ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICALS OF VETIVER

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ABSTRACT

World Health Organization (WHO, 1984) noted that majority of the world's population depends on traditional medicine for primary health care. Medicinal and aromatic plants are widely used as medicines that constitute a major source of natural organic compounds . In recent years, there is an increasing trend in research of bioactive compounds extracted from various herbs and aromatic plants. The plant extract and essential oils have been screened for their potential uses as an alternative remedies for the treatment of many infectious diseases for many years. In this paper, the antimicrobial and bioactive principles of vetiver leaves are presented. The extraction of active compounds from the plant materials using chloroform hot extraction method yielded maximum compounds. The phytochemical study showed tht the plant grown in normal condition yielded more extracts than the other treated ones. Phytochemicals like saponins, flavanoids, phenols were present in both root and leaf extracts and tannins were absent in both the root and leaf extracts. The wax content was found to be more in plants, which were grown in dye, contaminated soil for soil remediation. Leaf contained 0.78 g and root contained 0.65g of wax. This strongly proves that vetiver under stress condtion it secretes more wax inorder to adapt with rooting media. The root and leaf fractions controlled more number of pathogenic bacteria and fungi. The maximum zone of inhibition was found to be 25 mm in *P.aeruginosa* followed by *S.aureus* (24 mm). In *C.albicans* the zone of inhibition was 28 mm and in *C.neoformens* it was 22 mm. The purified 5th fraction of root and 8th fraction of leaves grown in the normal condition showed the maximum antibacterial activity against pathogenic bacteria and fungi. MIC

• Presenting author

of these fractions found to be 10 mg / mL and inhibitory concentrations was varied between 5-7.5 mg/mL. Since the fractions were not a single compound the inhibition concentration were found to be little high. The GCMS and NMR analysis proved that there were 23 chemicals identities from 5^{th} fraction of root extract and 12 from 8^{th} fraction of leaves. Among these compounds based on the peak area it was found that cedren –13-ol-8, 1-cyclohexane, 2- methyl –2- (3 methyl-2-oxobutyl, Ledene oxide (II) were common for both the fractions, but 1-Butyn-3-one, 1-6,6-dimethyl – 1,2-epoxy cyclohexyl and tricyclo (5,1.00 (2,4) oct- 5 –ene- 5- propanoic acid, 3,3,8,8 – tetramethyl were seen only in root fraction and 4,7- octadecadiynoic acid, methyl ester was seen only leaf extract.

INTRODUCTION

Curing of diseases and restoration of health always been major objectives of humanity. The use of plants as medicines goes back to early man. The great civilizations of the ancient Chinese, Indians and North Africans provided have written evidence of man's ingenuity in utilizing plants for treatment of a wide variety of diseases. The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant. There is still much more one can learn from investigating the medicinal plants. Intensive use of antibiotics is often resulted in the development of resistant strains (Sydney *et al.,* 1980). Because of this drug resistance, the search for the new antibiotics continues unabated. In this connection plants continue to be a rich source of therapeutic drugs. The active principles of many drugs are found in plants or are produced as secondary metabolites.

The promotion of vetiver grass as an income-generating crop and its development from merely being an agricultural waste to economical partial substitutes or raw materials would reduce environmental deterioration and deforestation. It would also reduce the importation of pulping paper, save energy, restore and maintain the fertility of natural resources and finally bring about a better economy and living standards. Generally

vetiver leaves are being used for roof thatch , vetiver hut (Lavania, 2003), pre-fabricated vetiver – clay blocks (Hengsadeekul and Nimityongskul, 2003), vetiver – clay composite storage bin , cement replacement material (Nimityongskul *et al.*, 2003), fiber board, straw bale against insects, bale building and as containers (Thiramongkol and Babpraserth, 2002). Vetiver leaves can be used for cellulosic ethanol production, mushroom culture, furnace fuel, carbon sequestering and handicrafts and aromatic oil production. Vetiver grass with beautiful form and aesthetic value, it sometimes used as an ornamental plant in landscaping (Truong *et al.*, 2002) or as a decorative potted plant. Although works on biomass, soil erosion, wastewater treatment, genetic modification, engineering aspects and other economic uses of the plant vetiver (*C.zizanioides*) have been studied, less research has been done in the area of value added products, antimicrobial and bioactive compounds and medicinal properties of root of *C. zizanioides* had been published, the antibacterial activity against human pathogens of all the bioactive compounds from leaves of *C. zizanioides* is yet to be studied. Therefore, it is reasonable to analyze the antimicrobial properties of root and leaves of *C. zizanioides* against selected human pathogens. Hence this study has been carried out with the intention of isolating the bioactive compounds from the extracts and finding out the antimicrobial properties of the root and leaves of *C. zizanioides*. Hence the work has been undertaken.

MATERIALS AND METHODS

Leaves and roots of *C.zizanioides* grown in farmyard manured soil (E1), plant grown in municipal wastewater (E2) and dye contaminated soil (E3) were collected, were cut into pieces and kept for shade dry at room temperature (28°C ± 2 °C) for about four days. The dried leaves and roots were powdered in the pulverizer separately. The dried leaf and root powder were taken for cold and hot extraction.

Cold extraction method

The powdered roots and leaves of *C.zizanioides* (40 g of each) of samples were soaked in 200 mL of hexane, chloroform and ethanol separately for 48 hrs. The extracts

of roots and leaves were filtered using Whatman filter paper (No.1) and were kept in Rotary vacuum digital bath with temperatures of 60°C for hexane, 56°C for chloroform and 78°C for ethanol respectively. The extracts of roots and leaves were transferred into the pre weighed petri plates for faster evaporation. Weights of the extracts were taken.

Hot extraction method

Simultaneously hot extraction method using Soxhlet apparatus was carried out with hexane, chloroform and ethanol. The hot extraction process for roots and leaves was lost for 5 h and the weight of the extract was recorded.

Removal of wax from the extracts (both cold and hot methods) of root and leaves

The extracts of roots and leaves from 40 g of plant materials in each solvent were taken from the cold and hot extraction process and dissolved in acetone (25 mL) in the beakers separately. The mouth of the beakers were covered with plastic sheets and kept them in the sonicator for 40 minutes. After 40 min, they were kept in the deep freezer (-20°C) immediately for 2 hours. Then they were filtered separately using Whatman (No.1), after rinsing thoroughly with acetone. The wax from the filter papers was weighed.

Antimicrobial activity by disc diffusion method

The *in vitro* antimicrobial activity of the sample solution was studied by disc diffusion method. Test microorganisms used were *Escherichia coli, Salmonella typhi, Klebseilla pneumonia, Vibrio cholera, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus fecalis,* E*nterococcus fecalis, Candida albicans, Cryptococcus neoformens.* An inoculum of each organism was suspended in nutrient broth and incubated for 18 h at 37 °C were used for testing the antibacterial and anti fungal activity. Muller Hinton agar . (g/L : Beef infusion -300, Casamino acids/acids hydrolysate of casein-17.5, Starch-1.5, Agar-17,Distilled water -1L) plates were seeded with 18 h broth cultures of different bacteria while Sabouraud's

Dextrose agar (g/L: Peptone-10, Dextrose sugar -40, Agar-20,Distilled water-1L) plates were seeded with 18 h broth culture of *Candida albicans* and *Cryptococcus neoformens*. In each of these plates, for bacteria, 30 µg of pre-loaded disc +ve (chloramphenicol), -ve (ethanol) and for fungi Ketoconozole as positive control and ethanol as negative control . Root and leaf extracts were placed and allowed to diffuse at room temperature (28°C ± 2 °C) for 2 h. The plates were then incubated at 37 °C for 18 to 24 h for bacterial pathogen and 3 days for fungal pathogens. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in triplicate and the mean of the diameter of the inhibition zones was calculated

TLC and Column chromatography

The spots were collected individually and the silica gel with the compounds were dissolved in chloroform and filtered after a thorough rinsing with chloroform, to get the pure compound. From root extract six bands and from leaf extract 12 bands were collected from TLC and tested for antimicrobial activity against human pathogenic bacteria and fungi.

The active bands of TLC were further purified by column chromatography. Ten ml of each fraction from root and leaves were collected separately and checked for the Rf value in TLC. When similar Rf values were found in the fractions, they were pooled and evaporated under vacuum. The purified fractions were tested against pathogenic bacteria and fungi by well diffusion method.

Minimum Inhibitory Concentration and Inhibitory Concentration (MIC and IC_{50})

MIC and inhibitory concentration (IC_{50}) of the purified column chromatographic fractions against pathogenic bacteria and fungi were determined by broth dilution method using microtitre plate technique and pour plate technique using Chloramphenecol as +ve control (for bacteria), Ketoconozole (for fungus) and ethanol as –ve control.

A single colony of each pathogen was revived in nutrient broths. A fresh 1 % inoculum was transferred to nutrient broth. The pathogen was challenged with the root and leaf extracts at varying concentration ranging between 10mg – 0.01mg to determine MIC. The challenged culture was then incubated at 37 °C. Under aseptic condition 200µL of the challenged bacterial and fungal culture were transferred to 96 wells Microtitre (ELISA) reader plate. OD readings were recorded at 655nm after every 0, 1,12 and 24 h to determine the cell density. From the OD readings obtained after various incubations the MIC and IC_{50} for the root and leaf extracts were determined.

Phytochemical analysis

The extracts of the three samples thus obtained were subjected to phytochemical screening such as, saponins, tannins, titerpenes, alkaloids, flavonoids, rotenoids and phenol following the methodology of Gibbs (1974) and Harborne (1998).

The fractions were further purified with different solvents like methanol, iso-propanol and benzene. The purified fractions were analyzed in Gas Chromatography – Mass Spectrometry (GCMS). Qualitative identification of the different constituents was performed by comparison of the relative retention times and mass spectra with those of authentic reference compounds by retention indices (RI) and mass spectra. The chemical identities of the separated compounds were determined by matching their recorded mass spectra with the data bank mass spectra (NIST and WILLEY libraries) provided by the instrument software and by comparing their calculated retention indices with literature values measured on columns with identical polarity. The structures of the compounds were further confirmed by chromatography of their authentic standards under the GC MS conditions mentioned above.

High-resolution NMR spectra of the extracted plant materials were acquired on Bruker spectrometer operating 300.1300265 MHz. The programme followed in NMR analysis was: Runtime Proton: 1.59 min, Carobon:1 hr 6 min, Pulse programme: zg30, Magnetic strength:7.05 Tesla, Probe :5mm dual type,Sample:25mg, Solvent ($CDCl_3$):0.5 mL

RESULTS

Bioactive compounds

The maximum yield % of root and leaves was recorded in the hot extraction using $CHCl_3$ and the minimum was recorded in the hexane hot extraction (Table 1). When compared to cold extraction method, the yield % was more in hot extraction method of both roots and leaves of *C.zizanioides*. The results showed that in chloroform extract, yield was 7.08 % and the leaves were 7.04 %. In other solvents, the yield was comparatively less.

Table 1 - Yield percentage of crude extracts of root and leaf of *C. zizanioides*

Sample		Cold Extraction				Hot Extraction			
		Hexane	$CHCl_3$	Ethanol	Water	Hexane	$CHCl_3$	Ethanol	Water
E1	Root	1.18	2.28	1.60	1.16	3.2	7.08	3.6	3.4
	Leaf	0.76	1.80	1.52	1.40	2.1	7.04	4.7	4.5
E2	Root	1.01	1.17	1.24	0.98	1.89	3.20	1.29	1.27
	Leaf	0.34	1.08	1.34	1.21	0.76	2.89	1.9	2.2
E3	Root	1.08	2.07	1.49	0.78	2.7	5.28	3.2	3.1
	Leaf	0.70	1.71	1.38	0.99	2.2	5.19	3.1	3.3

Wax content

The wax content was estimated in both the extracts of hot and cold methods. The hot $CHCl_3$ extract yielded more wax than the other solvents. Among the three extracts (E1, E2 and E3) E3 yielded maximum wax content, followed by E2 and E1. The hot root extract of CHCl3 yielded 1.37 g in E1, 1.4g in E2 and 1.58 in E3. General observation was made that the wax content was more in leaf than in root of *C.zizanoides* in all the treatments (E1 to E3) (Table 2).

Table – 2 The wax content of roots and leaves of *C.zizanioides*

Sample		Wax from cold extract (g)				Wax from hot extract (g)			
		Hexane	$CHCl_3$	Ethanol	Water	Hexane	$CHCl_3$	Ethanol	Water
E1	Root	0.18	0.29	0.11	0.06	0.23	0.35	0.18	0.12
	Leaf	0.83	1.10	0.68	0.11	0.98	1.37	0.75	0.22
E2	Root	0.23	0.31	0.22	0.20	0.68	0.78	0.52	0.58
	Leaf	0.80	0.9	0.5	0.4	0.93	1.48	0.67	0.62
E3	Root	0.28	0.46	0.36	0.29	0.72	0.78	0.56	0.65
	Leaf	0.82	0.98	0.62	0.53	0.97	1.58	0.68	0.78

Phytochemicals

The phytochemical screening tests revealed the fact that in the root and leaf extracts, saponins, flavanoids and phenols were present where as tannins were absent in both the extracts. Triterpenes and steroids were present in the root extract but absent in the leaf extracts. The results are given in table 3.

Table – 3 Phytochemicals present in root and leaf extracts of *C.zizanioides*

S.No	Phytochemicals	Root extract	Leaf extract
1	Saponins	+	+
2	Tannins	-	-
3	Triterpenes	+	-
4	Steroids	+	-
5	Flavanoids	+	+
6	Rotenoids	-	+
7.	Phenols	+	+

TLC

The extracts of root and leaves were subjected to TLC .The separated bands in the plates were calculated for Rf values, and the results are given in table 4. There were 6 and 12 spots for root and leaf extracts respectively . The Rf value for fraction 5 in the root extract was higher (0.4788) followed by fraction 6 (0.1267). The Rf value (1.0000) in the shoot extracts was maximum in fraction 1.There was no difference in RF value of compounds separation in E1, E2 and E3.

Table –4 Rf values of root and leaf extracts of samples *C.zizanioides*

Root extracts		Leaf extracts
Fraction number	Rf value	Rf value
1	0.0704	1.0000
2	0.0985	0.9134
3	0.1126	0.7788
4	0.0563	0.6730
5	0.4788	0.5961
6	0.1267	0.5384
7	-	0.4326
8	-	0.3461
9	-	0.3076
10	-	0.2211
11	-	0.1826
12	-	0.0288

Antimicrobial activity

The extracts ($CHCl_3$ hot) from roots and leaves of *C.zizanoides* were tested for their antimicrobial activity against pathogenic bacteria and fungi. Table 5 represents the

summary of the antimicrobial activity of crude extracts of roots and leaves of all the three samples (E1 to E3) with respect to each of the tested organism .

The $CHCl_3$ extract from E1 of roots showed promising activity against tested microorganisms when compared to the extracts (E2 and E3). The maximum zone of inhibition was 20 mm against *E.coli*; 24 mm against *S. aureus*, followed by *P. aeruginosa* (25 mm). In *K. pneumonia, S. typhi, S.faecalis* and *E. faecalis* the zone of inhibition was 21 mm. The zone of inhibition was 4 mm in E3, 3 mm in E2 and 5 mm against *V.cholerae* in the $CHCl_3$ root extract. The zone formation was almost absent in leaf extract against *V.cholerae.* In the crude hot extract of root *C. albicans* showed 28 mm zone of inhibition and against *C. neoformens* it was 22 mm. It was less in other extracts (E2 and E3).

Table –5 Antimicrobial activities of the crude hot $CHCl_3$ extracts of root and leaf of *C.zizanioides* from different treatments

Bacteria/ Fungi	+ve cont	-ve cont	E1		E2		E3	
			Root extract	Leaf extract	Root extract	Leaf extract	Root extract	Leaf extract
E.coli	25	9	20	18	12	10	16	15
K.pneumoniae	24	10	21	17	11	12	20	12
S. typhi	22	11	21	13	11	10	17	12
S. aureus	22	9	24	24	14	16	22	18
V.cholerae	20	NI	5	NI	3	NI	4	NI
S.faecalis	25	12	21	19	11	12	19	15
P.aeruginosa	26	11	25	15	12	11	22	13
E. faecalis	28	10	21	14	16	12	18	12
C. albicans	25	12	28	25.5	13	10	20	17
C.neoformens	23	13	22	20	10	8	18	16

NI- No inhibition

In E3, the maximum zone of inhibition of root extract against *S.aureus* (22 mm) followed by *K.pneumoniae*, *S. typhi* (20 mm and 17mm) and *S.faecalis* (19 mm), *E. faecalis* (18 mm), *E.coli* (16 mm). In the same way the zone of inhibition was found in other pathogenic bacteria in the leaf extract which showed moderate effect except *V.cholerae.* The anti fungal activity against *C.albicans* in the root extract of *C.zizanioides* was 20 mm and leaf extract was 17 mm. The inhibitory characters against *C.neoformens* in root and leaf extract were 18 mm and 16 mm.

Since the $CHCl_3$ extracts of roots and leaves showed promising results, chloroform extracts alone were used for further purification by TLC, Column Chromatography and Preparative Thin Layer Chromatography (PTLC). The purified fraction of roots and leaves were collected and tested for antibacterial activity. There were six fractions in the root hot extract. Among six fractions the 5th fraction was active against the growth of eight pathogens. The 5^{th} fraction of root showed significant inhibitory effect against *E.coli*, *S. aureus*, *C. albicans* and *C. neoformens* than other pathogens. The zone of inhibition against *E.coli* was 24 mm, *S. aureus* was 30 mm, *C. albicans* was 33 mm and *C. neoformens* was 28 mm. The zone of inhibition by fraction 1 was against 5 pathogens and fraction 4 was against 6 pathogens, whereas 4 pathogens were against fraction 3 and 6 **(**Table 6).

Table –6 Antimicrobial activity of purified root extracts of *C.zizanioides*

Bacteria / Fungi	Average zone of inhibition (mm)					
	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6
E.coli	20	17	5	21	24	12
K.pneumoniae	16	-	-	-	-	-
S. typhi	-	-	-	-	13	-
S. aureus	-	15	15	30	30	18
V.cholerae	-	-	-	-	-	-
S.faecalis	17	-	-	-	18	-
P.aeruginosa	-	-	-	31	18	-
E. faecalis	-	-	-	25	16	-
C.albicans	15	-	15	33	33	19
C.neoformens	5	5	17	28	28	19

In leaves, there were 8 fractions in which the 8th fraction showed more inhibitory effect against all the pathogenic bacteria except *V.cholerae*. Table 7 illustrates the maximum zone of inhibition against *E.coli* (22 mm) *C. neoformens* (32 mm) and *C.aibicans* 34 mm. The 4th fraction was inhibiting the growth of *E.faecalis* and the inhibitory zone formation was 23 mm. The zone formation was 22 mm against *P. aeruginosa* the 4th fraction .The same fraction showed the maximum inhibitory zone against *C. albicans* (24 mm) and *C. neoformens* (25 mm). The fraction 7 showed inhibitory effect against *C. albicans* and zone formation was 23 mm. The moderate effects were shown by the other fractions.

Table –7 Antimicrobial activities of purified leaf extracts of *C.zizanioides*

Bacteria / Fungi	Average zone of inhibition (mm)							
	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8
E.coli	11	5	10	20	17	14	12	22
K.pneumoniae	13	-	10	21	20	13	12	13
S. typhi	24	7	-	12	21	12	11	16
S. aureus	15	12	8	13	22	14	22	18
V.cholerae	-	-	-	-	-	-	-	-
S.faecalis	20	13	7	17	18	15	19	19
P.aeruginosa	15	20	12	22	17	17	17	17
E. faecalis	17	20	15	23	18	21	18	18
C. albicans	20	27	17	24	33	22	23	34
C.neoformens	20	27	18	25	34	24	26	32

Minimum Inhibitory Concentration (MIC) of root and leaf fraction

The MIC for both root and leaf fraction were determined between a concentration range of 10 mg / mL, 1 mg / mL, 0.1mg / mL and 0.01 mg / mL against the pathogenic bacteria and fungi (Fig. 9-10). From the O.D values recorded at 655 nm a graph was plotted to determine the viability of the pathogenic bacteria and fungi in the presence of the extracts as well as the antibiotic at varied concentrations as said above. From the graphical observation a marked reduction in the pathogenic bacteria and fungi were observed at 10-mg / mL concentration of the extracts. Hence the MIC was 10-mg / mL concentration of the extract and IC_{50} was 5mg /mL to 7.5 mg/ mL. Further screening of antimicrobial property, of the extract was performed at concentration below the MIC. No marked difference in the viability of the pathogens was observed.

IC_{50} for root and leaf fraction

IC_{50} for selected fractions against pathogenic bacteria and fungi was 5 to 7.5 mg/ml. For *E.coli* the IC_{50} was 5.43 mg / mL**,** for *S.typhi,* it was 5.7 mg / mL and for *K.pneumonia* it was 6.1mg / mL. Whereas *P.aeruginosa*, *S.aureus* and *S.faecalis* showed 5.6 mg / mL, 5.9 mg / mL and 6.3 mg / mL respectively. The percentage of inhibition at 50 for *E. faecalis* was 5.6 mg / mL and for *V.cholerae* it was 7.4 mg / mL. The IC_{50} for *C.albicans* was 5.4 mg / mL. For *C.neoformens* the inhibitory concentration at 50 was 5.2 mg / mL. The higher inhibitory concentration against *E.coli* was 5.43 mg / mL followed by *S.typhi* (5.4 mg / mL), *P.aeruginosa* (5.6 mg / mL). And the lower inhibitory concentration against *V.cholerae* was 7.4 mg / mL and *S.faecalis* was 6.3 mg / mL. Against pathogenic fungi *C.albicans* the minimum inhibitory concentration was 5.4 mg / mL whereas, *C.neoformens* showed minimum inhibitory concentration was 5.2 mg / mL.

Among all the pathogenic bacteria, the higher inhibitory concentration was exhibited against *P.aeruginosa* and *S.faecalis* 5.2 mg / mL each. The lower inhibitory concentration against *K.pneumoniae* was 7 mg / mL followed by *E.coli* (6 mg /mL and *S.typhi* (6 mg/ mL). For *S.aureus* and *E. faecalis* the inhibitory concentration was 5.4 mg / mL and 5.7 mg / mL. The inhibitory concentration on the leaf extract against *C.albicans* and *C.neoformens* was 6.9 mg / mL and 5.5 mg / mL.

GC MS Analysis

GC MS study of *C.zizanioides* revealed the qualitative identification of different constituent. The most potent root 5th fraction was analyzed using GC-MS

The chemical identities of 23 compounds from root extract were determined by matching their recorded mass spectra with the data bank mass spectra (NIST and WILLEY libraries) provided by the instrument software and by comparing their calculated retention indices with literature values measured on columns with identical polarity. The structures of the components are displayed in the table 8.

Table – 8 GC analysis of the root 5th fraction

S.No	Compound	Retention time (min)	Area %
1	Cedren – 13-ol-8,	18.99	23.105
2	1-Cyclohexanone, 2 methyl-2- (3 methyl –2-oxobutyl)	18.73	19.833
3	1-Butyn-3-one, 1-(6,6-dimethyl-1, 2-epoxycyclohexyl	21.23	11.119
4	Tricyclo(5,1.0.0 (2,4) oct – 5-ene-5-propanoic acid, 3,3,8,8- tetramethyl-	20.01	9.951
5	Ledene oxide – (II)	17.96	6.505

The chemical identities of the separated 12 compounds from leaf 8th fraction were determined and the structures with maximum retention time and area % of the components are displayed in the table9.

Table – 9 GC analysis of the leaf 8th fraction

S.No	Compound	Retention time (min)	Area %
1	1-Cyclohexanone, 2 methyl-2- (3 methyl –2-oxobutyl)	14.72	21.105
2	Cedren – 13-ol-8,	14.93	20.139
3	4,7 – Octadecadiynoic acid, methyl ester	15.40	20.705
4	Ledene oxide – (II)	14.26	4.798

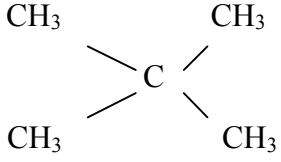
NMR Analysis

Root

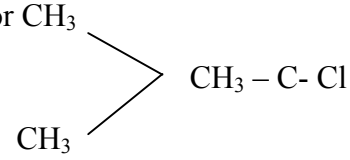
The fraction 5 of root part of the plant, which eluted with chloroform, was subjected to NMR ^{13}C and ^{1}H. The ^{13}C – NMR spectrum indicated 15 carbon signals.

In ^{1}H – NMR spectrum it was 8 proton signals.

Carbon ^{13}C NMR ($CDCl_3$) 300 MHz

δ Value ppm	Possible Carbons
14.13	Aliphatic carbons CH_2, CH_3 chain
23 and 35	Carbons of 5 membered ring
25,24,35.5	Cyclohexane
27	CH_2 in cylcic ring
29,31	CH_2
19	CH_2
105	Carbon of 3rd in 2 methyl pyrrole
77	Carbon attached to OH group
179	COOH (or) Furon ring with carboxaldehyde
181	may be C=O | O H
155,156	COO
38,36,22,26	Methyl group 
123,125,129	May be aromatic carbons
40	May be epoxide
47	May be N $(CH_3)_3$

Proton ^{1}H NMR ($CDCl_3$) 300 MHz

δ Value ppm	Possible Protons
7.2	Solvent peak ($COCl_3$)
3.96 – 4.09	Pentet Aliphatic C4 proton
δ 3.7	CH_2 proton
3.4 to 3.8	Proton attached to Cl atom.
3.1	Halogen attached proton
2.7	Proton attached to C=O Cpds
1.6	$R_2 – CH – C- Cl$ or CH_3  $CH_3 – C- Cl$ CH_3

Leaf

The fraction 2 of leaf part of the plant, which eluted with chloroform, was subjected to NMR ^{13}C and ^{1}H. The ^{13}C – NMR spectrum indicated 7 carbon signals. In ^{1}H – NMR spectrum it was 11 proton signals.

Carbon ^{13}C NMR ($CDCl_3$) 300 MHz

δ Value ppm	Possible Carbons
0.7 to 0.9	Aliphatic protons
1.2 to 1.4	R – CH_2 – R
2.1	OH proton
4.6	Amino or amide Nk2
5.3 and 5.8	Aromatic CH protons
3.5	Methoxy OCH_3
2.3	CH- 
6.4, 6.5, 6.6, 6.7	Aromatic ring protons (H)
9.3, 9.6, 9.7	Amine may be Primary Amines
10.2	CHO
7.5, 7.7	Aromatic Protons

Proton ^{1}H NMR ($CDCl_3$) 300 MHz

δ Value ppm	Possible Protons
0.7	R-CH_3
0.8	Methyl Protons CH_2 – CH_2 – R
1.2 to 1.4	R- CH_2 -R
1.57	R-C=C=C-H
2.3	CH -  phenyl
3.5	Methoxy protons

DISCUSSION

The antimicrobial activity of plant extracts has been recognized for many years. However, few investigations on vetiver have not done against human pathogens like *E.coli, S.aureus, P.aeruginosa, E.faecalis, S.faecalis,V.cholerae, S.typhi, K.pneumoniae,* and pathogenic fungi *C.albicans* and *C.neoformens.* In the present study, root and leaf extracts of 3 samples of *C.zizanioides* grown in 3 different treatments were investigated for anitmicrobial activity. The hot $CHCl_3$ extracts yielded more yield % than the cold.This may due to some compounds like wax, which is temperature dependent. The yield % in E1, was maximum than E2 and E3. The reason may be that in E1 was extracted from the plants grown in normal condition, where as E2 was from the plant grown in wastewater treatment and E3 was extracted from the plant grown in dye contaminated soil. There was no significant difference in phytochemicals of E1, E2 and E3. But the wax content in E3 and E2 was more than E1. The reason may be the plant might have produced more wax to tolerate the stress conditions faced during the treatments, but this was not the case in E1. The antimicrobial activity was significantly observed in E1 extract than in E2 and E3.

Since E1 supported the antimicrobial activity, further purification of the extracts and antimicrobial activity were carried out only in E1. The purified fractions of root extracts were tested against *E.coli, S.aureus, P.aeruginosa, E.faecalis, S.faecalis, V.cholerae, S.typhi, K.pneumoniae,* and pathogenic fungi *C.albicans* and *C.neoformens.* The purified fractions of leaf extracts were tested against *E.coli, S.aureus, P.aeruginosa, E.faecalis, S.faecalis, S.typhi, K.pneumoniae,* and pathogenic fungi *C.albicans* and *C.neoformens*. The results of antimicrobial activity of E1 revealed the maximum zone of inhibition of root in *S.aureus* and *K.pneumoniae* than other tested organisms. Similar results were obtained by Hammer *et al.*(1999). They proved that twenty of the plant oils and extracts were found to have antimicrobial activity against *C.albicans , S.aureus* and *E.coli*. Gangrade *et al.,* (1990); Hammer *et al.,* (1999); Leupin *et al*., (2000) also reported the susceptibility of *S. aureus* to vetiver oil.

The purified fractions of root and leaf of *C.zizanioides* was investigated using a broth microdilution method, for activity against *E.coli, S.aureus, P.aeruginosa, E.faecalis, S.faecalis, V.cholerae, S.typhi, K.pneumoniae,* and pathogenic fungi *C.albicans* and *C.neoformens.* The lowest inhibitory concentration was 10 mg/mL concentrations. This observation was similar to the findings of Putiyanan and Nanathachit, (2000). It was found to be 1,628 µg/mL in second components and 0.78 µg/mL for purified combined column chromatographic fractions. This may be the addition or synergistic effect of many compounds in purified fraction. Hammer *et al.* (1999) reported that the MIC were 0.03% (v/v) theyme oil against *C.albicans* and *E.coli* and 0.008% (v/v) vetiver oil against *S.aureus.*

Putiyanan and Nanathachit (2000) reported that there was antibacterial activities against *S.aureus, E.coli,* and *P.aeruginosa* at 10 % w/v concentrations and antifungal activity against *T.mentagraphytes* at as low as 1% w/v concentration.

GCMS analysis of purified fractions of root contains 23 active compounds and the leaves contain 12 active compounds. The active compounds identified were like cedren-13-ol-8, 1-cyclohexanone, 2, methyl.2-(3 methyl 1-2 –oxobutyl) present in both leaf and root of vetiver.

FUTURE STUDIES

The active constituents need to be isolated and identified. The results from this investigation provide preliminary data for future development and it supports the snotion that the plant (vetiver) contains may compounds of pharmaceutical importance.

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He has been the University Nominee for Academic Council, Board of Examination, and Affiliation Committee to various institutions, Member of various scientific bodies, and member of Editorial Board of Journals. His area of specialization is Environmental Microbiology and Environmental Biotechnology. He loves green environment and has planted about 2.0 lakh trees, constructed rainwater harvesting structures in Bharathiar University Campus and inculcated Environmental Awareness to the students.