio of hydroalcoholic extract and solvent was 60:40 of water and ethanol. From there result sample give positive result in ZOI and bacterial strains were sensitive towards extract from minimum inhibitory concentration **[15]**.

MATERIALS AND METHODS

Collection of samples:

The sample doob grass (*Cynodon dectylon*) was collected from Lucknow (UP).

Preparation of plant extract by solvent extraction method:

Plant samples were collected and firstly washed with distilled water then air dried. Then the samples were separated in the leaves and stem portions and dipped in 1:10 ratio with respective solvents such as methanol, petroleum ether and distilled water, then kept in dark for 24 hrs.

The extracts were allowed for evaporation of solvents once filtered by using whatmann filter paper No.1 and weighed bowls. The extracts got dried; 300µl of Dimethyl Sulphoxide (DMSO) was added and scratched. The scratched extract was then transferred to micro-centrifuge tube **[16, 17]**.

Antibiogram analysis:

It is the method which is used for testing the efficacy of antibiotics by introducing an antibiotic into the middle of the bacteria-laden petri dish. A clear zone indicates the bactericidal activity. The greater is the diameter of the zone, the higher the efficacy of the antibiotic towards the bacteria **[18]**.

Minimal inhibitory concentration (MIC) test of sample:

MIC test was carried out by using broth dilution method. The extracts were serially diluted in broth containing bacterial cultures and incubated at 37°C for 24 hours. Further the MIC value was calculated once OD was taken at 620 nm wavelength **[19]**.

Phytochemical analysis:

For carbohydrates:

Take 0.5ml extract and add 0.5ml Fehling A and then add 0.5ml Fehling B. then boil gently, when the precipitate is obtained then it means result is positive.

For Phenols and Tannins:

Took 0.1 ml of grass stem methanol extract. Added 0.2 ml of 2% $FeCl_3$. Blue- green or black coloration indicate positive result.

For Saponins:

Take 0.1ml extract and add 0.4ml distilled water, then shake it, if froth is present then result is positive.

For Steroids:

Take 0.1ml extract and add 0.2ml chloroform then add 0.2ml sulphuric acid, when red colour in lower chloroform is obtain then result is positive.

For Flavonoids:

Take 0.1ml extract and add 0.2ml sodium hydroxide of 2%, then yellow colour is obtained, and then adding 0.2ml HCl of 1N.

For Alkaloids:

Take 0.1ml extract and add 0.5ml HCl of 5%, then heated on boling water bath on 70°C/10min. add 0.5ml few drops of 5% NaOH, after some time turbidity and yellow precipitate is obtained.

For Terpenoids:

Take 0.1ml extract and add 0.5ml acetic acid and then 0.5ml chloroform. After some time add 0.5ml concentrated sulphuric, add slowly and red violet colour was observed for the presence of terpenoid.

For Catecholictanins:

Take 0.1ml extract and add 0.5ml distilled water, then add 0.1ml ferric chloride of 2%, and then result is obtained.

For Gallictanins:

Take 0.1ml extract and add 0.5ml distilled water, then add 0.5ml ferric chloride of 2%, when blue color obtained then it give positive result.

For Protein estimation test:

Take two clean test tubes and take extract and put extract in one test tube where one is empty, then add 0.5ml distilled water, in empty test tube and 0.4ml in extract test tube. Add 2.5ml Bradford reagent in both test tube, keep in dark place for 30min. After 30 min. later, take OD, when it give positive then it means protein are present and when it give negative result that means protein is not present.

RESULTS

Sample collection and extract preparation:

Figure 1: Collected sample of *Cynodon dactylon* (Doob grass)

Antibiogram analysis of extracts:

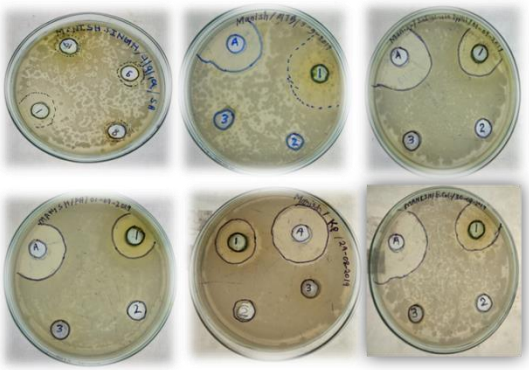

Figure 2: Zone of inhibition of grass with methanol on *E. coli, K pneumonia, P aeurogenosa, S typhi, M tuberculosis, S aureus.*

Table 1: Antibiotic sensitivity test of the extracts against bacterial pathogens.

Pathogen	Zone of inhibition (mm)			
	A	S1	S2	S3
E. coli	30	22.5	0	0
K pneumonia	25.5	19	9.5	0
P aeurogenosa	21.5	20	0	0
S typhi	22.5	22.5	0	0
M tuberculosis	25.5	28.5	0	0
S aureus	36	18	0	0

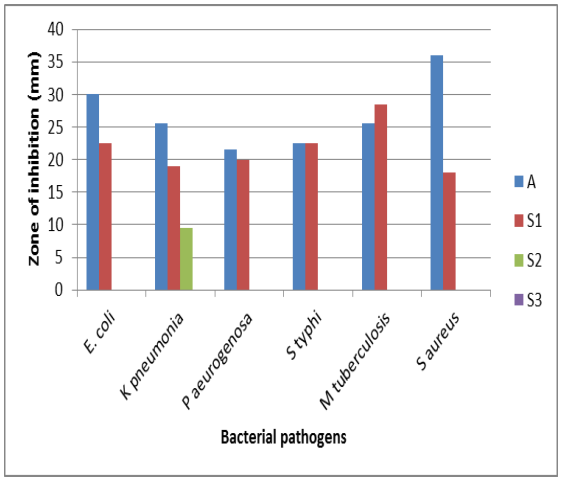

Figure 3: Graphical representation of the antibacterial activity of extracts against bacterial pathogens.

Where, A = AMPICILLIN (CONTOL), S1 = GRASS STEAM+ METHANOL, S2= GRASS STEAM+ PETROLEUM ETHER, S3= GRASS STEAM+ DISTILLED WATER

Comparison of methanolic extract of doob grass with Rifampicin against *M tuberculosis*:

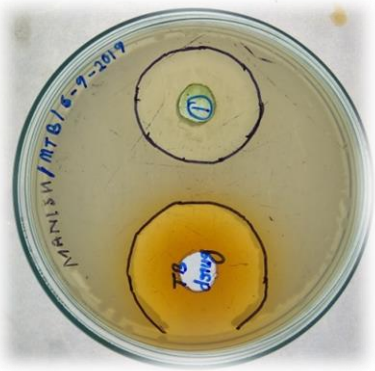

Figure 4: Zone of inhibition of methanol extract and Refampicin on *M tuberculosis.*

Table 2: Antibiotic sensitivity test of the methanol extracts and Refampicin on *M tuberculosis.*

Pathogen	**Zone of inhibition (mm)**	
	Rifampicin	S1
M tuberculosis	30	26

Minimal inhibitory concentration (MIC) test of sample result:

Minimal inhibitory concentration (MIC) test:

Table 3: MIC value of methanolic extract against *M tuberculosis*

SAMPLE	MIC(mg/ml)
Blank	0
Methanol extract	0.462

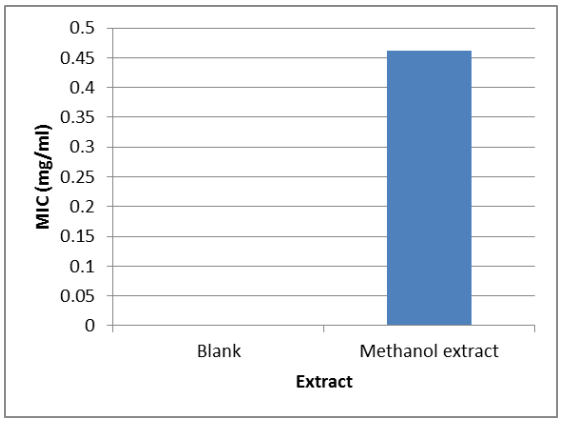

Figure 5: Graphical representation of MIC value of methanolic extract against *M tuberculosis*

Phytochemical analysis:

Table 4: Phytochemical analysis result

S. No.	TEST	RESULT
1.	Carbohydrate	Positive
2.	Phenol and tannin	Positive
3.	Saponin	Positive
4.	Steroids	Positive
5.	Flavonoids	Negative
6.	Alkaloids	Positive
7.	Terpenoids	Positive
8.	Catecholic tannins	Positive
9.	Gallictaninns	Negative
10.	Protein	Negative

Figure 6: Phytochemical tests of methanolic extract of Doob grass

DISCUSSION AND CONCLUSION

With the modernization of the world and advancement in technology we have a great increase in medicines.

Herbal drugs have various applications in pharmaceuticals and also are widely used in cosmetics. However, the preparation of biocompatible and non-toxic herbal drugs is a very important now a days. I choose doob grass for the extraction of active metabolites. The extraction was done by using solvent extraction method. The antimicrobial activity of these extracts was checked against *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Mycobacterium tuberculosis, K pneumonia* and *Pseudomonas aeruginosa* by using agar well diffusion method. Where it was found that the best results were shown against the *Mycobacterium tuberculosis*. After getting the best results the activity was compared with the control drug that is rifampicin, which is used for the treatment of the Tb.

The concentration of the drug was calculated by using broth dilution method against the *Mycobacterium tuberculosis.* Last but not the least the phytochemical analysis was done to find out the metabolites present in the plant Based on above research work it can be concluded that *Cynodon dactylon* can be the good source for the medicinal drug and can be explored further with the nanoparticles in order to increase the antimicrobial effect.

Further work also includes the further purification of the metabolites responsible for antibacterial properties using sophisticated purification procedure, and the nanoparticle preparation. Pharmacologically evaluation of extracts with the nanoparticles of the various metal ions, for human consumption and also investigation of Phytochemical responsible for antibacterial properties.

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