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IN VITRO ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *VETIVERIA ZIZANIOIDES* ROOT

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ABSTRACT

Objective: To evaluate the antibacterial activity of ethanolic extract of roots of *Vetiveria zizanioides.*

Method: Ethanolic extract of roots of *Vetiveria Zizanioides* was prepared. Standard cultures of *Escherichia coli* NCIM 2118; *Bacillus subtilis* NCIM 2063, *P. aeurogenosa* NCIM 2036 and *Staphylococcus aureus* NCIM 2079 were used for the study. The antibacterial tests used were the agar well plate method. Ciprofloxacin was used as the positive control.

Results: EEVZ does not show any activity against *B.subtilis*. However, the activity was less than the standard Ciprofloxacin. The extract shows increasing inhibitory activity with increase in concentration (150-750µg).

Conclusions: From our investigation, for screening ethanolic extract of *Vetiveria zizanioides* the results obtained confirmed therapeutic potency of some plants used in traditional medicine. The plants could be potential source of new antimicrobial agent.

INTRODUCTION: About 80% of the world's population uses folk medicine in traditional medicine states World Health Organisation. Since ancient time plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health (Kirbag *et al*., 2009). India is one of the richest countries in the world with regard to diversity of medicinal plants. For centuries, plants have been used throughout the world as drugs and remedies for various diseases since they have great potential for producing new drugs of great benefit to mankind. There are many approaches to search for new biologically active principles in higher plants. Even though pharmacological industries have produced a number of antibiotics in last three decades, resistance to these drugs by micro organism has been increased. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. It is expected that plant extract showing target sites other then the dose used by antibiotics will be active against drug resistant microbial pathogens[1].

Micro organisms are heterogeneous group of several distinct classes of living beings. Bacteria are prokaryotic microorganisms, which do not contain chlorophyll. They are unicellular and does not show true branching, expect in the so called higher bacteria (*Actinomycetales*) [2]. To treat chronic and infectious diseases plants used in traditional medicine contain a wide range of ingredients[6]. Many plant leaves have antimicrobial principles such as tannins, essential oils, and other aromatic compounds[7]. *Vetiveria zizanioides* is a densely tufted grass, profoundly used in Ayurvedic medicine Gramineae. The plant is distributed throughout the plains of India, ascending up to an elevation of 1200m. The chemical constituents present in the plant are Vetiverol, Vetivone, Khusimone, Khusimol, Vetivene, Khositone, Terpenes, Benzoic acid, Tripene-4-ol, β- Humulene, Epizizianal, vetivenyl vetivenate, iso khusimol , β- vetivone, vetivazulene. The plant used as digestive, carminative stomachic, constipating, haematinic, expectorant, antispasmodic, antiasthmatic, antigout, anthelmentic, antimicrobical and diuretic. The roots are used for cooling to the brain, and also used in treatment of ulcers. In addition to these, the plant is used for anemeia, amenorrhea, dysmenorrhea and helmenthiasis. *Vetiver* oil posses sedative property and has been traditionally used in aromatherapy for relieving stress, anxiety, nervous tension and insomnia. Neither the root nor the stem is an antidote to either snake-venom or scorpion-venom.

MATERIALS AND METHODS:

Materials: Sabouraud dextrose agar (SDA), Mueller Hinton and Muller Hinton broth were purchased from Himedia laboratories Pvt. Ltd., Mumbai, while dimethylsulfoxide (DMSO) was purchased from Qualigens fine chemicals, Mumbai. Ofloxacin and flucanazole infusion were purchased from Cipla Pharmaceuticals, Mumbai. All other chemicals used in the study are of analytical grade purchased from respective suppliers.

Collection and Authentication: *Vetiveria zizanioides* roots were collected from coimbatore district, Tamilnadu during the month of June 2008. The plant material were identified and authenticated by Dr. K. Manivannan, professor and head, Department of Horticulture, Annamalai University, Chidambaram, India and the voucher specimen no. Hort/56/2008 has been preserved in our department for further reference.

Preparation of the extract: Fresh roots of the plant were collected and dried in shade under room temperature, powdered mechanically and sieved through No. 22 mesh sieve. The finely powdered roots were kept separately in an air

tight container until the time of use. About 600 gm of powder was soaked with 3 litres of ethanol for 12 h and then macerated at room temperature using a mechanical shaker for 4h. The extract was filtered off and the marc was again soaked with the same volume of ethanol for 12 h and then further extracted for 4h and filtered. The filtrates were then combined concentrated under reduced pressure and evaporated at 40^0 C. The percentage yield of the *Vetiveria zizanoides* (EEVZ) was 27.4% w/v.

Phytochemical screening of the extract: Phytochemical screening was carried out for the ethanol extracts of *Vetiveria zizanioides* for the presence of phytochemical constituents' like alkaloids, flavonoids, saponins, terpenoids, tannins and phenolic.

Antimicrobial tests:

In vitro* Antibacterial activity of ethanolic extract of *Vetiveria zizanioides:

Test organism: The test organisms used were *Escherichia coli* NCIM 2118; *Bacillus subtilis* NCIM 2063, *P. aeurogenosa* NCIM 2036 and *Staphylococcus aureus* NCIM 2079. All the stock cultures were obtained from NCIM, Pune, India.

Culture media and inoculums: Muller Hinton broth (MH) and Nutrient broth (NB) media (Hi-Media Pvt. Ltd., Bombay, India) were used for NCIM. The cultures used were incubated at 37^oC for 24 hrs.

Bacterial inoculums: All organisms were grown overnight (24 hr) at 37^o C on Nutrient Agar (NA) or Muller Hintor Agar (MHA) and harvested during the stationary growth phase. A direct suspension of organisms was prepared in 5 ml sterile distilled water. Inoculum was standardized by matching the turbidity of the culture to 0.5 Mc Farland standards. This was produced by mixing 0.5ml of 0.048 M $Bacl_2$ (1.175% w/v Barium Chloride dehydrate) with 99.5 ml of 0.36 N H_2SO_4. This produced an inoculating suspension of approximately 10^8 CFU per ml, which is then diluted in fresh broth to achieve final inoculum of approximately 105 CFU/ml.

Methods:

Antibacterial assay of the *Vetiveria zizanioides* roots extracts: The agar well diffusion method was carried out to evaluate antibacterial activity[1]. Test organism was spread on Muller-Hinton agar plates. The standard inoculums (NCIM cultures) were evenly spread on the surface of the medium then wells of 6mm diameter were punched into the agar medium and filled with 60 µl (5mg/ml) of *Vetiveria zizanioides* plant extract of various concentration (150-750µg) were dissolved in DMSO. Six wells were made, in each well different concentration of extract is added and 10µl of Ciprofloxacin (which is used as standard) was filled in and this plate is kept in refrigerator for 20 minutes for diffusion. The plates were incubated for 24 hours at 37^oC. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

RESULT:

Phytochemical screening: Preliminary phytochemical screening of ethanolic extract of *Vetiveria zizanioides* (EEVZ) revealed the presence of alkaloids, flavonoids, saponins, terpenoids, tannins and Phenolics (**Table 1**).

TABLE 1: PHYTOCHEMICAL SCREENING OF THE EXTRACT (EEVZ)

Phytochemical constituents	EEVZ
Alkaloids	+
Flavonoids	+
Terpenoids	+
Tannins	+
Phenolics	+
Saponins	+

Antibacterial assay of the *Vetiveria zizanioides* root extracts on NCIM cultures: The antibacterial activities of EEVZ under study by agar well plate

method were shown in **table 2** and **3**. The antibacterial activity is measured by zone of inhibition (mm). Totally four bacterial strains (two gram positive *S.aureus, B.subtilis* and two gram negative bacteria *P. aeurogenosa, E.coli*) were used in this investigation.

TABLE 2: ZONE OF INHIBITION (MM) OF EEVZ AT VARIOUS CONCENTRATIONS ON GRAM POSITIVE BACTERIA

Organism	Concentration (EEVZ) µg	Zone of Inhibition (mm)	Standard (Ciprofloxacin)
S.aureus	150	18 mm	31 mm
	300	20 mm	
	450	23 mm	
	600	24 mm	
	750	25 mm	
B.Subtilis	150	-	34 mm
	300	-	
	450	-	
	600	-	
	750	-	

TABLE 3: ZONE OF INHIBITION (MM) OF EEVZ AT VARIOUS CONCENTRATIONS ON GRAM NEGATIVE BACTERIA

Organism	Concentration (EEVZ) µg	Zone of Inhibition (mm)	Standard (Ciprofloxacin)
P.aeurogenesa	150	11 mm	30 mm
	300	12 mm	
	450	14 mm	
	600	16 mm	
	750	18 mm	
E.coli	150	12 mm	36 mm
	300	14 mm	
	450	16 mm	
	600	18 mm	
	750	20 mm	

EEVZ showed better growth inhibition against *S. aureus, P. aeurogenosa and E. coli* (25mm, 18mm, 20mm) respectively at 750 µg. However, the activity was less than the standard Ciprofloxacin. EEVZ does not show any activity against *B.subtilis*. The extract shows increasing inhibitory activity with increase in concentration (150-750µg).

DISCUSSION: Herbal medicines are a valuable and readily available resource for primary health care and complementary health care system undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that had to be discovered[5]. Though large numbers of plants are constantly being screened for their antimicrobial effects still there is a search for natural antibiotic. Many plant genetic resources have been analyzed for their active constituents possessing antibacterial activities[3]. There are two possibilities that may account for the higher antibacterial activity of ethanolic extracts are the nature of active constituents (alkaloids, flavonoids, essential oil, terpenoids, tannins, etc.) and the other is the capacity of ethanol may have yielded a great number of active constituents responsible for antibacterial activity[8].

EEVZ is known to posses flavonoids, alkaloids, terpenoids, saponins, tannins and phenols which either individually or combination exert antimicrobial activity. Our study showed that EEVZ inhibited gram negative bacteria than gram positive bacteria. Flavonoids are found to be effective antimicrobial substance against a wide range of micro organisms, probably due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell wall; more lipophilic flavonoids may disrupt microbial membrane. Antibacterial activity of tannins may be related to their ability to inactivate microbial adhesion enzymes and cell envelope transport proteins, they also complex with polysaccharides[10]. The presence of tannins present in the roots of *Vetiveria zizanioides* implied that tannin may be the active compound which may be responsible for *in vitro* antibacterial activity in this study. Tannin in the plant extract was found to possess antibacterial activity[4].

CONCLUSION: Herbs are integral part of nature; plants contain natural substance that can promote health. From our investigation, for screening ethanolic extract of *Vetiveria zizanioides* the results obtained confirmed therapeutic potency of some plants used in traditional medicine. Plant based medicines contains rich antioxidants and are traditionally used in different parts of the world. The hypothesis of obtaining plant based medicine is beneficial to human health based on the active profile exposed through various *in vitro* assays it can be concluded that the ethanolic extract of *Vetiveria zizanioides* shows significant antibacterial activities. Further investigations on the isolation and identification of bioactive components on the plant would help to ascertain its potency. This could be further exploited by *in vivo* study systems to increase the overall activity.

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