

ORIGINAL RESEARCH ARTICLE

Screening of Antimicrobial Activity of Vetiver Extracts against Certain Pathogenic Microorganisms

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**ABSTRACT**

In the present study, the Vetiver (leaves, roots) extracts were prepared by using various solvents *viz.*, methanol, chloroform, hexane by Soxhlet apparatus. The extracts were evaporated to dryness and stored in a refrigerator at 4°C for future use. The antifungal, antibacterial activity of Vetiver extracts (10.00 mg/ml) were determined by Disc diffusion method. The Minimum inhibitory concentration (MIC) of the extracts (40, 20, 10, 5, 2.50, 1.25 mg/ml) were tested by broth dilution method. The Ampicillin (5 µg/ml) and Flucanazole (100 units/disc) were used as positive controls for bacteria and fungi respectively. The disc containing 5% DMSO alone used as blind control. The highest mean zone of inhibition (29 mm and 32 mm) was observed in methanol vetiver extracts of leaves and roots respectively against *Staphylococcus aureus*. Among the tested fungal cultures, *Aspergillus niger* exhibit a highest mean zone of inhibition (30 mm and 32 mm) against Vetiver leaves and root extract. The highest minimum inhibitory concentration (MIC) was obtained in methanol extracts of Vetiver against *Staphylococcus aureus* and *Aspergillus niger*.

**Key words:** Vetiver, Antibacterial activity, Antifungal activity, Disc diffusion and MIC.

**1. INTRODUCTION**

Vetiver is native to Indian and is found in wild state throughout the Indian subcontinent encompassing temperature to tropical climate. For various economic purposes including extraction of essential oil, the roots are dug out from wild resources in northern India and in several other countries. Haiti Island in the Caribbean Sea is the major source for the supply of vetiver oil to the world market, followed by Java, China, Japan, India etc. *Vetiveria zizanioides* (L.) Nash (Poaceae), popularly known as khus grass, has been known in India since ancient times. It is the major source of the well-known oil of vetiver, which is used in medicine and in perfumery<sup>[1]</sup>. In India, the roots have been used for making screens, mats, hand fans, and baskets. Different morphological parts of the grass are used for various ailments, such as boils, burns, epilepsy, fever, scorpion sting, snake bite, and sores in the mouth. The root extract is used for headache and toothache, the leaf paste is used for lumbago, sprain, and rheumatism, the stem decoction for urinary tract infection, the leaf juice as an anti-

helminthic, the vapors for malarial fever, and the root ash is given for acidity relief<sup>[2,3]</sup>.

The vetiver oil is traditionally known as “Vetivert oil” in trade, and is obtained from the aromatic roots of vetiver. The annual world trade in vetivert oil is estimated around 250 tons, with Haiti, Indonesia, China, Japan, India, Brazil being the main producers. USA, Europe, India and Japan being the main consumers<sup>[4]</sup>. Vetiver oil is a light to dark brown, olive, or amber viscous oil having a deep smoky, earthy woody odor with a sweet persistent undertone. The colour and scent can vary according to the source. Poorer grades with darker color and have smoky back notes are also produced in China and Java by subsistent farmers with primitive equipment<sup>[5]</sup>. Vetiver oil owes several beauty benefits and emotional effects. It balances the activity of the sebaceous oil glands, has deodorizing properties and helps normalize oily skin and clear acne. It replenishes moisture in dry & dehydrated skin and has a rejuvenation effect on mature skin, as well as cuts/ wounds/ irritated and inflamed skin. Vetiver oil reportedly prevents stretch marks. The oil strengthens the

central nervous system and is helpful in overcoming depression, insomnia, anxiety, stress, tension and nervousness.

Vetiver oil is a light to dark brown, olive, or amber viscous oil having a deep smoky, earthy-woody odor with a sweet persistent undertone. The color and scent can vary according to the source. Poorer grades with darker color and have smoky back notes are also produced in China and Java by subsistent farmers with primitive equipment<sup>[6]</sup>. Vetiver oil has a rather powerful smell but is very pleasant when diluted. It blends well with oils of sandalwood, rose, violet, jasmine, opopanax, patchouli, oakmoss, lavender, clary sage, mimosa, cassia, and ylang. It is high-priced oil as it is used extensively in fine perfumery and cosmetic products. In dilute state, it smells like sandalwood oil. It is used exclusively in the preparation of compound perfumes, in which the oil, an account of its low volatility, normally used as a base to fix other high-value volatile oils like rose oil, lavender oil, and jasmine oil<sup>[7]</sup>.

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant material

The plant materials (leaves and roots) of *Vetiveria zizanioides* were collected from the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar.

### 2.2. Preparation of extracts

The agar plate disc diffusion method was followed<sup>[8]</sup> for testing antibacterial activity. The extracts of fresh matured roots and leaves of *Vetiveria zizanioides* were prepared (1 kg) after drying the leaves, roots were chopped into small pieces and then mixed with 3 litres of chloroform, methanol, hexane were used separately for the extractions in the Soxhlet apparatus. The extracts were evaporated to dryness reduced pressure using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use<sup>[9]</sup>.

### 2.3. Collection of Bacterial and Fungal cultures

Six different bacterial cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. The collected bacterial cultures were *Staphylococcus aureus* (MTCC – 3159), *Escherichia coli* (MTCC – 3878), *Bacillus subtilis* (MTCC – 1825), *Salmonella typhi* (MTCC – 3215), *Pseudomonas aeruginosa* (MTCC – 6324) and *Klebsiella pneumoniae* (MTCC - 5123).

Four different fungal cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. The collected fungal cultures were *Candida albicans* (MTCC – 1863),

*Saccharomyces cerevisiae* (MTCC-2627), *Aspergillus flavus* (MTCC – 4962), *Aspergillus niger* (MTCC – 4285) and *Trichophyton mentagrophytes* (MTCC – 1886).

## 2.4. Cultures Maintenance

### 2.4.1. Maintenance of test bacterial cultures

The test bacterial isolates were sub-cultured and maintained on Nutrient agar slants and stored in refrigerator at 4°C.

### 2.4.2. Bacterial inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards and then used for the determination of antibacterial activity.

### 2.4.3. Maintenance of test fungal cultures

The test fungal isolates were sub-cultured and maintained on Sabouraud's dextrose agar slants and stored in refrigerator at 4°C.

### 2.4.4. Fungal inoculum preparation

Fungal inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Sabouraud's dextrose broth and incubated at 28°C for 2 days (yeasts) and 3 days (moulds) till a moderate turbidity was developed.

## 2.5. Disc preparation

### 2.5.1. Preparation of disc for antibacterial activity

Disc of 5 mm diameter were prepared using Whatman filter paper No.1. These were sterilized in the Hot air oven at 160°C for 1 hour. The solvent extracts of roots and leaves of *Vetiveria zizanioides* (Chloroform, Methanol and Hexane) were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 20µl of different solvent vetiver (roots ,leaves)extracts at 10 mg/ml to check their antibacterial activity. The paper discs which contain 5% DMSO were act as a negative control and the paper discs containing Ampicillin (5µg/disc) act as a positive control.

### 2.5.2. Preparation of disc for antifungal activity

Disc of 5 mm diameter were prepared using Whatman filter paper No.1. These were sterilized in the hot air oven at 160°C for 1 hour. The solvent extracts of *Vetiveria zizanioides* Chloroform, Methanol and Hexane) were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 20µl of different solvent vetiver extracts at 10 mg/ml to check their antifungal activity. The paper discs which contain 5% DMSO were act as a negative control and the

paper discs containing Flucanazole (100 units/disc) act as a positive control.

## 2.6. Antibacterial activity

### 2.6.1. Disc Diffusion Method

The antibacterial disc diffusion assay was carried out on Muller Hinton Agar plates following the method described by Bauer *et al.* [10]. Petri plates were prepared by pouring 20 ml of Mueller Hinton agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plated were allowed to dry for five minutes. After drying, the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with agar surface. The Ampicillin (5 µg/disc) was used as positive control and the 5% DMSO was used as a blind control in these assays. The plates were incubated at 37°C for 24 hours. The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

### 2.6.2. Minimum inhibitory concentration for bacteria

MIC of the vetiver extracts were tested in Muller Hinton broth for bacteria by broth macro dilution method [11]. Vetiver extracts were dissolved in 5% DMSO to obtain 128 mg/ml stock solutions. Vetiver (leaves, roots) extracts was prepared at different concentrations *viz.*, 40, 20, 10, 5, 2.5, 1.25 mg/ml. 50 µl of standardized suspension of the test organism was transferred on to test tube. The control tube contains only organism and devoid of vetiver extracts. The culture tubes incubated at 37°C for 24 hours. The lowest concentrations which did not show any growth of tested organism after macroscopic valuation was determined as minimum inhibitory concentration (MIC).

## 2.7. ANTIFUNGAL ACTIVITY

### 2.7.1. Disc Diffusion Method

The antifungal activity of vetiver extracts were determined by disc diffusion method proposed by Bauer *et al.* [10]. The petriplates were prepared by pouring 20 ml of Sabouraud's dextrose agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plates were allowed to dry for 5 minutes. After drying the disc with extract were placed on the surface of the plate with sterile

forceps and gently pressed to ensure contact with the agar surface. The Flucanazole (100 units/disc) was used as the positive control and 5% DMSO was used as blind control in these assays. The plates were incubated at 28°C for 48 hours (yeast) and 72 hours (molds). The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

### 2.7.2. Minimum inhibitory concentration for fungi

MIC of the vetiver extracts against fungal isolates were tested in Sabouraud's dextrose broth by broth macro dilution method [11]. The vetiver extracts were dissolved in 5% DMSO to obtain 128 mg/ml stock solutions. Vetiver (leaves, roots) extracts was prepared at different concentrations *viz.*, 40, 20, 10, 5, 2.5, 1.25 mg/ml. 50 µl of standardized suspension of the test organism was transferred on to each tube. The control tube contains only organism and devoid of vetiver extracts. The culture tubes were incubated at 28°C for 48 hours (yeast) and 72 hours (moulds). The lowest concentrations which did not show any growth of tested organism after macroscopic valuation was determined as minimum inhibitory concentration (MIC).

## 3. RESULTS AND DISCUSSION

### 3.1. Antibacterial activity of different extracts of vetiver leaves

Crude leaf extract of *Vetiveria zizanioides* showed antibacterial activity against six pathogenic bacteria at 10 mg/ml concentration. The methanol leaf extract of vetiver against *Staphylococcus aureus* showed the highest mean zone of inhibition (29mm) was recorded, followed by *Klebsiella pneumoniae* (25 mm), *Escherichia coli* (20mm), *Pseudomonas aeruginosa* (18mm), *Streptococcus faecalis* (17mm) and *Salmonella typhi* (15mm) (**Table 1**). Chloroform leaf extract of vetiver against *Staphylococcus aureus* showed the highest mean zone of inhibition (26 mm), followed by *Klebsiella pneumoniae* (23 mm), *Escherichia coli* (18mm) *Pseudomonas aeruginosa* (16mm), *Streptococcus faecalis* (14mm) and *Salmonella typhi* (12mm). Hexane leaf extract of vetiver against *Staphylococcus aureus* showed that the highest mean zone of inhibition (24mm) followed by *Klebsiella pneumoniae* (21 mm), *Escherichia coli* (16mm), *Pseudomonas aeruginosa* (14 mm), *Streptococcus faecalis* (13mm) and *Salmonella typhi* (10mm). In all the three extracts, *Staphylococcus aureus* showed the maximum zone of inhibition ranged

between 24 to 29 mm. The lowest mean zone of inhibition in all the three extracts were *Salmonella typhi* ranged between 10 to 15 mm.

The MIC value of methanol leaf extract of vetiver against *Staphylococcus aureus* were 2.50 µg/ml, *Escherichia coli* and *Klebsiella pneumoniae* were 5.00 µg/ml, *Streptococcus faecalis* and *Pseudomonas aeruginosa* were 10.00 µg/ml, *Salmonella typhi* were 20.00µg/ml. MIC value of chloroform leaf extract of vetiver against *Staphylococcus aureus* were 5.00µg/ml, *Escherichia coli* and *Klebsiella pneumoniae* were 10.00µg/ml, *Streptococcus faecalis*, *Pseudomonas*

*aeruginosa* and *Salmonella typhi* were 20.00µg/ml. MIC value of Hexane leaf extract of vetiver against *Staphylococcus aureus* were 10.00µg/ml, *Escherichia coli* and *Klebsiella pneumoniae* were 20.00µg/ml, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Salmonella typhi* were 40.00µg/ml (Table 1). All the three extracts MIC value of *Staphylococcus aureus* were ranged between 2.50 to 10.00µg/ml and that of *Shigella flexneri* were ranged between 20.00 to 40.00µg/ml.

Table 1: Antibacterial activity of different extracts of Vetiver leaves

S. No	Microorganisms	Mean zone inhibition (mm) 10mg/ml				Minimum inhibition concentration (µg/ml)			
		Chloroform	Methanol	Hexane	*Positive control	Chloroform	Methanol	Hexane	*Positive control
1	<i>Staphylococcus aureus</i>	26 ± 0.3	29 ± 0.2	24 ± 0.3	34 ± 0.1	5.00	2.50	10.00	5.00
2	<i>Escherichia coli</i>	18 ± 0.2	20 ± 0.4	16 ± 0.1	25 ± 0.2	10.00	5.00	20.00	10.00
3	<i>Streptococcus faecalis</i>	14 ± 0.1	17 ± 0.3	13 ± 0.4	21 ± 0.3	20.00	10.00	40.00	20.00
4	<i>Salmonella typhi</i>	12 ± 0.4	15 ± 0.3	10 ± 0.5	20 ± 0.4	20.00	20.00	40.00	20.00
5	<i>Klebsiella pneumoniae</i>	23 ± 0.6	25 ± 0.1	21 ± 0.2	31 ± 0.2	10.00	5.00	20.00	5.00
6	<i>Pseudomonas aeruginosa</i>	16 ± 0.5	18 ± 0.5	14 ± 0.6	22 ± 0.5	20.00	10.00	40.00	10.00

\*Ampicillin (5µg); Mean ± SD

### 3.2. Antibacterial activity of different extracts of vetiver root

Crude root extract of *Vetiveria zizanioides* showed antibacterial activity against six pathogenic bacteria. The methanol root extract of vetiver against *Staphylococcus aureus* showed that highest mean zone of inhibition (32mm) followed by *Klebsiella pneumoniae* (27 mm), *Escherichia coli* and *Pseudomonas aeruginosa* (25 mm) *Streptococcus faecalis* (22mm) and *Salmonella typhi* (19 mm) (Table 2). Chloroform root extract of *Vetiveria zizanioides* against *Staphylococcus aureus* showed the highest mean zone of inhibition (29 mm) followed by *Klebsiella pneumoniae* (24 mm), *Escherichia coli* (22 mm), *Pseudomonas aeruginosa* (21mm), *Streptococcus faecalis* (19 mm) and *Salmonella typhi* (17 mm). Hexane root extract of vetiver against *Staphylococcus aureus* showed the highest mean zone of inhibition (27 mm) followed by *Klebsiella pneumoniae* (22 mm), *Escherichia coli* (21 mm), *Pseudomonas aeruginosa* (20 mm), *Streptococcus faecalis* (17 mm) and *Salmonella typhi* (15 mm). In all the three extracts *Staphylococcus aureus* showed the highest mean zone of inhibition

ranged between 31 to 27 mm. The lowest mean zone of inhibition was *Salmonella typhi* ranged between 19 to 15 mm.

MIC value of methanol root extract of vetiver against *Staphylococcus aureus* were 1.25µg/ml, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were 2.50µg/ml, *Streptococcus faecalis* and *Salmonella typhi* were 5.00µg/ml. MIC value of chloroform root extract of vetiver against *Staphylococcus aureus* were 2.50µg/ml, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were 5.00 µg/ml, *Streptococcus faecalis* and *Salmonella typhi* were 10.00µg/ml. The MIC value of Hexane root extract of vetiver against *Staphylococcus aureus* were 5.00µg/ml, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were 10.00 µg/ml, *Salmonella typhi* and *Streptococcus faecalis* were 20.00 µg/ml. The highest MIC value in all three root extracts of vetiver against *Staphylococcus aureus* ranged between 1.25-5.00µg/ml. The lowest MIC value exhibited by *Salmonella typhi* ranged between 5.00-20.00 µg/ml (Table 2).

Table 2: Antibacterial activity of different extracts of Vetiver roots

S. No	Microorganisms	Mean zone inhibition (mm) 10mg/ml				Minimum inhibition concentration (µg/ml)			
		Chloroform	Methanol	Hexane	*Positive control	Chloroform	Methanol	Hexane	*Positive control
1	<i>Staphylococcus aureus</i>	29 ± 0.3	32 ± 0.3	27 ± 0.4	38 ± 0.2	2.50	1.25	5.00	5.00
2	<i>Escherichia coli</i>	22 ± 0.5	25 ± 0.5	21 ± 0.2	30 ± 0.3	5.00	2.50	10.00	5.00
3	<i>Streptococcus faecalis</i>	19 ± 0.2	22 ± 0.4	17 ± 0.7	27 ± 0.1	10.00	5.00	20.00	10.00
4	<i>Salmonella typhi</i>	17 ± 0.4	19 ± 0.3	15 ± 0.3	24 ± 0.1	10.00	5.00	20.00	20.00
5	<i>Klebsiella pneumoniae</i>	24 ± 0.7	27 ± 0.7	22 ± 0.4	32 ± 0.6	5.00	2.50	10.00	5.00
6	<i>Pseudomonas aeruginosa</i>	21 ± 0.6	25 ± 0.6	20 ± 0.5	31 ± 0.5	5.00	2.50	10.00	5.00

\*Ampicillin (5µg); Mean ± SD

### 3.3. Antifungal activity of different extracts of vetiver leaves

Crude methanol leaf extract of vetiver showed maximum activity against *Aspergillus niger* (30mm) followed by *Trichophyton mentagrophytes* (25 mm), *Aspergillus flavus* (16 mm), *Candida albicans* (14 mm) and *Saccharomyces cerevisiae* (12 mm) (Table 3). Chloroform leaf extract of vetiver showed maximum activity against *Aspergillus niger* (27 mm) followed by *Trichophyton mentagrophytes* (23 mm), *Aspergillus flavus* (14 mm), *Candida albicans* (13 mm) and *Saccharomyces cerevisiae* (10 mm). Hexane leaf extract of vetiver showed maximum activity against *Aspergillus niger* (26 mm), followed by *Trichophyton mentagrophytes* (22 mm), *Aspergillus flavus* (13mm), *Candida albicans* (11mm) and *Saccharomyces cerevisiae* (9mm). In all the three leaf extracts of vetiver *Aspergillus niger* showed maximum zone of inhibition ranged between 30 to 26 mm. *Saccharomyces cerevisiae* exhibits a minimum zone of inhibition ranged between 12 to 9 mm.

The MIC value of methanol leaf extract of vetiver against *Aspergillus niger* were 1.25 µg/ml followed by *Trichophyton mentagrophytes* were 5.00 µg/ml, *Aspergillus flavus* were 5.00 µg/ml, *Candida albicans* and *Saccharomyces cerevisiae* were 10.00 µg/ml. MIC value of chloroform leaf extract of vetiver against *Aspergillus niger* were 5.00 µg/ml, *Aspergillus flavus* and *Trichophyton mentagrophytes* were 10.00µg/ml, *Candida albicans* and *Saccharomyces cerevisiae* were 20.00µg/ml. Hexane leaf extract of vetiver showed MIC value of 10.0µg/ml against *Aspergillus niger*, followed by *Aspergillus flavus*, *Trichophyton mentagrophytes* and *Candida albicans* were 20.00 µg/ml, *Saccharomyces cerevisiae* were 40.00µg/ml. In all the three leaf extract of vetiver MIC value was highest against *Aspergillus niger* ranged between 1.25 to 10.00µg/ml lowest MIC value were recorded between 10.0to 40.00 µg/ml by *Saccharomyces cerevisiae* (Table 3).

Table 3: Antifungal activity of different extracts of Vetiver leaves

S. No	Microorganisms	Mean zone inhibition (mm) 10mg/ml				Minimum inhibition concentration (µg/ml)			
		Chloroform	Methanol	Hexane	*Positive control	Chloroform	Methanol	Hexane	*Positive control
1	<i>Aspergillus flavus</i>	14 ± 0.3	16 ± 0.2	13 ± 0.4	22 ± 0.3	10.00	5.00	20.00	10.00
2	<i>Aspergillus niger</i>	27 ± 0.4	30 ± 0.4	26 ± 0.3	36 ± 0.2	5.00	1.25	10.00	5.00
3	<i>Trichophyton mentagrophytes</i>	23 ± 0.2	25 ± 0.6	22 ± 0.1	31 ± 0.4	10.00	5.00	20.00	5.00
4	<i>Candida albicans</i>	13 ± 0.6	14 ± 0.3	11 ± 0.2	20 ± 0.1	20.00	10.00	20.00	10.00
5	<i>Saccharomyces cerevisiae</i>	10 ± 0.4	12 ± 0.5	9 ± 0.5	18 ± 0.4	20.00	10.00	40.00	20.00

\*Fluconazole (100 units/disc); Mean ± SD

### 3.4. Antifungal activity of different extracts of vetiver roots

Crude methanol root extract of vetiver against *Aspergillus niger* showed maximum zone of inhibition 32 mm followed by *Trichophyton mentagrophytes* was 27mm, *Aspergillus flavus* was 18 mm, *Candida albicans* was 16 mm and *Saccharomyces cerevisiae* was 14 mm (Table 4). Chloroform root extracts of vetiver against *Aspergillus niger* showed maximum zone of inhibition was 29 mm followed by *Trichophyton mentagrophytes* was 25 mm, *Aspergillus flavus* was 16 mm, *Candida albicans* was 15 mm and *Saccharomyces cerevisiae* was 12 mm. Hexane root extract of vetiver against *Aspergillus niger* was 28 mm followed by *Trichophyton mentagrophytes* was 24 mm, *Aspergillus flavus* was 14 mm, *Candida albicans* 13 mm and *Saccharomyces cerevisiae* was 11mm. All the three root extracts of vetiver *Aspergillus niger* showed maximum zone of inhibition ranged between 32 to 28 mm minimum zone of inhibition exhibited by *Saccharomyces cerevisiae* between 14 to 11mm.

MIC value of methanol root extract of vetiver against *Aspergillus niger* was 1.25 µg/ml followed by *T. mentagrophytes* was 5.00µg/ml, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces cerevisiae* was 10.00µg/ml. Chloroform root extract of vetiver was tested against *Aspergillus niger* MIC value was 5.00µg/ml followed by *Trichophyton mentagrophytes* and *Aspergillus flavus* were 10.00µg/ml, *Candida albicans* and *Saccharomyces cerevisiae* were 20.00µg/ml. Hexane root extract of vetiver was tested against *Aspergillus niger*, the MIC value was 10.00µg/ml followed by *Trichophyton mentagrophytes* and *Aspergillus flavus* were 20.00µg/ml, *Candida albicans* and *Saccharomyces cerevisiae* were 40.00 µg/ml. In all the three root extracts of veiver *Aspergillus niger* showed the highest MIC value ranged between 1.25 to 10.00 µg/ml. The lowest MIC value exhibited by *Candida albicans* and *Saccharomyces cerevisiae* ranged between 10.00 to 40.00µg/ml (Table 4).

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards

this goal is the *in vitro* antibacterial activity assay, antifungal properties of plants<sup>[12,13]</sup>. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. In the present study, the methanol leaf, root extracts of *Vetiveria zizanioides* showed the maximum activity against *Staphylococcus aureus*, followed by, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Salmonella typhi*. In all the three extracts, methanol showed the maximum activity against the vetiver leaf and root extracts followed by chloroform and hexane. In all the extracts, hexane extracts of vetiver leaf and root showed the minimum activity.

Mahesh and Satish<sup>[14]</sup> supported that the methanol extracts of leaf, root of *Acacia nilotica*, *Sida cordifolia*, *Tinospora cordifolia*, *Withania somnifera* and *Ziziphus mauritian* showed the activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas fluorescens*. Khesorn Nantachit et al.<sup>[15]</sup> reported

that the crude methanolic root extract of *Vetiveria zizanioides* showed antimicrobial activity against four pathogenic bacteria and four pathogenic fungi at 1-10% W/V concentration. *Staphylococcus aureus* showed that maximum activity against *Vetiveria zizanioides* leaf, root extracts. In our study also, methanolic leaf and root extracts of vetiver showed maximum activity against *Staphylococcus aureus*. Crude root and leaf extracts of *Vetiveria zizanioides* were tested against the *Aspergillus niger*, *Trichophyton mentagrophytes*, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces cerevisiae*. Methanolic root, leaf extract of vetiver against *Aspergillus niger* showed maximum activity followed by *Trichophyton mentagrophytes*, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces cerevisiae*. Minimum Inhibitory Concentration (MIC) of methanolic extract of vetiver against *Staphylococcus aureus* was 2.5 µg/ml, *Trichophyton mentagrophytes* was 1.25µg/ml.

Table 4: Antifungal activity of different extracts of Vetiver roots

S. No	Microorganisms	Mean zone inhibition (mm) 10mg/ml				Minimum inhibition concentration (µg/ml)			
		Chloroform	Methanol	Hexane	*Positive control	Chloroform	Methanol	Hexane	*Positive control
1	<i>Aspergillus flavus</i>	16 ± 0.3	18 ± 0.1	14 ± 0.3	23 ± 0.1	10.00	10.00	20.00	10.00
2	<i>Aspergillus niger</i>	29 ± 0.4	32 ± 0.3	28 ± 0.2	37 ± 0.4	5.00	1.25	10.00	5.00
3	<i>Trichophyton mentagrophytes</i>	25 ± 0.2	27 ± 0.5	24 ± 0.1	31 ± 0.2	10.00	5.00	20.00	5.00
4	<i>Candida albicans</i>	15 ± 0.6	16 ± 0.7	13 ± 0.2	21 ± 0.5	20.00	10.00	40.00	10.00
5	<i>Saccharomyces cerevisiae</i>	12 ± 0.7	14 ± 0.4	11 ± 0.4	18 ± 0.3	20.00	10.00	40.00	20.00

\*Fluconazole (100 units/disc); Mean ± SD

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