International Standard Serial Number (ISSN): 2249-6807

*International Journal of Institutional Pharmacy and Life Sciences 6(1): January-February 2016*

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

**Life Sciences**

**Research Article……!!!**

Received: 11-01-2016; Revised: 08-02-2016; Accepted: 09-02-2016

# ANTIMICROBIAL ACTIVITY OF *ZIZIPHUS MAURITIANA* AND *CYNODON DACTYLON* LEAVES EXTRACTS

Padma Singh[1] and Pooja Dhiman[2]*

1. Department of Microbiology, Kanya Gurukul girls campus, Gurukul Kangri University Haridwar, (Uttrakhand)-249407
2. G-14, A.N.Khosla Bhawan, IIT Roorkee (Uttrakhand)

**Keywords:**

*Ziziphus mauritiana*, *Cynodon dactylon*, Antimicrobial activity

**For Correspondence:**

**Pooja Dhiman**

G-14, A.N.Khosla Bhawan, IIT Roorkee (Uttrakhand)

**E-mail:**

poojadhiman.0023@gmail.com

## ABSTRACT

*Ziziphus mauritiana* and *Cynodon dactylon* having tremendous medicinal properties. Both the weeds are reported to possess bioactive compounds, recognized for traditional use and medicinal importance .In the present study the different extracts ( aqueous, acetone and ethanol) of *Z. mauritiana* and *C.dactylon* leaves were prepared and tested against *Aspergillus fumigatus, Mucor spp., Staphylococcus aureus* and *E. coli* by agar well diffusion assay .

Three different concentration (200, 400, 600mg/ml for fungi) and (200, 250 and 300mg/ml for bacteria ) of the extracts were apply for their antimicrobial potential against the isolates. The acetone extract of *Z.mauritiana* and aqueous extract of *C. dactylon* showed maximum antifungal activity against *A.fumigatus* (i.e. 47.05% & 88.33%) and ethanol extract of *Z. mauritiana* and *C.dactylon* showed maximum antifungal activity (37.5% &39.42%) against Mucor spp. at 600mg/ml out of other extracts. For bacterial isolates aqueous extract and ethanol extract of *Z.mauritiana* and *C. dactylon* showed maximum antibacterial activity against *E.coli* (i.e. 79.71% & 47.01%) and ethanol extract (79.71%) and acetone extract (71.01%) showed maximum antifungal activity against *S. aureus* out of other extracts at 300mg/ml. Hence these extracts reflected presence of variety of compounds and solvents which are responsible for antimicrobial activity against the pathogens.

## INTRODUCTION

Many plants produces special subustances in their roots, leaves, flower or seeds that help them to survive. This ability to synthesise a wide variety of chemical compounds is used to perform important biological function as well as to defend against attack from predator such as insect, bacteria, fungi etc. plants are the only source for natural drugs. Many of the powerful drugs used in modern medicine are original from plants. These drugs are relatively cheaper and safer than synthetic or modern drug.

*Rhamnaceae* comprises about 40 species distributed in warm-temperate and subtropical regions out of which *Z. mauritana* Lam., very common. Carbohydrates, starch, proteins, sugar, mucilage and vitamins are abundantly present in Ziziphus species. *Z. mauritana* is generally grown in dry places[1]. *Z. mauritiana was* reported few pharmacological reports on antioxidant, antisteroidogenic activity[2]. Aditionally its fruits extracts cause neurotransmitter release which probably related to presence of ascorbic acid and the leaves may potentially be safe for use as sedative drug[3]. The antimicrobial activity of extracts of leaves of *Z. mauritiana* was screened for some pathogenic strains[4]. *C. dactylon* is commony known as Durva or doob grass. *C. dactylon* is a weed which belongs to the family Poaceae. It is said to have many medicinal properties including antiemetic, anti diabetic, diuretic, antiinflammatory, hepatoprotective activity as well as treatment of urinary tract infections, prostatitis, syphilis, and dysentery[5,6]. The present study was undertaken to provide scientific validation for the traditional use of two very common weeds *Z. mauritiana* and C.*dactylon* as antimicrobial agent.

## MATERIALS AND METHODS

### Collection of plant material

Leaves of the plants (*Ziziphus mauritiana* and *Cynodon dactylon*) were collected from the Jwalapur Pul by pass, Haridwar Uttrakhand in polythene bags. The leaves were shade dried for about two weeks. The dried leaves were griended to form fine powder by mean of a blender. The powder is placed in plastic bags and kept in a cool and dry place for further use.

### Extraction (Maceration Process)

10 gram of leaf powder was mixed with 200ml of each distilled water (for aqueous extract), 95% ehanol ( for ethanol extract) and 95% acetone ( for acetone extract) separately on water bath at $50^0$C. After 48hr it was then filtered using No.1 Whatman filter paper[7] and later evaporated to till the volume becomes 1/4$^{th}$ of the original[8].

**Isolation of the test organisms**

The isolation of test organism was carried out from Ganga river by serial dilution method. For fungal isolation1 ml of aliquot were added from dilution $10^{-3}$, $10^{-4}$, $10^{-5}$ to the Sabouaurd's agar Media and for bacterial isolation 1 ml of aliquots were added from dilution $10^{-4}$, $10^{-5}$, $10^{-6}$ to the Nutient Agar media. The fungal spp were identified by Lactophenol cotton blue staining and bacteria by Gram Staining technique. Mannitol salt agar and Mac conkey Agar media was used for the selective isolation of *Staphylococcus aureus* and *E. coli*. Sugar fermentation, catalase, starch hydrolysis, citrate utilization, indole production, methyl red test, voges- Proskauer test were performed for the biochemical identification of Bacterial isolates.After identification all the isolates was set to 0.5 Mc Farland Standard.[9] .

**Drug Sensitivity test**

Five different antifungal drugs Fluconazole, Ketoconazole, Co-trimoxazole, secnidazole and terbinafine ( 10mg/ml) were tested for *Aspergillus fumigates* and *Mucor spp*. Multidrug disc (Ampicilin, tetracycline, Gentamicin, Piperacillin, Chloramphenicol, Amikacin, Gatifloxacin, ceftizomin, Ciprofloxacin,and ofloxacin) was used for *Staphylococcus aureus* and *E. coli*.

**Sensitivity testing of extracts against isolates**

For *Aspergillus fumigatus* and *Mucor spp* Different concentration of both plant extracts (i.e. 200mg/ml, 400mg/ml, 600mg/ml) and for Staphylococcus aureus and E. coli 200mg/ml, 250mg/ml, 300mg/ml were tested for their antimicrobial potential by agar well diffusion method[10].after incubation the zone of inhibition was measured with positive control using the following formula-

$$percent\ inhibition = \frac{\text{mean of samples}}{mean\ of\ positive\ control} \text{x}100$$

**Minimum inhibitory concentration**

The Minimum Inhibitory Concentration was determined using tube dilution technique[19].1 ml of Different concentrations of the extracts were introduced into 9 ml of nutrient broth in test tubes. About 0.1 ml of the culture set to 0.5 Mc farland standard was added and incubated accordingly. The least concentration of the extract that did not permit turbidity in the broth was taken as the minimum inhibitory concentration.

**Minimum fungicidal concentration and minimum bactericidal concentration**

Streak plate technique was employed for MFC and MIC . A freshly prepared nutrient medium was inoculated from the tube having least concentration that showed no visible growth and incubated for 24 h at 37ºC for bacteria and $27^0$C at 48 h for fungi. The lowest

concentration in which no growth occurs on the solid medium was accepted as the minimum fungicidal and bactericidal concentration.

**Phytochemical screening**

Phytochemical screening of extracts was performed for Tannin, Alkaloids, Flavanoids, Phytosterols, Phenols, Carbohydrates, proteins etc.[11,12,13].

**Observations**

**Table 1: Antibacterial activity of leaf extract of *Z. Mauritiana* against *S. aureus* and *E coli***

| ORGANISM | *S. aureus* | | | | *E. coli* | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Conc. mg/ml** | **Zone of inhibition (mm)** | | **% Inhibition** | **Zone of inhibition (mm)** | | **% inhibition** |
| | | **Mean** | **Std Error** | | **Mean** | **Std Error** | |
| Aqueous | 200 | 20.0 | ±2.0 | 74.07 | 21.0 | ±1.0 | 56.75 |
| | 250 | 23.5 | ±1.5 | 74.60 | 24.0 | ±0.0 | 57.14 |
| | 300 | 26.5 | ±2.5 | 76.81 | 26.5 | ±1.5 | 58.88 |
| Ethanol | 200 | 20.5 | ±0.5 | 75.92 | 12.5 | ±1.5 | 33.78 |
| | 250 | 25.0 | ±2.0 | 79.36 | 14.5 | ±2.5 | 34.52 |
| | 300 | **27.5** | **±3.5** | **79.71** | 18.0 | ±1.0 | 40.0 |
| Acetone | 200 | 16.0 | ±3.0 | 59.25 | 14.0 | ±0.0 | 37.83 |
| | 250 | 19.5 | ±1.5 | 61.90 | 16.0 | ±1.0 | 38.09 |
| | 300 | 23.5 | ±0.5 | 68.11 | **18.5** | **±1.5** | **48.11** |
| positive control (Erythromycin for *S. aureus*) and chloramphenicol for *E.coli*) | 200 | 27.0 | ±3.0 | - | 37.0 | ±2.0 | - |
| | 250 | 31.5 | ±1.5 | - | 42.0 | ±1.0 | - |
| | 300 | 34.5 | ±2.5 | - | 45.0 | ±3.0 | - |

**Table 2: Antibacterial activity of leaf extract of *C. dactylon* against *S.aureus* and *E. coli.***

| ORGANISM | *S. aureus* | | | | *E. coli* | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Conc. mg/ml** | **Zone of inhibition (mm)** | | **% Inhibition** | **Zone of inhibition (mm)** | | **% inhibition** |
| | | **Mean** | **Std Error** | | **Mean** | **Std Error** | |
| Aqueous | 200 | 14.5 | ±3.5 | 53.70 | 6.0 | ±1.0 | 16.21 |
| | 250 | 18.5 | ±1.5 | 58.53 | 9.0 | ±1.0 | 21.42 |
| | 300 | 20.5 | ±1.0 | 59.42 | 13.0 | ±2.0 | 28.88 |
| Ethanol | 200 | 13.0 | ±2.0 | 48.14 | 15.0 | ±0.0 | 40.54 |
| | 250 | 15.5 | ±1.5 | 49.20 | 18.0 | ±1.0 | 42.85 |
| | 300 | 17.5 | ±1.5 | 50.72 | 21.5 | ±1.5 | **47.77** |
| Acetone | 200 | 18.5 | ±0.5 | 68.15 | 12.0 | ±2.0 | 32.43 |
| | 250 | 22.0 | ±2.0 | 69.84 | 15.5 | ±1.5 | 36.90 |
| | 300 | 24.5 | ±2.5 | **71.01** | 20.5 | ±1.5 | 45.55 |
| Positive control (Erythromycin for *S. aureus*) and chloramphenicol for *E.coli*) | 200 | 27.0 | ±3.0 | - | 37.0 | ±2.0 | - |
| | 250 | 31.5 | ±1.5 | - | 42.0 | ±1.0 | - |
| | 300 | 34.5 | ±2.5 | - | 45.0 | ±3.0 | - |

**Table 3: Antifungal activity of leaf extract of *Z. mauririan* against *A.fumigatus* and *Mucor spp.***

| ORGANISM | *A. fumigatus* | | | | *Mucor spp.* | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Conc. mg/ml** | **Zone of inhibition (mm)** | | **% Inhibition** | **Zone of inhibition (mm)** | | **% inhibition** |
| | | **Mean** | **Std Error** | | **Mean** | **Std Error** | |
| Aqueous | 400 | - | - | - | 8 | ±1.0 | 15.05 |
| | 500 | - | - | - | 10.5 | ±1.5 | 23.33 |
| | 600 | 8 | ±1.0 | 26.66 | 12.5 | ±0.5 | 27.77 |
| Ethanol | 400 | - | - | - | 18 | ±0.0 | 34.61 |
| | 500 | 10.5 | ±0.5 | 29.57 | 19.5 | ±0.5 | 27.19 |
| | 600 | 14 | ±1.0 | 39.43 | 15.5 | ±0.5 | **37.5** |
| Acetone | 400 | 11 | ±1.0 | 25.88 | 10 | ±1.0 | 22.22 |
| | 500 | 16.5 | ±0.5 | 38.82 | 11.5 | ±0.5 | 20.17 |
| | 600 | 20 | ±1.0 | **47.05** | 13 | ±1.0 | 25.00 |
| Positive control (Terbinafine for *A. fumigatus)* (Fluconazole for *Mucor spp*) | 400 | 30 | ±2.0 | - | 45 | ±3.0 | - |
| | 500 | 35.5 | ±1.0 | - | 52 | ±1.5 | - |
| | 600 | 42.5 | ±3.0 | - | 57 | ±2.5 | - |

**Table 4:Antifungal activity of leaf extract of *C. dactylon* against *A.fumigatus* and *Mucor spp.***

| ORGANISM | *A. fumigatus* | | | | *Mucor spp* | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Conc. mg/ml** | **Zone of inhibition (mm)** | | **% Inhibition** | **Zone of inhibition (mm)** | | **% inhibition** |
| | | **Mean** | **Std Error** | | **Mean** | **Std Error** | |
| Aqueous | 400 | 11 | ±1.0 | 36.66 | 5.5 | ±0.5 | 10.57 |
| | 500 | 18 | ±00 | 60.00 | 10.5 | ±1.5 | 23.33 |
| | 600 | 26.5 | ±2.5 | 88.33 | 13.5 | ±0.5 | 30 |
| Ethanol | 400 | - | - | - | 13.5 | ±0.5 | 23.68 |
| | 500 | - | - | - | 19 | ±0.5 | 36.53 |
| | 600 | - | - | - | 20.5 | ±1.0 | 39.42 |
| Acetone | 400 | - | - | - | 8 | ±0.5 | 17.77 |
| | 500 | 6.5 | ±1.5 | 15.29 | 17 | ±1.0 | 29.82 |
| | 600 | 13.5 | ±1.5 | 31.76 | 19 | ±2.0 | 33.33 |
| positive control (Terbinafine for *A. fumigatus)* (Fluconazole for *Mucor spp*) | 400 | 30 | ±2.0 | - | 45 | ±3.0 | - |
| | 500 | 35.5 | ±1.0 | - | 52 | ±1.5 | - |
| | 600 | 42.5 | ±3.0 | - | 57 | ±2.5 | - |

**Table 5: Determination of MIC and MBC**

| Extract | *Z. mauritiana* | | | | *C. dactylon* | | | |
|---|---|---|---|---|---|---|---|---|
| | ***E. coli*** | | ***S.aureus*** | | ***E. coli*** | | ***S.aureus*** | |
| | MIC (mg/ml) | MBC (mg/ml) | MIC (mg/ml) | MBC (mg/ml) | MIC (mg/ml) | MBC (mg/ml) | MIC (mg/ml) | MBC (mg/ml) |
| Aqueous | 0.1 | 10 | 0.01 | 10 | 10 | 100 | 10 | 100 |
| Ethanol | 1 | 100 | 0.1 | 1 | 1 | 10 | 1 | 100 |
| Acetone | 1 | 100 | 1 | 100 | 10 | 10 | 0.1 | 10 |

**Table 6: Determination of MIC and MFC**

| Extract | *Z. mauritiana* | | | | *C. dactylon* | | | |
|---|---|---|---|---|---|---|---|---|
| | ***A. fumigatus*** | | ***Mucor spp*** | | ***A.fumigatus*** | | ***Mucor spp*** | |
| | MIC (mg/ml) | MfC (mg/ml) | MIC (mg/ml) | MFC (mg/ml) | MIC (mg/ml) | MFC (mg/ml) | MIC (mg/ml) | MFC (mg/ml) |
| Aqueous | 0.1 | 10 | 0.01 | 10 | 0.1 | 0.1 | 10 | 100 |
| Ethanol | 1 | 100 | 0.1 | 1 | 1 | 100 | 1 | 100 |
| Acetone | 1 | 100 | 1 | 100 | 10 | 10 | 0.01 | 0.01 |

**Table 7: Phytochemical Screening of *Z. mauritiana* and *C. dactylon***

| S.No | Tests | ***Z. mauritiana*** | ***C. dactylon*** |
|---|---|---|---|
| 1 | Tannin | + | + |
| 2 | Alkaloids | + | - |
| 3 | Flavanoids | + | + |
| 4 | Phytosterols | + | + |
| 5 | Phenols | + | + |
| 6 | Carbohydrates | - | - |
| 7 | Proteins | + | ++ |

Abbreviations: MIC – minimum inhibitory concentration, MBC- minimum bactericidal concentration ,MFC- minimum fungicidal concentration, (+) = Positive result / (-) = Negative result / (++) Good Positive result, ZOI- zone of inhibition.

## RESULTS AND DISCUSSION

The present study carried out determine the antimicrobial activity of *Ziziphus mauritiana* and *Cynodon dactylon* against *Aspergillus niger, Mucor spp, Staphylococcus aureus* and *E. coli* According to Kanimozhi *et al*., 2012 study of antifungal activity of *C. dactylon* indicate maximum activity in ethanol extract against *A. niger* and *C. albicans* [14]but in our study we found its aqueous extract ( ZOI=26.5mm at 600mg/ml) was more effective against *A. fumigates* than acetone and ethanol extracts and in case of *Ziziphus mauritiana* Chowdary *et al.,* 2000 indicated maximum activity in ethanol extract against *A.flavus, A. niger* and *Alternaria alternate* at different concentration [15]but in our study its acetone extract gave maximum zone of inhibition i.e. 20mm at 600mg/ml against *A. fumigates* in comparision to aqueous and ethanol extract. On the other hand Mucor spp. gave maximum ZOI of 19.5 in ethanol extract out of aqueous and acetone. Whereas *C. dactylon* ethanol extract showed maximum ZOI (21.5mm and 24.5mm) against *E.coli* and *S. aureus* in comparision to aqueous and acetone extracts. *Z. mauritiana* aqueous extract gave maximum ZOI of 26.5mm against *E.coli* and ethanol extract gave 27.5mm ZOI against *S. aureus* at 300mg/ml. In Table 7 it is showed the presence of six different constituents in *Z. mauritiana* i.e. Alkaloids, phenols, proteins, Flavanoids, Polysterol, and Tannins but *C. dactylon* contain proteins, phenols, Flavanoids, Polysterol, and Tannins except Alkaloids and carbohydrate is absent.

## CONCLUSION

Plants consist of a wide range of phytochemical compounds, such as alkaloids, terpenoids, flavonoids, saponins,carbohydrate,proteins, phenols tannins, etc. which were utilised by the plants itself for defense mechanism from the external and internal injury and to maintain the plant all biological activities. This is accordance to the extraction yield's result most of the polar solvents able to resolve most of the plant bioactive constituents. Which enhance the activity of plant bioactive compound. These bioactive compound along with the solvent play a very significant role for the pathogens. These compounds have variously been reported to have antimicrobial activity and could be the reason for the activities recorded against these test organisms. The Plants chemicals have the potentiality of useful drugs if properly utilized[16,17,18]. The present study aimed to use these weeds against the pathogen because of antimicrobial potential of their extracts.

## REFERENCES

(1)Parmar P., Bhatt S., Dhyani S., Jain A.,"Phytochemical studies of the secondary metabolites of Ziziphus mauritiana Lam. Leaves", International journal of current pharmaceutical research, 2012;4(3):153-155.

(2)Nandkarni K.M., "Indian material medica", Popular Pr Bombay,2000 ;1315-1319.

(3)Waggas A.M., Al-Hasani, R.H., "Neurophysiological study on possible protective and therapeutic effects of Ziziphus spina-christi L. leaf extract in male albino rats treated with pentylenetetrazol", Saudi J. Biol. Sci, 2010;5(3): 1-6.

(4)Karon B., Ibrahim M., Mahmood A., Moyneen-ul-Haq, M.K.A., Chowdhary, U.M.M,

(5)Singh S.K., Kesari A.N., Gupta R.K., Jaiswal D., Watal G., "Assessment of antidiabetic potential of Cynodon dactylon extract in streptozotocin diabetic rats",. J Ethnopharmaco, 2007;114, 174-179. 8.

(6)Singh S.K., Rai P.K., Mehta S., Singh R.K., Watal G.,"Curative Effect Of Cynodon Dactylon Against Stz Induced Hepatic Injury In Diabetic Rats", Indian Journal Of Clinical Biochemistry,2009; 24 (4), 410- 413

(7)Abalaka E.M., Daniyan Y.S., Mann A ., "Evaluation of antimicrobial activity of two *Ziziphus spp*. On some microbial pathogens", Afr. J. Phar. Pharmacol 2010;4(4): 135-139.

(8)Gautam S., Jain K.A., Kumar A ., "Potential antimicrobial Activity of Ziziphus numularia against medicinally important pathogenic microorganism", A J Trad Med, 2011; 6 (6): 267-271 .

(9)Mc Farland J., "Nephelometer: An instrument for estimating the No. of bacteria in suspension used for calculating opsonic index for vaccines", Jour. American Med. Assoc,1907; 14:1176

(10)Kudi A. C., Uhoh J.U., Eduvie, L.O., Gefu, J., "Screening of some Nigerian medicinal plants for antibacterial activity", J E Pharma,1999 ;67:225-228

(11)Harborne J.H. "Phytochemical Methods" ,Chapman and Hill, Tokyo, Japan, 1973

(12)Gibbs R.D., "Chemotaxonomy of Flowering Plants", McGill Queen's University Press Montreal and London,1974;Vol.1,

(13)Sofowora A. "Medicinal Plants and Traditional medicine in Africa", John Wiley and Sons Ltd, 1982; 1st edition.131: 168 – 171. Trease GE, Evans WC. 1982. Phamacognosy.BailleneTindall, London: 735- 738.

(14)Kanimozhi D., Ratha B.V., "Evaluation of anti microbial activity of Cynodon dactylon", Int. J. Res. Pharm, 2012; Sci. 2(2):34-43.

(15)Chowdhary N.B., Padashetty N.S. "In vitro screening of antibacterial activites of leaves of Ber", Current Research,2000; 29:78-79.

(16 )Lee C.P., "Who's in the business of saving lives?" J. Med. Philos,2006; 31: 465-482

(17)Lam K.S., "New aspects of natural products in drug discovery".,Trends Microbiology,2007; 1(6): 279-289.

(18)Ogbunugafor H.A., Okochi V.I., Okpuzor J., Emeka P., "Tolerance and antiplasmodial screening of Ritchea longipedicellata in Plasmodium berghei", Biokemistri ,2008;20(1): 23-27.

(19)Kauffmann C.A. "Fungal infections", Proc. Am. Thorac soc,2006; 3:35-40.